Summer School

On

Recent Advances in Crop Improvement, Production and Post-Harvest Technology in Potato Research

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Potato (*Solanum tuberosum* L.) is one of the most important food crops after wheat, maize and rice, contributing to food and nutritional security in the world. This tuber crop of the family solanaceae has about 200 wild species. It originated in the high Andean hills of South America, from where it was first introduced into Europe towards the end of 16th century through Spanish conquerors. There the potato developed as a temperate crop and was later distributed throughout the world largely as a consequence of the colonial expansion of European countries. It was introduced to India by early 17th century probably through British missionaries or Portuguese traders.

Potato: The Crop and the Food

Potato is an annual, herbaceous, dicotyledonous and vegetatively propagated plant. It can also be propagated through botanical seed known as True Potato Seed (TPS). The potato tuber is a modified stem developed underground on a specialized structure called stolon. It contains all the characteristics of a normal stem like dormant bud (eye) and scaly leaf (eyebrow). Potato tuber is a bulky commodity which responds strongly to its prevailing environment thus needs proper storage.Potato is a highly nutritious, easily digestible, wholesome food containing carbohydrates, proteins, minerals, vitamins and high quality dietary fibre. A potato tuber contains 80 per cent water and 20 per cent dry matter consisting of 14 per cent starch, 2 per cent sugar, 2 per cent protein, 1 per cent minerals, 0.6 per cent fibre, 0.1 per cent fat, and vitamins B and C in adequate amount. Thus, potato provides more nutrition than cereals and vegetables. Keeping in view the shrinking cultivable land and burgeoning population in India, potato is a better alternative to deal with the situation.

Potato in India

In Europe the potato crop is grown in summer having long photoperiod of up to 14 hours and the crop duration of 140-180 days. The potato in Indian plains is, however, grown in completely contrasting situations. Nearly 85 per cent of the crop is grown during winters having short photoperiod (with about 10-11 hours sunshine) and the crop duration is also limited to 90-100 days because of short and mild winter. The mornings usually have fog, which further reduces the sunshine hours posing severe constraints on photosynthetic activity. Besides, the post-harvest period consists of long hot summer, which creates storage problems.

All these problems called for suitable varieties and technologies for growing potatoes under the sub-tropical conditions of India. This necessitated to initiate indigenous potato research and development programmes, and accordingly the Central Potato Research Institute (CPRI) came up in 1949 at Patna. The headquarters was later on shifted to Shimla in order to facilitate hybridization and maintenance of seed health. In 1971 the All India Coordinated Research Project (AICRP) on potato was initiated under the aegis of the Indian Council of Agricultural Research (ICAR) at the CPRI with an objective to coordinate potato research and development in the country across diverse agro-ecological regions. The success story of over five decades of potato research in India is phenomenal. Compared to the area, production and productivity in 1949-50, the increase over this period is 550 per cent, 1745 per cent and 178 per cent, respectively (Table 1). India now ranks fourth in potato area (1.48 million ha) and third in production (28.47 million tonnes) in the world with an average yield of 183,3q/ha.

Table 1: Area, Production & Yield of Potato in India

Year	*Area (million ha)*	*Production (million tones)*	*Yield (q/ha)*
1949-50	0.239	1.54	65.9
1959-60	0.362	2.73	75.5
1969-70	0.496	3.91	78.9
1979-80	0.685	8.33	121.5
1989-90	0.940	14.77	157.1
1999-00	1.340	24.71	184.4
2003-04	1.270	23.12	182.0
2005-06	1.400	23.90	170.6
2006-07	1.482	22.09	149.0
2007-08	1.553	28.47	183.3
2008-09	1.810	28.58	157.8
2009-10	1.840	36.58	199.2
2010-11	1.860	42.34	227.2
2011-12	1.910	41.48	217.5
2012-13	1.992	45.34	227.8
2013-14	2.02	46.39	229.2

It was only because of indigenously developed technologies that potato in India has shown spectacular growth in area, production and productivity during the last five decades. The major achievements of potato research in India are as under:

Varietal Improvement

So far 47 potato varieties have been bred for different agro-climatic regions of the country with 28 varieties alone for north Indian plains. Varieties have also been developed for north Indian hills and other special problem areas viz. Sikkim, north Bengal hills and south Indian hills. Of the 47 varieties developed, 19 possess multiple resistance to different biotic and abiotic stresses. Besides, nine varieties are suitable for processing purposes. These are Kufri Chipsona-1, Kufri Chipsona-2, Kufri Chipsona-3, Kufri Himsona, Kufri Frysona, Kufri Jyoti, Kufri Chandramukhi, Kufri Lauvkar and Kufri Surya. All these varieties fall in three maturity groups, i.e. early (70-80 days), medium (90-100 days) and late (110-120 days). The potato varieties developed by CPRI are grown not only in India but also in several neighbouring countries. The variety Kufri Chandramukhi is grown in Afghanistan, Kufri Jyoti in Nepal and Bhutan, and Kufri Sindhuri in Bangladesh and Nepal. Besides, five Indian hybrids are also commercially grown in Sri Lanka, Madagascar, Mexico and Philippines.

Seed Plot Technique

This technique was developed in 1970s to enable healthy seed potato production in the sub-tropical Indian plains under low aphid period. This technique aided by bio-technological approaches for virus elimination, micro-propagation and effective viral diagnostics has sustained the National Potato Seed Production Programme by producing about 2600 tonnes of breeder's seed annually. This breeder's seed is further multiplied to about 4,32,000 tonnes of certified seed by the State Departments of Agriculture/ Horticulture. Thus, the country saves about 484 million

US dollars because most Asian countries like Pakistan, Bangladesh and even China continue to import seed potatoes from Europe.

The decentralization of potato breeding from hills to plains in India through the seed plot technique enabled the development of varieties suited to different agro-climatic regions of the country. The area under seed potato production also increased by 12 times and enabled the availability of seed potato throughout the country in proper physiological state.

Tissue Culture

Efforts are being made to improve seed health standards and reduce the time required for production of breeder's seed by employing *in vitro* techniques of meristem culture and micro-propagation. Presently, about 5 per cent of Breeder's seed production programme is fed annually by microtubers produced through tissue culture. It is proposed to produce 100 per cent of breeder's seed through tissue culture propagated material in the years to come.

Agro-techniques

The development of package of practices for potato production in different agro-climatic zones has helped in improving potato productivity in these zones. The potato crop is input intensive and requires optimum cultural practices for achieving higher productivity. Optimum cultural practices depend on delineated phenological phases of crop growth and development viz. pre-emergence, emergence to tuber initiation, tuber initiation to tuber bulking and tuber bulking to termination of bulking.

The cultural practices are adjusted in the Indian plains in a way so that tuber initiation and development coincide with the period when night temperature is less than 20^{o}C and day temperature is below 30^{o}C. The phenological phase of tuber initiation to tuber bulking is mainly conditioned by nutrition and moisture. For this purpose, fertilizer and irrigation requirement in different agro-climatic zones have been worked out through multi-locational trials under AICRP (Potato). Termination of tuber bulking coincides with onset of foliage senescence. By manipulating the nutrition and moisture, the foliage senescence is delayed for ensuring continuation of linear tuber bulking phase resulting in higher yield.

Several profitable potato-based inter-cropping and crop rotations have also been identified for different regions of the country. Potato can be profitably intercropped with wheat, mustard and sugarcane. These cropping systems have helped in the maintenance of soil fertility and have improved the fertilizer economy, crop yield and gross returns. Besides, potato cultivation has also been mechanized in selected regions through the fabrication and development of cost-effective tools and implements.

Plant Protection

Effective management practices have been devised for the major potato diseases and insect-pests in India. Late blight is the most notorious disease of potato which occurs almost every year in the hills and plains. Besides chemical control measures, several late blight resistant varieties have been developed. Potato varieties have also been bred which possess resistance to wart and cyst nematodes. Cultural and biological control measures have also been developed to control the diseases and insect-pests. The development of late blight forecasting systems for hills and plains has enabled the early warning mechanism for the appearance of late blight disease.

Storage

In European countries, the potato crop is grown in summer and the main storage season is the cold winter. However, in India, 85 per cent of potato is produced in winter and stored during long hot summer. This requires storage of potatoes in cold stores at 2-4^{0}C, which involves substantial cost. It also leads to accumulation of reducing sugar in the potato tubers resulting in sweetening of potatoes. However, there are a number of traditional low-cost and non-refrigerated storage structures (essentially based on evaporative or passive evaporative cooling) in use in India with varying degrees of success. These traditional structures have been studied, validated and popularized for particular regions. In non-refrigerated storages, use of sprout suppressants has also been popularized to prevent excessive weight loss and shrinkage due to sprouting. The CIPC (isopropyl-N-chlorophenyl carbamate) is the most effective sprout inhibitor when applied @ 25 mg a.i. per kg tubers.

Processing and Value Addition

In addition to raw consumption, potatoes can be processed into several products like chips, French fries, cubes, granules and canned products. The primary determinants for potato processing include high dry matter and low reducing sugar content. A dry matter content of more than 20 per cent is desirable for chips, French fries and dehydrated products. Similarly, a reducing sugar content in tubers up to 100 mg/100g fresh weight is considered acceptable for processing. Nine varieties viz. Kufri Chipsona-1, Kufri Chipsona-2, Kufri Chipsona-3, Kufri Jyoti, Kufri Chandramukhi, Kufri Lauvkar, Kufri Surya and Kufri Himsona, Kufri Frysona have been developed for processing purposes. In India, potato processing in organised sector started about a decade ago, and the recent proliferation of this sector mainly results from the development of three indigenous potato processing varieties, viz. Kufri Chipsona-1 and Kufri Chipsona-3 by CPRI. These two varieties are now being used by the industries for processing into chips and French fries.

Computer Applications

Simulation modelling is now widely used in various disciplines to work out tactical decisions. CPRI has developed INFOCROP-POTATO model to simulate the potato growth and development, to determine the best growing period, to optimise management practices under different agro-ecological regions, and to forecast the accurate yield estimates. An expert system (Potato Pest Manager) has also been developed for decision support with respect to identification and management of diseases and insect-pests.

Transfer of Technology

Research achievements alone are not adequate to gauge the success of an agricultural system. The research information needs to be assessed and refined under various bio-physical and socio-economic situations through adaptive research before it is labelled as a technology. In this regard, the multi-locational trials under AICRP (Potato) and the TOT projects undertaken by CPRI such as Operational Research Project (ORP), Lab-to-Land Programme (LLP), Tribal Area Development (TAD) programme and Institution-Village Linkage Programme (IVLP) proved landmark in getting feedback from the field and development of appropriate technologies.

Transfer of technology to the end users is a complex task which consists of a number of components and dimensions. One of the important components is proper linkage between technology generating system and the client system. In this regard, innovative approaches like need assessment, participatory planning and implementation, and direct scientist-farmer interface facilitated faster dissemination of technologies and consequent adoption by the farmers/clients.

The CPRI has build up linkages with farmers through demonstrations, trainings, Kisan Melas, potato school on All India Radio, supply of literatures and other extension activities. Besides, studies have been conducted to measure the socio-economic impact and constraints in transfer of potato technology.

Potato Export

Although India contributes 7.55% to the total world potato production, its 0.7% share in world's potato export is quite insignificant. Indian potatoes are truly free from the prohibited disease like wart, black scruf, and pests like tuber moth and nematodes, which are the barometer for phytosanitary standards. India has also the natural advantage of exporting fresh table potatoes during January to June when supply from European countries dwindles. It can also supply fresh potatoes round the year because India has diverse agro-climates and potato is grown throughout the year in one or the other part of the country. Potato has a good future in India under the changed scenario of global economy. Globalisation has resulted in many developing countries becoming much more integrated into the international potato trade. With the phasing out of quantitative restrictions on agricultural commodities, the imports and exports of potato would be based on the differences in price and production cost between the importing and exporting countries involved. Due to low production cost in the country as a result of availability of cheap labour, India will have competitive advantage in the international potato trade.

Potato in the New Millennium

With the improvement in the living standard of people in India, the dietary habits will shift from cereals to vegetables. Under such a situation it is estimated that India will have to produce 49 million tonnes of potato by 2020. This target could be achieved only by improving the productivity level. The productivity of potato in India is quite low (183.3q/ha) as compared to that of Belgium (490q/ha), New zealand (450q/ha), UK (397q/ha) and USA (383q/ha). This is due to shorter crop duration in India. There is a wide ranging variations in the agro-ecological setting of different parts of the country, which results in wide variations in the productivity levels of different states (Table 2). Therefore, all our efforts may be put in to develop location-specific and problem-specific varieties and technologies.

Table 2: Statewise Area, Production & Yield of Potato in India during 2012-13

States	Area (,000 Hectares)	Production (' 000 Tonnes)	Yield (q/ha)
Uttar Pradesh	603.76	14430.28	239.0
West Bengal	386.61	11591.30	299.8
Bihar	322.50	6640.60	205.9
Assam	99.77	975.27	97.7
Madhya Pradesh	108.87	2299.00	211.2
Punjab	85.25	2132.31	250.1
Gujarat	81.27	2499.73	307.6
Jharkhand	47.21	659.61	139.7
Karnataka	44.40	698.30	157.3
Haryana	29.47	676.01	229.4
Others	183.2	2741.20	149.7
Total	**1992.2**	**45343.60**	**227.6**

Source: Directorate of Economics & Statistics, Govt. of India.

Most of the people in India have either no knowledge or wrong notions about the nutritive value of potato. With low fat (0.1 per cent) and calorie contents, it does not cause obesity. Due to misconception the potato consumption, the per capita consumption of potato in India is only about 16 kg/year. On the other hand, the per capita consumption in Europe is 121 kg/year and as high as 136 kg/year in Poland. Hence, there is ample scope for improving the consumption of potatoes in India. For this purpose, a publicity compaign like eggs and milk needs to be launched through mass media such as television, radio and newspapers highlighting its nutritional value. Moreover, the possibility of using surplus potatoes as animal feed also needs to be explored.

The surplus potatoes in a season are stored in cold stores at 2-4^{o}C in the country. This makes stored potatoes just unfit for processing and loses preference for table purposes due to accumulation of sugar content. To avoid sweetening potato are required to be stored at 10-12^{o}C. Only seed potatoes should be cold stored at 2-4^{o}C. This would release atleast 60 per cent of cold storage space that can be converted to store potatoes for processing and table purposes at 10-12^{o}C with CIPC treatment leading to considerable savings on energy and storage costs.

Processing is a fast growing sector in the potato world economy. Due to increased urbanization, rise in per capita income and expanding tourism, the demand for processed potato products in India and international market has risen at a fast pace. However, in India, processing of potatoes constitutes less than 2 per cent of the total annual production as compared to 60 per cent in USA, 47 per cent in the Netherlands and 22 per cent in China. Hence, there is great scope to expand the potato processing industries in India and also to diversify the processing to produce flour, cubes, granules, flakes and starch.

Under the changed global scenario, the potato production and utilisation pattern is changing very fast. These changes harbour many opportunities which could be tapped through effective extension system. The use of modern information and communication technologies (ICT) to create awareness is highly pertinent in the contemporary times. This would enable us to reach directly to the end users by eliminating the intermediate channels which create distortion of information. Efforts are also needed to devise market-based extension strategies in order to promote entrepreneurship among potato growers with regard to potato production and marketing.

Disease and Pest Management in Seed Potato Production

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Seed potato crop can be affected by many diseases and pests. The major diseases which reduce quality of the seed are caused by viruses, fungi and bacteria. Potato pests which act as vectors for the spread of the viruses play an important role. The losses caused by potato viruses are not confined to the year when infection takes place but continue to affect the crop as long as the diseased tubers are used as seed. The virus infected degenerated seed cannot make full use of the inputs like fertilizers and water thus produce low yield and poor quality of the tubers. Therefore, it is very important to manage diseases and pests in disease free seed potato production facility. Major viruses such as PVX, PVS, PVA, PVM and PVY, ToLCNDV and GBNV need to be managed. Fungal diseases such as black scurf and bacterial diseases such as common scab hold special importance in seed potato production since the maximum permissible limit of the infected tubers is only 5% in seed certification and hence need to be managed within the limit. Potato pests such as aphids, whiteflies, leafhoppers and thrips act as vectors for the spread of the viruses from plant to plant and thus must be managed in any successful seed production programme. Seed potato production is not permitted in land infested by wart (*Synchytrium endobioticum*), cyst forming nematodes (*Globodera* spp.), in area infested with brown rot (*Ralstonia solanacearum*) and common scab (*Streptomyces scabies*). The description and management of major diseases and pests that affect seed potato production is given here.

Viruses: Potato virus X (**PVX):** Plants infected with PVX often remain symptomless. When symptoms are expressed, there is a pattern of light and dark green on leaflets, the lighter, small, irregular blotches occur between the veins. The yield loss vary between 10-25%. PVX can interact with PVY and PVA to cause more severe symptoms such as rugose mosaic (PVX+PVY) and crinkle mosaic (PVX+PVA) resulting in higher yield loss. The major mode of transmission of PVX is through the infected seed tubers mainly through cutting of tubers into pieces using an unsterilized knife, through mechanical injury of tubers, sprouts and foliage. PVX spreads easily through worker's contaminated hands, clothes, implements, farm machinery, seed trays etc. PVX can spreads rapidly in poorly cared crop resulting in upto 50% or more infection with in 3-4 cycles of cultivation. Since PVX is readily transmitted through contaminated hands, clothes of workers, farm machineries/ tools therefore, unnecessary cultivations should be avoided. Use of herbicides can reduce the number of cultivations required. Use of 3 % trisodium phosphate or sodium hypochloride solutions (1:50 dilutions) can inactivate this virus in hands, tools and knives used to cut tubers. General practices of sanitation and roguing to remove infected plants from the field can reduce the inoculum.

Potato Virus S: PVS is contact and non-persistently aphid transmitted virus. PVS like PVX is generally symptomless on the majority of potato cultivars. However, symptoms such as rugosity, mosaic, stunting, bronzing, vein deepening, early drop of leaves, and fine necrotic spots on lower leaves have also been reported occasionally in some cultivars.

Potato Virus M: PVM causes mottle, mosaic, crinkling, rolling of leaves and stunting of shoots. Severity of symptoms greatly depends on the combination of potato cultivars and PVM isolates. The main sources of PVM inoculum are infected seed tubers or seed material. It is experimentally sap transmissible. Aphids are the main vectors which transmits PVM in non-persistent manner from infected to healthy potato plants. The produce from infected plants carry virus in tubers which act as source of inoculum for the next year when used as seed and the cycle repeats.

Potato Virus Y (PVY): PVY is one of the most economically important virus of potato worldwide including India. Depending on cultivars and virus strains, it can cause up to 80 %

yield reduction. It has different strains. Strain PVY^O occurs worldwide. However, PVY^N and PVY^{NTN} strains are limited to certain countries, and therefore, are of quarantine significance.
Generally the symptoms of PVY vary with the strains of the virus and variety of potato. In general the affected plants are stunted with mild or severe mosaic and veinal necrosis. Original strain of PVY^O causes mosaic, necrotic spots, mild rugosity and drying of leaves whereas, PVY^C usually causes stipple-streak or necrotic veins / stars. PVY^{NTN} strains cause severe superficial tuber necrosis and may also causes necrotic foliar symptoms. Infected seed tubers or seed material is the main sources of PVY inoculum in tropical countries Solanaceous weeds may also serve as a reservoir of the virus. Aphids (*Myzus persicae*) probing on potato plants are potential vectors of PVY which transmit the virus to other healthy plants. The infected tubers produce the infected plant which might act as primary source of inoculum for the next year crop.
PVA: *Potato virus A* (PVA) causes mild mosaic symptoms somewhat similar to those caused by *Potato virus X* (PVX). However, unlike PVX the mottles caused by PVA appear on the veins and the infected leaves look shiny. Infected plants show an open habit. Severe leaf crinkle may be produced in combination with PVX. Control measures are similar to those for PVY which include planting of certified seed free from virus, growing the crop in aphid free period, elimination of sources of virus by roguing in seed crops, destroying self sown plants, early harvest of seed crops and the use of foliar insecticides with a repellent action.
PLRV: Potato leafroll virus (PLRV) is found wherever potatoes are grown. The infected plants may result in 50% or more reduction in yield. Depending upon the stage of infection, the infected plants show two type of symptoms *viz*., primary or secondary. The primary symptoms are confined to top young leaves, which usually stand upright, roll and turn slightly pale in certain cultivars. Most varieties, however, develop reddish/pink colour in top leaves starting at the margins, sometimes accompanied with slight rolling of the leaflets. Secondary symptoms develop when the plants are grown from infected seed tubers. Such symptoms are rather prominent in older leaves and absent or less pronounced on younger top leaves. Infected plants have characteristic pale, dwarfed, and upright appearance with rolling of lower leaves that turn yellow, brittle and leathery in texture. In some cultivars, a reddish or purple discolouration develops on the margins and underside of the leaves. The virus is tuber borne, transmitted efficiently by aphid in a persistent manner. The aphids normally need longer feeding periods on diseased and healthy plants. Primary infections are normally caused by the viruliferous aphids coming either from distant or nearby fields and/or diseased volunteer plants arising from the infected tubers. Potato plants infected with PLRV will produce infected tubers. If infected tubers are planted they will give rise to infected plants. The green peach aphid (*Myzus persicae*) is the most efficient and important vector of PLRV. The virus is also spread by *Aphis gossypii*. *Macrosiphum euphorbiae* which transmits potato strains less efficiently. The production of healthy certified seed – from virus free clonal material initially and its subsequent multiplication in areas of low risk for virus spread is the most important control measure. Elimination of sources of the virus through roguing of diseased plants in seed crops and killing of volunteer plants reduces the risk of spread of PLRV.

Tomato leaf curl New Delhi virus-potato apical leaf curl disease

In India, apical leaf curl disease of potato was observed in 1999 from Northern India. It is caused by *Tomato leaf curl New Delhi virus* (ToLCNDV). Heavy incidence of this disease has been recorded in Indo-Gangetic plains and Hisar area of India with significant yield losses in susceptible varieties. The affected plants show curling / crinkling of apical leaves with a conspicuous mosaic symptom. In case of seed borne infection the entire plant shows the symptoms of leaf distortion with mosaic accompanied with severe stunting of the plant. The symptoms are so severe that it can be identified easily, but such symptoms are expressed only when the concentration of virus is very high. However, in many cases the symptoms are

recovered as the maximum temperature fall below 28 °C. If the virus concentration is less the leaves show rolling which is just like symptoms caused by Potato virus M (PVM).

The disease usually appears in early planted crop and when a high population of whitefies is observed in field. The incidence of apical leaf curl is positively correlated with whitefly population and whitefly infestion period on potato crop. The disease incidence is less in late planted crop in all the cultivars due to lower population of whitefly. This begomovirus is transmitted by *B. tabaci* in a circulative and persistent manner. The virus is also transmitted through seed tubers. Removal of virus infected plants helps to minimize spread of the virus within the field. Application of mineral and vegetable oils has been found to inhibit virus transmission. This can also help to avoid development of insecticide resistance in whiteflies.

Groundnut bud necrosis virus

GBNV, a *Tospovirus*, causes stem necrosis disease in potato (PSND). Heavy incidence of stem necrosis has been recorded in some parts of Madhya Pradesh, Rajasthan and Pantnagar area in Uttaranchal. PSND is important in areas where thrips, the vector of GBNV, and the virus sources occur together. It is rather common in early crop of potatoes in Central/Western plains and plateau regions of India. Losses upto 29 per cent have been reported by PSND. Infected plants show extensive necrosis with formation of concentric rings or spots on leaves and stems. Primary infection shows as necrotic spots or rings on youngest leaflets. The spots may have concentric zonation and may be confused with early blight. Brown necrotic lesions develop on petioles and stems and within the stem. Death of one or more stem tops may occur or, in extreme cases, the whole plant may die. In secondary infection, plants arising from infected tubers are stunted and exhibit bunching of leaves. The leaflets are coarse and may desiccate and turn brown. Infected plants may appear normal and tubers may show distortion or cracking and remain small in size. Several thrips species belonging to the genera *Thrips* and *Frankliniella* act as the virus vectors in a persistent manner but they can acquire the virus only as nymphs, with 4-9 days of latency and about 1 hr inoculation access. Viruliferous thrips move to early crop of potatoes from other preceding crops and infect the potato crop. Early crops can escape disease by completing the susceptible young crop stage before the arrival of virus-containing vectors. Controlling weeds within and around potato crop reduces the thrips population which acts as potential reservoir of the virus. Soil application of granular systemic insecticides or foliar insecticides reduces vector population. The insecticides can be applied to the potato crop or border catch crops. Insecticides are effective when thrips are actively feeding as larvae or adults. Planting the crop towards end of October in NW Plains is helpful in reducing the disease by avoiding crop exposure to the vector. Use of systemic insecticide either as tuber dressing or/and foliar sprays also helpful in managing the disease.

Integrated strategies to mange major Potato Viruses

- Use disease free seed
- Plant seed crop in vector free period identified for the zone.
- Treat seed with imidacloprid @4ml/10 litre before planting
- Apply Phorate (Thimet) 10 G @ 10 kg /ha at earthing
- Spray imidacloprid @3ml/10 litre at emergence and at 30 days
- Spray an alternative insecticides such as thiamethoxam (25WG) @0.05% at 50 days
- Remove virus infected plants along with their tubers in least in three rouging at 30, 50 and 70 days.
- Spray mineral oil to manage the insect vectors.
- Spray demeton methyl (rogor or metasystox) 30 EC @) 1 litre/ha around 60-70 days of crop growth.
- Cut haulms when aphid population is likely to cross critical limit of 20 /100 compound leaves.
- Check and remove re-growths if any

Fungal & Bacterial Diseases: Tuber borne diseases such as black scurf and common scab are especially important in seed potato production because their maximum permissible limit in seed certification is limited to 5 percent.

Black scurf: Black scurf of potato caused by *Rhizoctonia solani* is a well known disease of potato with worldwide distribution. It causes damping off of seedlings, stem canker in growing plants and black scurf on potato tubers. The disease kill potato sprouts, delay crop emergence, reduce crop stand, affects tuber quality and marketability of the produce. The most common symptoms are on tubers as black irregular lumpy encrustations of fungal sclerotia which stick to the surface of tubers. Other symptoms on tubers could be cracking, malformation, pitting and stem end necrosis. Use of healthy seed free from sclerotia of the pathogen helps in disease management. Soil solarization with transparent polyethylene mulching during hot summer months in Indian subtropical plains has been found to be very effective for control of the soil borne part of the disease. Crop rotation offers an effective protection against soil borne inoculum of *R. solani*. Planting of potato should be carried out in relatively dry and warm soil to achieve rapid crop emergence. Shallow covering of seed tubers allows less opportunity for the fungus to attack the susceptible sprouts and thus less disease. Two to four year crop rotation with cereals and legumes leads to decline in the population levels of the *R. solani.* Cereals are good rotational crops since *R. solani* affecting cereals have different AG group of the pathogen and cannot affect potato. Various fungicides such as benomyl, thiabendazole, carboxin, pencycuron, azoxystrobin and fenpiclonil are effective for control of the disease. Seed treatment with 3% boric acid has been identified as a safe and effective chemical treatment for the control of black scurf. Seed treatment with 3 per cent boric acid as atomized application on infected tubers is more economical than the dip treatment for control of seed inoculum. An integrated disease management approach involves a combination of soil solarization carried out in Indian Plains during summer months together with seed treatment with 3 per cent boric acid before seed storage and incorporation of *Trichoderma viride* at planting of the crop.

Common Scab: Common scab appears on tuber surface in different forms. This could be just mild abrasions, star shaped corky depositions, concentric wrinkled layers of cork around a central black core, raised and rough corky pustules or 3 to 4 mm deep pits surrounded by hard corky tissue. Mulching during earlier part of season and frequent irrigation to maintain soil moisture approaching field capacity from tuber initiating stage to maturity of the crop is generally recommended for control of common scab. Soil solarization with transparent polyethylene mulching during hot summer season has been found effective for management of russet type of scab. The disease severity by soil solarization reduces to almost one third as compared to the unsolarized plots. Growing crops such as maize, cotton, grain sorghum, wheat, cabbage and onion in rotation with potato reduce incidence and severity of common scab. Seed treatment with 3% boric acid as dip for 30 minutes can be used for management of tuber borne pathogen. The disease can also be reduced by use of acidic fertilizers such as ammonium sulfate, single super phosphate; and potassium chloride. Lower disease incidence has been observed with higher concentration of water soluble aluminum in soil. Application of magnesium and manganese sulfate, and sulfur to potato crop has also been found to reduce the disease.

Common scab can be managed by summer cultivation, use of disease free certified seed, following crop rotation with non host crops, maintaining soil pH and maintaining soil moisture approaching field capacity at 7-10 days interval at tuber initiation stage can help in minimizing common scab.

Potato Pests important to seed potato production

Aphids: There are more than 15 species of aphids which infest potato. Among them *M. persicae* is known as green peach aphid or peach-potato aphid is the most important aphid. *Myzus persicae* is polyphagous and colonizes hundreds of plants belonging to more than 40 families. Aphids suck the sap from phloem of potato stems, leaves and roots using their stylets and inject viruses in the plants. Aphids have complex life cycles. Aphids molt four times. The mean number of offspring per wingless aphid range from 60 to 75. Optimum temperature for

reproduction is around 21°C. Adults and nymphs both suck the sap from leaves and many tiny adults could be seen on underside of the leaf. Important species known to infest potato are green peach aphid, *Myzus persicae*, cotton aphid, *Aphis gossypii*, bean aphid, *Aphis fabae*, buckthorn aphid, *Aphis nasturtii*, *Aphis spiraecola*, *Aphis nerii*, *Rophalosiphum rufiabdominalis*, *Rophalosiphum padi*, potato aphid, *Macrosiphum euphorbiae*, cabbage aphid, *Brevicoryne brassicae* and pea aphid, *Acyrthosiphon pisum*. In mid hills of Himachal Pradesh, Jammu and Kashmir and Uttarakhand, the aphid population starts appearing from 1st and 2nd week of June and reaches the critical levels of 20 aphids / 100 compound leaves by 4th week of July. In higher hills (> 2500m) of these states, the aphid population remains below the critical levels throughout the crop season which makes these hills highly suitable for seed potato production. The aphids in north western and northern plains appear by the second week of November and reach the critical level by third week of December. In case eastern UP, Madhya Pradesh and West Bengal the aphids appears in mid December and reaches the critical level by second and third week of December. The aphid population in Karnataka is high through the crop season. The low aphid areas and low aphid periods can also be used for seed production by integrating one or two sprays of systemic insecticide in the seed production schedule. Growing seed potato crop in aphid free or low aphid period by adopting seed plot technique and elimination of alternate hosts of both aphids and the viruses is very helpful in disease management. Row covers, reflective mulches or coarse net covers are helpful in delaying or reducing disease transmission. Spray of vegetable/mineral oil could inhibit virus acquisition, virus attachment to mouth parts and reduce probing behavior of aphids. Application of mineral oil to manage aphid population in the field is thus a promising tactic. However, use of oils should be avoided during hot weather. Yellow sticky traps have been developed and standardized for monitoring the aphid population in potato crop. According to a study when aphid count reaches 20 on 100 compound leaves recorded by visual count the corresponding figure for water traps and sticky trap would be 9.4 and 3.0 respectively. The haulm should be cut or chemically destroyed before critical aphids level reach 20 aphids/ 100 compound leaves. Among insecticides Oxydemeton methyl 25EC @ 1.25 l/ha and Imidacloprid 17.8SL @ 4ml/10 litres gives good control of aphids. However, it is unable to disrupt transmission of non-persistent viruses from the viruliferous aphids to the plants using the insecticides, therefore, total dependence on insecticides should be avoided in case of non persistant viruses.

Whitefly: Whitefly (*Bemisia tabaci*) has become one of the most important pests worldwide in subtropical and tropical agriculture as well as in greenhouse production systems. Whiteflies being polyphagous feed on a large number of plants. Over 900 host plants have been recorded for *B. tabaci*. It transmits about 111 types of viruses. Most of these belong to Gemini or the Carla virus groups. Yield losses caused by such viruses range from 15 to 100 percent. In India, *B. tabaci* is known to transmit a serious disease of potato caused by *Tomato leaf curl New Delhi virus-potato*. It is an emerging disease in northern and Indo-Gangetic plains. Whiteflies have six life stages viz. egg, four instars and the adult. The female lays up to 300 eggs singly on the underside of the leaf. The first instar known as the crawler, has legs and it is the only mobile instar that moves to look for feeding sites. The other instars are sessile and they complete their lifecycle on the same leaf. Completion of the life cycle at 25±2ºC takes 20 days. The whiteflies can complete 11-15 generations in year depending upon the weather conditions mainly temperature and humidity and the plant species. The whiteflies are known to survive on tomato, chilli, brinjal, capsicum, okra, cotton, tobacco, cucurbits, crucifers and a few weeds. In Indo-Gangetic plains of India population build up of whitefly is heavy in September and early October planted potato crop. The population however, declines with drop in temperature in November and December. Maintaining field sanitation by removing and destroying the weeds and crop residues, growing barrier crops like sorghum (*Sorghum bicolor*), Johnson grass (*Sorghum halopense*), corn (*Zea mays*) and elephant grass (*Pennisetum purpureum*) can also help in reducing the buildup of whitefly population. Avoid weed hosts, excess irrigation and nutrition. Removal and destruction of heavily infested plants, conservation of natural enemies such as

Parasitoids and predators are good cultural practices to reduce pest population. The use of inert ground covers such as plastics, sawdust, straw and rice husk mulches, has been attributed to interference with visual host-finding and suicidal attraction to the sun-heated mulch. Placing yellow sticky traps in field can reduce whitefly population in field. The ToLCNDV causing apical leaf curl disease of potato can be successfully controlled by managing the vector population with seed treatment of Imidacloprid 17.8SL (0.04%) and first foliar sprays of Imidacloprid 17.8SL (0.03%) at 85% germination and second of Thiamethoxam 25WG (5gm/10lit of water) after 10days of the first spray.Varieties Kufri Bahar, Kufri Chipsona-1 and Kufri Chipsona-2 are comparatively tolerant to whiteflies.

Leafhoppers: Potato leafhopper (*Empoasca fabae*) is a polyphagous pest distributed all over tropical and subtropical countries in the world. In India, the leaf hoppers are distributed in all potato growing regions of Indo-Gangetic plains. The hoppers infest potato crop grown early in the season. Adults and nymphs feed by inserting a needle-like beak into the plant and sucking out the sap. They also inject a toxin into the plant, which causes yellowing, browning, and curling of leaves. In potato, the leaf veins turn yellow, the leaf margins turn brown and brittle followed by drying of entire leaf. The adults and nymphs are somewhat wedge-shaped with heads that are slightly broader than the rest of their bodies. They move diagonally when disturbed. The feeding of leaves results in drying and the symptoms are known as hopper burn. Such symptoms can be easily identified wedge-shaped, triangular mark of burn, starting from the tip. It may cause cupping of the affected leaves. The severely infested field gives a burnt look appearing in a circular ring commonly known as "hoper burn". Yield losses can be tremendous if the pest is not controlled within 30-40 days of planting. Some leafhopper species are known to be vector of purple top roll of potato.This pest can tolerate a wide temperature range (8-29^{o}C). The pest appears with the onset of cloudy weather; however, the heavy rain can wash away the leaf hoppers. Early-season and red varieties of potato tend to suffer more damage than the long-season varieties. Crop rotation with suitable non-host crops and maintaining proper isolation distance in potato crop would reduce the population. A delay in planting to 25th September for early crop and to middle of October for the main crop would reduce the leaf hopper incidence. Judicious use of nitrogenous fertilizers and balanced plant nutrition checks the hopper multiplication. Early planted potato crop need to be protected from leafhoppers through foliar spray of insecticides. In seed crop, granular systemic insecticide such as Phorate10G@) 10.0kg/ha may be applied at the time of planting or at the time of earthing up. Dimethoate 30EC and methyl demeton 25 EC applied at the appearance of the pest, and phorate 10 G applied at planting can help in control of leafhoppers. Spraying late in the day or in the evening may provide better control than spraying early in the morning.

Thrips: Thrips such as *Thrips palmi* are small insects distributed in most potato growing in Indian plains. Thrips appear early in the season. The infestation is more in potato crop planted during first and second week of September. However, the delayed planting reduces the incidence of the thrips significantly. Nymphs feed in groups, particularly along the leaf midrib and veins of older leaves. Adults tend to feed on young growth and prefer to hide in complex plant parts especially on new leaves. Thrips are the vectors of tospo viruses such as GBNV which cause stem necrosis disease in potato. Eggs are colorless to pale white in colour, bean-shaped and are deposited in leaf tissue by a slit cut by the female. Larvae resemble the adults in general body form though they lack wings and are smaller. These are tiny, slender, fragile insects, with adults having heavily fringed wings. Life cycle is completed in about 20 days at 30^{o}C. Providing adequate irrigation is an effective control measure since dry weather helps them to thrive well. Thrips can also be controlled with the systemic insecticide such as Imidacloprid 17.8SL @0.3% which is also used to control aphids and whiteflies.

Indian table and processing potato varieties for different agro-climatic regions

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One of the most important factors governing productivity of a crop is the 'variety'. Thus breeding of improved cultivars is of paramount importance. Variety development, however, is a continuous process as new biotic and abiotic stresses continue to arise and the variety previously resistant or tolerant to such stresses may become susceptible due to the evolving of new strains/races of the pathogens or insects and also due to emerging abiotic factors. Performance of a variety depends on the agro-climatic conditions under which it is grown and also the purpose for which it has to be used. Thus the CPRI has been developing potato varieties suitable for cultivation under varying agro climactic zones of the country and also for different purposes i.e. table and processing.

Ecological zones and varietal requirements:

India has diverse soil types and agro-climatic conditions. Successful potato cultivation requires night temperatures of 15-20^{o}C with sunny days. Indian sub-tropical plains offer optimum conditions for potato cultivation, where 85-90 per cent of potatoes are grown during short winter days from October to Feb. The hills account for less than 5 per cent of the total potato production where the crop is grown during long summer days from April to September/October. The plateau regions of South-eastern, central and peninsular India constitutes about 6 per cent area where potato is grown mainly as rainfed or irrigated winter crop. On the basis of the diverse soil, climate and other agronomic features, the potato growing areas in India can be divided into eight zones (Table-1). These zones lay in two major potato growing areas i.e. north Indian hills and north Indian plains, while southern and north Bengal and Sikkim hills and plateaus are three special problem areas. The varietal requirements of these regions are given in Table 1.

Table 1. Potato growing zones in India and their varietal requirements

Zone	Varietal requirements
North-western hills	Long day adapted , highly resistant to late blight
Central hills	Long day adapted, highly resistant to late blight and bacterial wilt.
North-eastern hills	Long day adapted, highly resistant to late blight and bacterial wilt.
North-western plains	Short day adapted, early bulking, heat tolerance and moderate resistant to late blight, slow rate of degeneration. Tolerance to frost is an added advantage.
West-central plains	Short-day adapted, early bulking, moderate resistant to late blight and slow rate of degeneration. Tolerance to frost is an added advantage.
North-eastern plains	Short day adapted, early bulking, moderate resistant to late blight and slow rate of degeneration. Red skin tubers are preferred in some areas.
North Bengal hills and Sikkim	Medium maturity, resistance to late blight and immunity to wart. Red skin potatoes are preferred.

Plateau region	Early bulking, ability to tuberize under high temperatures, resistance to bacterial wilt, mites & potato tuber moth and slow rate of degeneration.
Southern hills	Long day adapted, early bulking, resistant to late blight and cyst nematode.

Quality attributes of potato for table and processing purposes: Marketability of potato produce is a function of its quality. Appearance, colour, size, shape and defects decide the quality for fresh potato. Total solids or dry matter is highly correlated with texture. On the basis of dry matter and texture, potatoes can be used for different purposes. A mealy texture is associated with high solids and a waxy texture with low solids. Mealy textured varieties are usually considered best for baking or French fries. Varieties with waxy texture are more often used for boiling or as salad. In India, mostly white, yellow or red skinned varieties with shallow or medium eyes are the choice of the consumers. Interest now seems to be shifting towards yellow fleshed varieties. More yellow flesh color is indicative of the higher level of vitamin A. Yellow fleshed varieties have a richer flavour than traditional white fleshed varieties and exhibit less darkening after cooking than some red skinned varieties. Specific characteristics of potato varieties for different purposes are listed in table 2.

The varieties should be widely adaptable, resistant to major diseases and pests, possess good keeping quality and can be used either for table or processing or both. Varieties should produce attractive, medium sized, shallow eyed, white, yellow or red skinned tubers, less physical injuries with good keeping and nutritional quality. For processing purposes varieties should possess high dry matter, low reducing sugars and less tuber defects for producing quality processed potato products. Low glycoalkaloids content and ability to withstand cold induced sweetening are added advantages.

Table 2. Requirement of potato varieties for different purposes

Characters	**Use requirements**			
	Table potatoes		**Processing**	
	Boiled	**Baking**	**French fries**	**Chips**
Tuber shape	Long-oval/round	Long-oval/round	Long-oval (>3 inch)	Round (2.5-3.3 inch)
Skin color	White/yellow/ Red	White/yellow /red	White/ yellow	White/ yellow
Eye depth	Shallow/ medium	Shallow/ medium	Shallow	Shallow
Flesh color	White/yellow	White/yellow	White/ yellow	White/ yellow
Texture	Waxy	Mealy	Mealy	Mealy
Uniformity	High	High	High	High
Defects	Minimum	Minimum	Minimum	Minimum
Dry matter (%)	18-20	>20	>20	>20
Reducing sugars*	-	-	<200mg	<100mg
Phenols	Less	Less	Less	Less
Glycoalkaloids *	< 15mg	< 15mg	< 15mg	< 15mg
Keeping quality	Good	Good	Good	Good
Damage resistance	High	High	High	High

*mg/100g fresh tuber weight

Potato Improvement

Basics: Cultivated tuber-bearing potato is a clonally propagated crop and maintained over generations through tuber seeds. The plant is tetraploid (2n=4*x*=48) with a complex tetrasomic inheritance combined with high degree of heterozygosity. Pure line breeding is not practiced in potato owing to heterozygosity and high degree of pollen sterility. Selfing of fertile clones results in inbreeding depression. Conventional potato breeding within the ploidy levels involves hybridization between superior clones followed by selection. The vegetative mode of propagation offers distinct advantages. It leads to the perpetuation of a specific gene-combination with precision over generations, thus allowing the breeders to select and maintain, with ease, outstanding segregants in breeding programme and obtain indefinite number of genetically identical individuals. Various hybridization methods like distant crossing, some time followed by back-crossing, bi-parental cross, multiple cross and poly-cross are usually utilized in potato breeding. Besides the above traditional approaches, non-conventional methods are also used in potato breeding and germplasm improvement programmes taking advantages of diversity in reproductive biology like synaptic mutants, unilateral sexual polyploidization, haploidy, stylar barriers, endosperm barriers, endosperm balance number (EBN) etc. Many a times, di-haploids are evolved for production of homozygous lines or for pre-breeding at diploid level for transferring desired traits through transgression.

Early attempts: From its initial status of a garden vegetable in Western India in early 17th century, potato cultivation spread to diverse eco-zones in India over the next two and a half centuries. Early potato introductions in India were *S. tuberosum* ssp. *andigena*. There was enormous confusion regarding the identity and nomenclature of these introductions as these were known by different local names in diverse dialects. As a result, during the initial periods of potato research in India, efforts were directed towards identification of such local "*desi* varieties". Based on the studies on various morphological features, duplicate samples were eliminated, and subsequently a few samples were got identified with the help of Potato Synonym Committee, National Institute of Agricultural Botany, England. These efforts led to the identification and characterization of 16 non-European varieties, which came to be known as *desi* or indigenous samples or varieties. These indigenous samples represent survivors of earlier introductions and chance selections in the Indian agro-climates. A list of these indigenous varieties with their salient attributes is presented in Table 3.

Table 3: Indigenous potato varieties/samples in India

Varieties/samples	Salient features
Agra Red, Chamba Red, Coonoor White, Coonoor Red, Darjeeling Red Round, Desi, Dhantauri, Gola Type A, Gola Type B, Gola Type C, Phulwa, Phulwa Purple Splashed, Sathoo, Red Long Kidney, Shan and Silbilati	Heat and drought tolerant, therefore cultivated predominantly in the Indian plains; tolerant to degenerative viruses; due to physiological advantages can be stored in country stores during hot Indian summers

Source: Pushkarnath (1969). *Potato in India-Varieties*, Indian Council of Agricultural Research, New Delhi, 493, pp.

Among these, Phulwa, Darjeeling Red Round and Gola, were found to be the most popular ones. These types though no more the mainstream varieties under cultivation now in our country, yet they enjoy consumer preference in small pockets atleast in Eastern India. Besides the indigenous, 38 European varieties were identified from whatever were under cultivation in India before independence. These are referred to as exotic varieties. Not all exotic varieties, however, were commercially important. Only 16 of these had some commercial value (Table 4). These exotic

European varieties were naturally long-day adapted and, therefore, their cultivation was restricted to the hills of the Indian sub-continent.

Table 4: Exotic potato varieties in India

Varieties	Salient features
Ally, Arran Counsal, Ben Cruachan, Craig's Defiance, Dunbar Cavalier, Great Scot, Italian White Round, Late Carman, Magnum Bonum, Majestic, Northern Star, President, Raeburn's Gregor Cups, Red Rock, Royal Kidney and Up-to-Date	Long-day adapted, therefore suitable for the Indian hills only; multiplication was characterized with progressive accumulation of degenerative viral diseases; physiological limitations on tuber storage and utilization in hot Indian summers

Source: Pushkarnath (1969). *Potato in India-Varieties,* Indian Council of Agricultural Research, New Delhi, 493pp.

National breeding Programme: During earlier phase varietal improvement for potato was a challenge to breeders in India because:

i) The introduced European varieties were all long day adapted,
ii) Their multiplication in Indian conditions was characterized by progressive accumulation of viral diseases resulting in concomitant decrease in yield, and
iii) Limitations in tuber storage and utilization in hot and humid Indian conditions.

The task was further complicated by unique reproductive features of the plant since it flowers only under long days. This condition is available in the higher elevations and hence potato hybridization was initiated at Kufri (Shimla), Himachal Pradesh. Initial attempts for breeding high yielding potato hybrids for sub-tropical plains and temperate hills was unsuccessful owing to quick degeneration of hill bread progenies in the plains during evaluation and also dormancy of hill potatoes. These bottlenecks did not allow any clonal evaluation in the plains during the appropriate season.

A regular potato breeding programme in India was started in 1949 by Central Potato Research Institute (CPRI). Its headquarters were shifted from Patna, Bihar, to Shimla, Himachal Pradesh in 1956. With perfection of seed plot technique in 1963, it now became possible to raise, maintain and evaluate segregating populations in the plains under disease-free low aphid periods. The hybridization continued to be done in high hills at Kufri. This approach brought in positive results from potato improvement programme and also revolutionized the potato seed production system in the country.

Indian potato varieties

Concerted breeding efforts of potato varietal improvement programmes at Central Potato Research Institute has led to development of 51 improved potato varieties for cultivation under diverse agro-climatic zones of the country. Presently 23 varieties are under cultivation and occupy nearly 95% of the total potato area in India. Prominent among them are Kufri Jyoti in the hills and state of West Bengal, Kufri Badshah in Gujarat, Kufri Bahar in Uttar Pradesh and Kufri Pukhraj in plains of India. Varieties for specific problem areas are Kufri Kanchan for Darjeeling hills where wart is a serious problem and Kufri Swarna for Nilgiri hills where cyst nematodes are serious pests. Varieties specifically suitable for processing are Kufri Chipsona-1, Kufri Chipsona-3, Kufri Chipsona-4 for making chips and Kufri Frysona for French Fries. The salient features of some important varieties along with their distinguishing morphological features helpful in their identification are described below.

Important Table varieties

Kufri Jyoti: It is a medium maturing widely adapted variety suitable for cultivation in hills, plains as well as plateau regions of India. It is moderately resistant to early and late blight and immune to wart. It has white cream, ovoid tubers with shallow eyes and cream flesh. Its canopy is compact and stem green with red brown pigment highly scattered throughout. Leaflet is ovate, flowers are white and sprouts red-purple. It is an early bulker with slow rate of degeneration.

Kufri Bahar: It is a medium maturing variety suitable for cultivation in north Indian plains. It is immune to wart and tolerant to gemini virus. It has white cream, ovoid tubers with medium-deep eyes and white flesh. Its canopy is semi-compact and stem green. Leaflet is ovate-lanceolate, flowers are white and sprouts green. It is an early bulker.

Kufri Badshah: It is a medium maturing variety suitable for cultivation in north Indian plains as well as plateau regions of India. It is resistant to early blight, late blight and PVX. It has white cream, ovoid tubers with shallow eyes and cream flesh. Its canopy is semi-compact and stem green with red brown pigment highly scattered throughout. Leaflet is ovate-lanceolate, flowers are white and sprouts red-purple.

Kufri Pukhraj: It is an early to medium maturing variety suitable for cultivation in northern plains as well as plateau regions of India. It is resistant to early blight, moderately resistant to late blight and immune to wart. It has yellow, ovoid tubers with medium-deep eyes and yellow flesh. Its canopy is semi-compact and stem green with purple pigment highly scattered throughout. Leaflet is ovate-lanceolate, flowers are white and sprouts purple. It is an early bulker and suitable for low-input eco-system.

Kufri Khyati: It is an early maturing variety suitable for cultivation in northern plains of India. It is field resistant to early blight and late blight. It has white cream, ovoid tubers with medium-deep eyes and cream flesh. Its canopy is semi-compact and stem green with purple pigment lightly scattered throughout. Leaflet is ovate-lanceolate, flowers are white and sprouts red-purple. It is an early bulker and suitable for high cropping intensity.

Kufri Sadabahar: It is a medium maturing variety suitable for cultivation in Uttar Pradesh and adjoining areas. It is moderately resistant to late blight. It has white cream, ovoid tubers with shallow eyes and white flesh. Its canopy is compact and stem green with purple pigment highly scattered throughout. Leaflet is ovate-lanceolate, flowers are white and sprouts red-purple.

Kufri Chandramukhi: It is an early maturing variety suitable for cultivation in northern plains as well as plateau regions of India. It has white cream, ovoid tubers with shallow eyes and white flesh. Its canopy is semi-compact and stem green with red-brown pigment highly scattered throughout. Leaflet is ovate-lanceolate, flowers are red-violet and sprouts red-purple. It has very good cooking quality.

Kufri Ashoka: It is an early maturing variety suitable for cultivation in northern plains of India. It has white cream, ovoid tubers with medium-deep eyes and white cream flesh. Its canopy is semi-compact and stem green. Leaflet is ovate-lanceolate, flowers are red-violet and sprouts red-purple.

Kufri Jawahar: It is an early maturing variety suitable for cultivation in northern plains as well as plateau regions of India. It is moderately resistant to late blight and immune to wart. It has white cream, round tubers with medium-deep eyes and cream flesh. Its canopy is compact and stem green. Leaflet is ovate, flowers are white and sprouts red-purple. It has slow rate of degeneration and is suitable for inter-cropping.

Kufri Anand: It is a medium maturing variety suitable for cultivation in northern plains of India. It is moderately resistant to late blight and immune to wart. It has white cream, oblong tubers with shallow eyes and white flesh. Its canopy is semi-compact and stem green with purple pigment lightly scattered throughout. Leaflet is ovate, flowers are red-violet and sprouts red-purple.

Kufri Sutlej: It is a medium maturing variety suitable for cultivation in northern plains of India. It is moderately resistant to late blight and immune to wart. It has white cream, ovoid tubers with

shallow eyes and white flesh. Its canopy is semi-compact and stem green with purple pigment lightly scattered throughout. Leaflet is ovate-lanceolate, flowers are white and sprouts green.

Kufri Sindhuri: It is a late maturing variety suitable for cultivation in northern plains of India. It is moderately resistant to early blight. It has red with stippled white cream, round tubers with deep eyes and cream flesh. Its canopy is open and stem green with purple pigment highly scattered throughout. Leaflet is lanceolate, flowers are red violet and sprouts purple. It is suitable for low-input system.

Kufri Lalima: It is a medium maturing variety suitable for cultivation in northern plains of India. It is moderately resistant to early blight. It has red, round tubers with deep eyes and white flesh. Its canopy is semi compact and stem red-purple with green pigment lightly scattered throughout. Leaflet is ovate-lanceolate, flowers are red-violet and sprouts red-purple.

Kufri Arun: It is a medium maturing variety suitable for cultivation in north Indian plains. It is moderately resistant to late blight. It has white red, ovoid tubers with medium-deep eyes and cream flesh. Its canopy is semi-compact and stem red-purple with green pigment highly scattered throughout. Leaflet is lanceolate, flowers are red-violet and sprouts red-purple.

Kufri Kanchan: It is a medium maturing variety suitable for cultivation in north Bengal hills as well as Sikkim. It is moderately resistant to late blight and immune to wart. It has red, ovoid tubers with shallow eyes and cream flesh. Its canopy is semi-compact and stem red-purple with green pigment highly scattered throughout. Leaflet is ovate-lanceolate, flowers are blue-violet and sprouts pink.

Kufri Girdhari: It is a medium maturing variety suitable for cultivation in Indian hills. It is highly resistant to late blight. It has white cream, ovoid tubers with shallow eyes and white flesh. Its canopy is open and green stem. Leaflet is ovate-lanceolate, flowers are white and sprouts pink.

Kufri Himalini: It is a medium maturing variety suitable for cultivation in north Indian hills. It is resistant to late blight. It has white cream, ovoid tubers with medium-deep eyes and cream flesh. Its canopy is semi-compact and stems green with red pigment only at base. Leaflet is ovate, flowers are red-violet and sprouts pink.

Kufri Lauvkar: It is an early maturing variety suitable for cultivation in plateau regions of India. It has white cream, round tubers with medium-deep eyes and cream flesh. Its canopy is semi-compact and stem green with purple pigment lightly scattered throughout. Leaflet is ovate, flowers are white and sprouts red-purple. It is heat tolerant.

Kufri Surya: It is an early maturing variety suitable for cultivation in northern plains as well as plateau regions of India. It is immune to wart. It has white cream, oblong tubers with shallow eyes and cream flesh. Its canopy is semi-compact and stem green with purple pigment lightly scattered throughout. Leaflet is ovate-lanceolate, flowers are red-violet and sprouts red-purple. It is heat tolerant.

Kufri Gaurav: It is an early maturing variety suitable for cultivation in north Indian plains. It has white cream, ovoid tubers with medium-deep eyes and white cream flesh. Its canopy is semi-compact and green stem. Leaflet is ovate-lanceolate, flowers are white and sprouts green. It is nutrient–use efficient variety.

Kufri Garima: It is an early maturing variety suitable for cultivation in north Indian plains and plateau regions. It has attractive light yellow, ovoid tubers with shallow eyes and light yellow flesh. Tubers do not show deformities like cracking or hollow heart. Its canopy is compact, stem predominantly green with red-brown pigment only at base. Leaflet width medium and ovate-lanceolate, flowering profuse, corolla white and sprouts red purple.

Important Processing varieties:

Kufri Chipsona-1: It is a medium maturing variety suitable for cultivation in north Indian plains. It is resistant to late blight. It has white cream, ovoid tubers with shallow eyes and white cream flesh. Its canopy is semi-compact and stem green. Leaflet is ovate-lanceolate, flowers are white and sprouts green. It has high dry matter and low reducing sugars and produces light colour chips.

Kufri Chipsona-3: It is a medium maturing variety suitable for cultivation in north Indian plains. It is resistant to late blight. It has white cream, ovoid tubers with shallow eyes and white flesh. Its canopy is semi-compact and stem green with red-brown pigment only at base. Leaflet is ovate-lanceolate, flowers are white and sprouts red-purple. It is suitable for making chips as well as French Fries because it has high dry matter and low reducing sugars.

Kufri Chipsona-4: It is a medium maturing variety suitable for cultivation in Karnataka, West-Bengal and Madhya Pradesh. It is field resistant to late blight. It has white cream, round tubers with shallow eyes and white flesh. Its canopy is compact and stem green with red-brown pigment lightly scattered throughout. Leaflet is lanceolate, flowers are white and sprouts red-purple. It has high dry matter and low reducing sugars, and thus suitable for making chips.

Kufri Frysona: It is a medium maturing variety suitable for cultivation in north Indian plains. It is field resistant to late blight and immune to wart. It has white cream, long-oblong tubers with shallow eyes and white flesh. Its canopy is open and stem green with purple pigment highly scattered throughout. Leaflet is ovate-lanceolate, flowers are red-violet and sprouts red-purple. It has high dry matter and low reducing sugars and suitable for making French Fries.

Variety improvement programme of CPRI for over 50 years has been instrumental in fourteen-fold increase in total production and three-fold increase in yield per unit area in the country. Many Indian varieties have found favour in foreign countries as well. These are: I 654 as CCM-69.1 in Mexico, I-822 as cv.Krushi in Sri Lanka, I-1035 as cvs. Montonosa in Philippines and Mailaka in Madagascar, I-1039 as cvs. India in Bolivia and Red Skin in Vietnam and I-1085 as cvs Sita in Sri Lanka and BSUP-04 in Philippines. Further, Indian potato varieties enjoy a high degree of consumer preference in our neighbourhood. There is enormous scope for export of potato for seed and table use to these countries. But Indian potato varieties so far had an extremely limited evaluation on foreign soils. Therefore, a systematic study on adaptability of varieties in the Indian ocean rim countries, the middle East, S.W. Asia, CIS countries and Eastern Europe needs to be the major thrust of any further potato development programme.

Suggested readings

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Breeding potato varieties for biotic and abiotic stresses

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The productivity of potato crop is constantly threatened by assorted biotic and abiotic stresses, which affects the quality and quantity of tubers, the main food storage organ. Among various biotic stresses, fungi (*P.infestans, Alternaria solani, Rhizoctonia solani*, *Verticillium dahliae*), viruses (PVY, PLRV, PVX, PVM, PVA, PVS), bacteria (*Erwinia species, Streptomyces scabies*, *Pseudomonas solanacearum*) and nematodes (*Globodera pallida* and *Globodera rostochiensis)* poses serious losses and remains as major impediment in potato production worldwide. Management of biotic stresses include both protective approaches such as breeding for resistance and therapeutic approach like chemical control. Employing durable resistant varieties is an important contrivance in the management strategy of biotic stresses. This reduces recurring chemical fungicide sprays, thus minimising the impact on environment, besides rendering economic benefits. The sub-sections in the chapter summarises conceptual information on significant biotic stresses, detailing their area of distribution, races/pathotype, mechanism & sources of resistance, screening methodology, economic importance and various breeding strategies adopted worldwide to scuffle the diseases caused by respective pathogens, with special state of past, present and future panorama of Indian breeding programme.

Late blight

Late blight is caused by *Phytophthora infestans* which is highly variable and therefore, breeding varieties against it has been a seesaw story. Pathological variants (races) could be detected universally after the resistance genes from *S. demissum* were transferred to the commercial cultivars. In 1909, R.N. Salaman demonstrated the heritable nature of resistance to late blight in wild species *S. edinense*. Resistance to blight can occur both in foliage and tubers. However, breeders have largely neglected the latter. Broadly resistance can be grouped into two types: (i) race-specific resistance (also called vertical resistance or major gene resistance, qualitative or discontinuous resistance) and (ii) race non-specific (also called horizontal resistance or minor gene resistance, field resistance, polygenic resistance, quantitative resistance or partial resistance).

The race-specific resistance based on gene-for-gene relationship was initially identified in hexaploid (2n=6x=72), wild species *S. demissum*. It is expressed in the form of hypersensitive response of the tissue to all races of *P. infestans* that did not possess the corresponding virulence to the resistance genes (R-genes). Specific resistance is conditioned by a series of major dominant genes each of which is brought into action by distinct pathotypes; currently eleven such genes (ex-demissum) are recognized. *S. stoloniferum* has also been found to possess similar, if not identical, resistance genes. However, the R-genes wherever deployed were defeated in due course of time. In India, the process of transfer of R-genes from *S. demissum* background was started in mid-fifties and the first set of late blight resistant varieties was released for commercial cultivation in 1968. Of these, cv. Kufri Jyoti (possessing R-genes 3.4.7) became most popular which is still grown in several parts of the country. Since then a lot of varieties carrying R-genes have been bred and deployed across the country. In cv. Kufri Jyoti, matching virulences (3.4.7) were detected immediately after 5-6 years of its cultivation both in North-eastern and North-western hills. Both frequency of matching virulences, their combinations and disease increased rapidly making it completely susceptible by 1988. In India, this problem has been avoided by making adjustments in screening methodology. The seedlings in F_1C_1 are challenge-inoculated with the most complex race (8-9 gene complex). The seedlings showing either complete susceptibility or immunity are discarded. The selected seedlings possess both R-gene resistances coupled with a high degree of field resistance.

Race non-specific resistance is a quantitative and multifaceted trait, probably governed by many genes, it is, therefore, difficult to analyse in Mendelian ratios. Field resistance to late blight operates mainly through four factors, *viz.* infection efficiency, incubation period, colonization rate and sporulation efficiency. Many host factors, environmental aspects, edaphic, nutritional and climatic have an effect on these four components of resistance. Besides, components of field resistance to tuber blight include the depth in the soil at which the tubers are produced, the ease with which the spores are washed down from the canopy into the soil, the rapidity of periderm formation and the resistance to wounding. Although, at the phenotypic level, both types of resistances can be easily identified, yet at the genotypic level these are almost similar. Genetic analysis of resistance to late blight using DNA markers showed that major genes for resistance (R-gene) are closely linked to the factors controlling quantitative resistance suggesting that there is no real difference between qualitative and quantitative resistance to late blight as far as the nature of the genes involved. The differences observed at the phenotypic level may be the result of various allelic and non-allelic interactions. Thus a complex picture emerges, which renders both selection for and evaluation of blight resistance a slow process.

Several wild *Solanum* species possesses high degree of resistance to late blight. Species like *S. bulbocastanum*, *S. demissum*, and *S. stoloniferum* had clones, which possessed low infection frequency. Besides clones of *S. bulbocastanum* and *S. demissum* developed only small lesions, whereas clones of *S. stoloniferum* possess high degree of resistance to tissue colonization. Umaerus demonstrated that *S. demissum* is a treasure of resistance. Besides R-genes, it also possesses field resistance, which operates primarily through low infection frequency at seedling stage. In German-Dutch potato collection nothing encouraging was found in *S. tuberosum* ssp. *andigena* and the primitive cultivars except an accession of *S. phureja*. However, resistance was detected in wild diploid Mexican species like *S. pinnatisectum*, *S. bulbocastanum*, *S. polyadenium* and *S. verrucosum*. It was also detected in Bolivian and Aregentinian species, including *S. chacoense*, *S. berthaulti*, *S. microdontum* and *S. vernei*. Although, initially *S. tuberosum* ssp. *andigena* was thought to be devoid of any resistance to late blight but, there has been renewed interest in it as andigena potatoes respond to selection for resistance and produce a number of highly resistant clones. Resistance has also been reported in the Russian diploid sources, including *S. polytrichon*, *S. simplicifolium* and *S. microdontum*, which showed resistance to tuber blight as well.

Viruses

Viral diseases are an important constraint for potato crop because of their systemic distribution in the host and are mainly responsible for the degeneration of seed stocks. At least twelve viral diseases are known to infect potato crop in India and elsewhere. Among them, viruses PVX, PVY, PVS, PVA, PVM and leaf roll (PLRV) are important. Introducing resistant cultivars is one of the most efficient ways of reducing the losses caused by viruses. Resistance genes to different potato viruses have been identified in many wild potato species. Some of these genes have been incorporated in many of the recently released potato cultivars.

The nature of resistance against viruses is of several types: i) tolerance, ii) resistance to infection, iii) hypersensitivity usually giving field immunity, and iv) extreme resistance or immunity. Tolerance to viruses in potatoes is usually considered a dangerous type of resistance. Resistance to infection can be defined as the type in which only a small percentage of infection appears in the field and is governed by polygenes. Hypersensitivity and immunity are on the other hand due to mostly single dominant genes. Out of the four types of resistance, immunity gives almost complete elimination of virus and is preferable over other types. However, in recent years, more emphasis is being given to vector resistance where the resistance sought is against the vectors (aphids and other vectors), the carrier of viruses and not against viruses themselves.

There are three main groups of strains of PVY viz. PVY^O (common strains), PVY^N (tobacco veinal necrosis strains) and PVY^C (stipple streak strains). The strain-specific resistance is controlled by the resistance gene N_Y while the extreme resistance is controlled by the gene R_Y. *Ny* genes are found in large number of cultivars including Pentland Crown, Pentland Ivory, King Edward and Cana and in hybrids derived from wild species like *S. chacoense*, *S. demissum* and *S. microdontum*. The sources of *Ry* gene are *S. stoloniferum*, *S. hougasii* and *S. tuberosum* ssp. *andigena*. Accordingly the genes are named as Ry_{sto}, Ry_{hou} and Ry_{adg}. *Ry* is inherited as a single dominant gene hence easy to breed.

Mild, moderate and severe strains of PVA occur. They differ in severity of symptoms produced in potato cultivars. The gene *Na*, present in many cultivars, protects the plant from infection under natural pressure from PVA by means of a hypersensitive response. The gene *Na* is linked to gene Nx_{tbr}, which controls the resistance to PVX. PVX strains can be separated according to their serological reaction into two main pathotypes: 1 and HB. Cockerham (1970) identified several genes conferring hypersensitivity viz. $Nx_{tbr,}$ $Nb_{tbr,}$ Nx_{chc} and Rx^n_{acl} . Similarly, genes conferring extreme resistance are known to occur in several species (Rx_{adg}, Rx_{acl} and $Rx_{(scr)}$). Strains of PLRV differ in the severity of the symptoms they produce on potato and on test plants. Resistance to PLRV has been found to be oligogenic and has been detected in *S. brevidens* and *S. tuberosum*. Transfer of resistance genes from *S. brevidens* has been achieved through protoplast fusion but from *S. tuberosum* it has been possible only through bridge crosses. Among the hexaploid somatic hybrids derived from *S. tuberosum* and *S. brevidens* by protoplast fusion, some hybrids with high PLRV resistance were obtained.

The development of cultivars with multiple virus resistance remains a challenge for the breeders. This may be because the breeder must select for many important characters, therefore, introducing even a few genes for resistance to viruses becomes a difficult task. At CPRI, parental lines having virus resistance in duplex/triplex/tetraplex form have been developed. The progeny of triplex/tetraplex parents are being crossed with nulliplex parents to produce almost cent per cent immune clones. After the early evaluation for viral resistance, an evaluation for horticultural traits could be done at the later clonal generations.

Bacterial wilt

Bacterial wilt or brown rot, first reported in India in 1892, is the most destructive of all bacterial diseases. Incidence of bacterial wilt is wide spread in all mid hill regions of the country and pockets of Assam, Meghalaya and Maharasthra. The disease damages the crop in two different ways - premature wilting of standing crop and rotting of tubers in fields, transit and stores. It is primarily seed borne, but survives equally well in soil. Host resistance is hard to find because of lack of co-evolution of the host and the bacterium, high variability in the bacterium and instability of the host resistance.

Resistance to bacterial wilt is a partially dominant character and is more of a polygenic type. Inheritance of resistance and its expression is complex and both additive and non-additive gene actions are involved, but the latter component is more important. A gene-for-gene relationship is not applicable to bacterial wilt. Certain genes other than those 'for resistance alone' have turned out to have the novel (pleiotropic) effects in conferring the resistance once the potato plant has come into contact with pathogen under a certain set of environmental conditions. These genes were eventually called 'genes for resistance' once a certain level of resistance was detected. The major or minor status of these genes depends on the particular genotype of the pathogen, and the particular environmental conditions that influence their expression. Attempts to transfer resistance from wild *Solanum* sp. into common potato result in excessive recombination, resulting in breakdown upon intercrossing. Non-strain specificity and race-cultivar specificity are the common features required for resistance to bacterial wilt. Thus, the host genotype x pathogen genotype interaction in potato-*R. solanacearum* system seems to be artifactual. Both the host and pathogen are sensitive to environmental changes. Therefore, host

genotype x pathogen genotype interaction may also be a result of host genotype x environment and / or pathogen x environment interaction.

The resistance in the clones of species like *S. phureja* mainly and a few other *Solanum* species have been exploited extensively in the South American countries, but the Indian isolates of the bacterium has proved to be strongly virulent making these sources ineffective. Resistance in *S. phureja*, the only species where it has been studied in detail, is strain- and temperature-specific and breaks down under the warm climates. Nematode injury also leads to its break down. A collection of nearly 500 clones of *Solanum* species which carry low to moderate degree of resistance i.e. *S. phureja, S. microdontum, S. canasense, S. stenotomum, S. pinnetisectum, S. sparsipilum, S. kurtzianum, S. jamesii, S. polytrichon, S. vernei, S. acaule* and *S. stoloniferum* and inter-specific hybrids between a number of above species and also resistant varieties developed so far in other parts of the world, *viz.* Prisca, Cruza, Caxamarca, Molenera and Ampola were screened against different isolates of the pathogen. All these cultivars/cultures proved susceptible to Indian isolates except for the one clone of diploid *S. microdontum* showing a moderate level of resistance. The efforts made to transfer the useful resistance from this source into the *tuberosum* background *via* dihaploids resulted in the development of two promising meiotic tetraploids. However, in field tests these were also found to be susceptible.

Wart

Wart disease of potato caused by a phycomycetous fungus, *Synchytrium endobioticum* (Schilb) was first reported from India in 1953 in North Bengal Hills. To avoid its further spread to other parts of the country, this area was brought under domestic quarantine in 1959. This has helped in containing this dreaded disease in Darjeeling district only. The disease causes cauliflower like growths on tubers, stolons and stem bases. The heavy infection of disease causes rottage of entire produce and results in total loss of crop. Cultivation of wart immune varieties on a long-term basis is the only viable alternative. The resistance genes are available in a number of varieties of *S. tuberosum*. Besides, a number of wild species like *S. boliviense, S acaule, S microdontum, S. demissum, S. sparsipilum, S. polytrichon, S. simplicifolium, S. chacoence* f.sp. *boergerii, S. vernei* and *S. spegazzinii* are known to have resistance to the disease. Monogenic dominant mode of inheritance for at least the control of necrotic response has been proved. However, modifying genes are also present which condition the nature and extent of response. Systematic breeding programme for wart resistance started in 1964. The crosses between wart immune/resistant *tuberosum* parents result in recovery of quite high percentage of resistant clones than between resistant x susceptible parents. Using Adina x Ultimus, several late blight resistant and wart immune hybrids were developed. One of them was released for commercial cultivation under the name Kufri Sherpa in 1983, which did not become popular because of its poor keeping quality, unattractive dull white skin, round tubers with medium deep eyes. Indian cultivars, *viz*. Kufri Jyoti, Kufri Chamatkar, Kufri Muthu, Kufri Sheetman, Kufri Bahar, Kufri Khasigaro and Kufri Kumar are immune to the race of *S. endobioticum* prevalent in the Darjeeling hills. These cultivars except Kufri Jyoti also didn't establish in the area because of local preference for varieties with red skin tubers. To develop a red tuber variety, Pimpernel was used as one of the parents. One hybrid from cross SLB/Z 405a x Pimpernel was selected and has since been released as cultivar Kufri Kanchan. This variety is immune to wart and possesses high degree of field resistance to late blight.

Nematodes

About 90 species of nematodes belonging to 38 genera have been reported to be associated with potatoes. Among these, the root knot nematodes and potato cyst nematodes have been recognized as the major pests. At least nine species of Root-knot nematode (*Meloidogyne spp.*) are known to infect potatoes. Among these, *M. incognita* is the most important throughout the world followed by *M. javanica*. The dominant root-knot nematode species affecting potato both

in hills and plains is *Meloidogyne incognita* while *M. javanica* infestation is restricted to mid hills and plains.

Heavily infested plants are stunted with yellowish leaves while no visible symptoms of nematode injury are seen under low infestation levels. The galls on potato roots are small and often go unnoticed. Wart-like structures are formed due to tuber infection, which reduces the commercial value and keeping quality of tubers. The second stage juveniles (hatched out from the egg masses laid by females) infect the young roots resulting in the formation of giant cells and the formation of syncytium and development of the nematode in the roots causes formation of galls or root knots. The nematode infection on tubers is characterized by the formation of typical wart like pimples on the outer skin.

Potato cyst nematodes, *Globodera* spp. also known as Golden nematode or potato root eelworms, is considered as one of the major pests throughout the world. Quarantine or regulatory actions are imposed against them in most countries. In India, the potato cyst nematode was first detected in 1961 by Jones at Ootacamund in Tamil Nadu. The efforts to locate the source of resistance to cyst nematodes though began in 1968, a systematic breeding programme at CPRI was started in 1971. Over 2000 genotypes comprising group tuberosum, group andigena and tuber bearing *Solanum* species were screened and resistance was located in 20 accessions of 14 wild tuber bearing species *S. ehrenberjii, S. vernei, S. chacoense, S. phureja*, *S. demissum*, *S. gourlayi*, *S. microdontum*, *S. sucrense*, *S. tarijense*, *S. acaule*, *S. fendleri*, *S. multidissectum*, *S. oplocense*, *S. sparsipilum* and some accessions of *S. tuberosum* ssp. *adigena*. Simultaneously, efforts were also made to procure resistant breeding lines to both the species from the Netherlands and USDA. A parental line VTn^2 62.33.3 (*S. tuberosum* x *S. vernei* hybrid) received from the Netherlands, having resistance to both the species, was extensively used in crosses with late blight resistant cultivar Kufri Jyoti resulting in release of hybrid Kufri Swarna for cultivation in cyst nematode infested areas of Nilgiri hills

High temperature stress

The potato has long been considered a crop for cool and temperate climates. Higher temperatures inhibit yield by overall reduction of plant development due to heat stress or by reduced partitioning of assimilates to tubers. Tuberization is reduced at night temperatures above 20^OC with complete inhibition of tuberization above 25^OC. Exposure of potato plants to heat stress alters the hormonal balance in the plants. As a result most of assimilated carbon is partitioned to above ground vegetative parts at the cost of the tubers. Aspects of heat tolerance that are considered important and should be taken into account in breeding programme includes ability of the plants to tuberize at night temperature of 22^O C and above, low shoot/root ratio at high temperature, and early maturity of the crop.

To obtain harvestable tubers, the plants must be induced to tuberize, and must also be stimulated to partition major part of the dry matter to the below ground plant parts. Temperature has an immediate effect on assimilate partitioning. It has been established that heat sensitive cultivars accumulate more starch in leaves and the rate of transport of sucrose to tubers is low in comparison to tolerant genotypes grown under heat stress. This may be either due to lower photoassimilate synthesis or also due to utilization of substantial amount of photoassimilate by shoots itself. High temperatures are known to strongly reduce the harvest index in potato. Stolon initiation is significantly influenced by temperature; it has been found that under high temperature the stolon development is delayed. Branching and growth of stolons is stimulated but tuberization is delayed. High temperatures delay, impede or even inhibit tuber initiation. Potato tuber formation is badly affected due to high night temperatures of above 20°C and therefore, the most important characteristic of a heat tolerant potato genotype should be its ability to tuberize under high night temperatures of above 20°C. Better foliage growth and higher LAI are also characteristics of heat tolerance. In addition, higher tuberisation in the leaf bud cuttings, low shoot/root ratio, early maturity, higher rate of photo-assimilate partitioning from

leaves to tubers, higher cell membrane thermo-stability, higher stability of chlorophyll, slower rate of elongation of internodes and low GA/ABA ratio during tuberization may also be important features of a probable heat tolerant genotype. One of these attributes viz. tuberisation at high night temperatures of 22°C is being well exploited for selection of seedlings for development of heat tolerant genotype, one such genotype named as Kufri Surya has already been developed, which has potential to tuberize up to 22°C temperature and few more are in advanced stages of trial for release as a variety.

To breed heat tolerant genotypes for Indian conditions, crosses were made amongst known heat tolerant and local high yielding genotypes. The known heat tolerant genotypes used in the breeding programme were LT-1, LT-2, LT-5, LT-7, LT-8, LT-9, DTO-28, DTO-33 (received from CIP) Katahdin, Desiree and Kufri Lauvkar. The progenies were screened for heat tolerance by their ability to form tubers within one month after shifting to high temperatures. The selected genotypes were multiplied and further selected at early planting in Indo-Gangetic plains at Modipuram and Jalandhar under heat stress.

Drought Stress

Drought is a major limiting factor for potato production in the world influencing yield as well as tuber quality. Drought may occur due to erratic rainfall, inadequate irrigation techniques and lack of water supply. Even with good irrigation practices, water stress may occur because of high transpiration rates especially during mid-day, when root system cannot completely meet the transpiration requirements of the plant. Drought have chronic effects on plant growth and may lead to premature senescence of the plant. Stomata of potato leaves close at relatively lower water deficits (leaf water potential or LWP of -3 to -5 MPa) resulting in reduced transpiration, whereas, in crops like sorghum, soybean etc stomatas close at -11 to -13 MPa LWP. Adaptation to water stress may involve several physiological and morphological characters and their importance may vary according to the type of water stress experienced by the plants.

Water stress may affect potato plants through its effect on radiant energy interception, conversion of light energy into dry matter, partitioning of assimilates and tuber dry matter concentration. Water stress in known to reduce both the rate and the duration of growth of leaves along with reduction in number of leaves, therefore, the radiant energy interception is decreased. Drought has adverse effects on the functioning of photo system II, and thus the rate of photosynthesis, though conversion of light energy into dry matter content is not very sensitive to water stress. Prolonged drought reduces the activity of light harvesting complex and down regulation of carboxylating enzymes. The partitioning of dry matter is also affected by drought, though, genotypic differences do occur. The potato root system is shallow and under water stress conditions it is not able to extract water from soil effectively as compared to cereal crops. Thus the potato plants respond by increasing root: shoot ratio in view of increasing dry matter production under drought hence the balance of growth is favoured towards root growth. The work on Indian potato cultivars has also shown that the most critical stage in crop production under water stress is the stolon initiation stage, which represses the yield by 30-65%.

Adaptation to water stress conditions may be either through drought avoidance or drought tolerance and it may involve several different physiological and morphological characters, the relative importance of which may vary according to the type of water stress experienced by the crops. Potato cultivars considered to be drought tolerant have been found either to have greater threshold of soil moisture deficit or are less sensitive to soil moisture deficit and sometimes both. Such cultivars sustain leaf expansion with increasing soil moisture deficit and also develop relatively large rooting system for better exploitation of available soil moisture. The work on recovery of leaf growth after a period of water stress in Indian potato cultivars has shown that plant response varies with the cultivars, in some cultivars minimum reduction in leaf growth occurred and the recovery from stress on re-watering enhanced the leaf growth. Some cultivars showed moderate reduction in growth and re-watering caused sufficient recovery of leaf growth,

whereas, other cultivars had great reduction in growth and re-watering could not result in sufficient recovery of leaf growth. This shows that leaf growth response varies with the genotype and their tolerance limit.

Frost Tolerance

The problem of frost is known in almost every country where potatoes are grown. Temperatures below $-2^{O}C$ in the field can produce partial or complete loss of the crop. In temperate zones, frosts can occur during spring when the crop is establishing itself, or during autumn when the crop is maturing. Higher crop losses occur in tropical highlands and subtropical plains where frosts can occur any time during the crop growth period. In India more than 80% of the potatoes are grown during winter in plains and the crop is prone to frosts during the months of December and January. Based on the field observations, two types of frosts are often distinguished. "White frost" occurs when there is a decrease in temperature and relative humidity is high. "Black frost" occurs under low temperatures and much drier conditions, hence more damaging and severe because plant tissue is darkened immediately. Acclimation or hardening may increase the resistance to frosts in many plants. Exposure of the plants to prolonged low temperature is effective in increasing resistance to frost injury in *S. tuberosum, S. multidisectum, S. chomatophilum, S. acaule and S. commersonii*. Genetic variability exists in the genus *Solanum* with respect to frost injury. *S. acaule* has the ability to withstand extracellular ice formation up to $-5^{O}C$, which gives this species frost tolerance. This species can be used in the breeding programmes to transfer frost tolerance to *S. tuberosum*. In North India, frost occurs in most years during December and January in the plains of Punjab and Eastern UP. Cultivars Kufri Sheetman and Kufri Dewa released by the Central Potato Research Institute possess resistance to frost. High degree of frost resistance was observed in other 28 hybrids from crosses involving *S. acaule*.

Fertilizer Use efficiency

The potato is considered to be heavy feeder on nutrients and requires high inputs of NPK and water for optimum production. This not only increases the cost of production but also causes environmental pollution. The application of high rates of N and K fertilizers and irrigation water on coarse-textured soils on which the shallow-rooted crop is often grown can result in loss of N and K, which represents an economic loss to the grower and may cause environmental degradation of groundwater. A nutrient efficient potato can produce higher yields per unit of nutrient, applied or absorbed even at a limited nutrient supply (Graham, 1984). Such genotypes could reduce N fertilization and nitrate leaching (Duynisveld et al., 1988; Sharifi et al., 2007). The recovery of applied phosphorus by potato crop is not more than 15-20% (Trehan et al., 2008). Numerous studies have demonstrated the existence of considerable variation for nutrient efficiency among crop species and cultivars within species, which suggests genetic control of inorganic plant nutrition (Errebhi et al., 1999; Trehan et al., 2005). Although plant breeders seldom select for nutrient use efficiency (NUE), breeding programs that develop lines that produce high yields may result in unconscious selection of genotypes that use nutrients more efficiently (Batten, 1993).

At CPRI, studies on nutrient use efficiency have shown that potato cultivars showed wide variation in agronomic use efficiency (AUE), nutrient uptake efficiency (NUE) and physiological use efficiency (PUE) with respect to nitrogen, phosphors and potassium. Kufri Pukhraj was the most N, P and K efficient cultivar among ten cultivars tested in the absence as well as presence of green manure. The efficient cultivars gave higher tuber yield under nutrient stress (i.e. with less dose of N, P and K fertilizer) than less efficient cultivars. The main cause of higher nitrogen efficiency in the presence of green manure was the capacity of a genotype to use/absorb more N per unit green manured soil i.e. the ability of the root system of a genotype to

acquire more N from green manured soil (NUE). The variation in potassium and phosphorus efficiency of different potato cultivars was due to both their capability to use absorbed K and P to produce potato tubers (PUE) and to their capacity to take up more K and P per unit soil (NUE). Breeders should combine parameters/characters (NUE and PUE) responsible for high nitrogen, phosphorus and potassium efficiency to breed multi-nutrient efficient potato cultivar. Recently CPRI has released potato variety Kufri Gaurav having higher nutrient use efficiency.

Quality traits

Potatoes represent a non-fattening, nutritious and wholesome food, which supply important nutrients to the human diet. Tubers contain significant concentrations of vitamin C and essential amino acids. They are also a valuable source of at least 12 essential vitamins and minerals. Besides being important in human diet, potatoes are also used as animal feed and as raw material for starch and alcohol production. Potato quality parameters change according to the specific market utilization types, and are often referred to two major categories. The first category groups "external quality", aspects comprising skin colour, tuber size and shape, eye depth. These traits are deemed very important for fresh consumption where external traits are most likely to influence consumer's choice. The second category comprises "internal quality" aspects including nutritional properties, culinary value, after-cooking properties or processing quality. Internal quality is given by traits such as dry matter content, flavour, sugar and protein content, starch quality, type and amount of glycoalkaloids. Although quality is one of the most important characteristics of potato, it is probably the most poorly defined and least researched at the genetic level Dale and Mackay (1994). There are several factors affecting tuber quality. They include the genetic makeup of the cultivar, crop maturity, agronomic practices, environmental conditions, storage temperatures, the presence of pests and diseases Traits that are genetically controlled can be grouped as biological traits (proteins, carbohydrates, vitamins, minerals, reduced amounts of toxic glycoalkaloids), sensorial traits (flavour, texture, colour) and industrial traits (tuber shape and size, dry matter content, cold sweetening, oil absorption, starch quality).
Breeding potato for quality traits requires a continuous flow of new genes and allelic diversity into the *S. tuberosum* gene pool. The genetic improvement of this crop is hampered by its tetrasomic inheritance, high level of heterozigosity, and incompatibility barriers. However, recent advances in plant biotechnology have significantly improved the possibilities of producing novel genetic variability and efficiently perform selection, expecially when biotechnologists pool resources with breeders. Equally important is the fact that basic studies have contributed to elucidate our knowledge on the genetics, biochemistry and physiology of several quality traits, making breeding efforts less empirical and more predictable.

In potato genetic engineering techniques have been applied to produce routinely and several transformation protocols are currently available. Data published by Dunwell (2000) indicated that the potato ranks second, after corn, in the list of plant species for which field trials were carried out in the United States. In the past most potato genotypes were transformed with genes for herbicide or insect resistance. However, much emphasis is now being attached to quality traits. The production of starches with modified amylose to amilopectin ratio represents a good example of the possibilities offered by genetic engineering in improving potato quality traits. Lloyd et al. (1999) provided evidence that transgenic potato lines where the activity of ADP-glucose pyrophosphorylase (AGPase) was reduced through antisense technology had a significant reduction of amylose. They also observed that in AGPase antisense plants, amylopectin accumulated shorter chains and that the size of starch granules was reduced. By contrast, the simultaneous antisense inhibition of two isoforms of starch branching enzymes (SBE A and B) to below 1% of the wild type activity gave transgenic lines with increased amount of amylose (Schwall et al., 2000). The amylose content of their transgenic lines was comparable to that of the high-amylose corn starch reported by Shi et al. (1998).

Great attention has been attached to improve the essential amino acid composition of tubers and expecially their lysine, tyrosine, methionine and cysteine content. Chakroborty et al. (2000) transformed a potato genotype with the gene *AmA1* from *Amaranthus hypocondriacus,* encoding a protein with a nutritionally balanced amino acid composition. The amino acid profile in tubers of both types of transgenic plants showed a 1.5- to 8-fold increase in all essential amino acids to the wild type.

Genetic engineering has been recently used to improve carotenoid content of tubers. In particular, to overcome the zeaxanthin deficiency of human diet, Römer et al. (2002) down-regulated the synthesis of zeaxanthin epoxidase specifically in tubers through antisense technology and co-suppression approaches. Both strategies achieved decreased conversion of zeaxanthin to violaxanthin, in transgenic tubers with a corresponding increase of zeaxanthin content of 4- to 130-fold. Due to the use of a tuberspecific promoter, leaf carotenoid content of all transformants was very similar to the control plants, and thus photosynthesis was not negatively affected by lack of violaxanthin. One main constraint in the use of wild species is that, together with useful traits, they can transfer characteristics that are undesired from the commercial standpoint. In the case of *Solanum* species, traits such as long stolons, deep eyes, negative quality traits can be transmitted. As reported recently by Pavek and Corsini (2001), transmission of undesired traits has very much limited the use of potato genetic resources. Therefore, after interspecific crosses, time consuming evaluation and selection are necessary to eliminate unwanted wild-type genes and restore the cultivated improved phenotypes. Marker-assisted selection is perhaps the most powerful approach that uses DNA markers efficiently for selection of interspecific hybridization by reducing the linkage drug in terms of time and space. The use of DNA markers can be ascribed not only to the use of markers tightly linked to target genes (positive assisted selection), but also in the use of markers specific for the wild donor parent to perform selection against the wild genome (negative assisted selection; Barone, 2004).

A very exciting development in the context of efficient selection has been the generation of a molecular-linkage map based on functional gene markers involved in carbohydrate metabolism and transport (Chen et al. 2001). Using diploid mapping populations for which molecular maps were already available, the authors performed CAPS, SCAR, and RFLP marker assays for 69 functional genes previously studied and identified (among the others *AGPase*, *SssI*, *GbssII*, *Dbe*, *UGPase*, *Ppc*, and *Cis*). This work allowed the identification of 85 genetic loci covering a considerable amount of the potato genome. The availability of this molecular-function map allowed a candidate-gene approach to be used for studying starch and other sugar-related agronomic traits in potato. Chen et al. (2001) compared the QTL map for starch content previously published (Schäfer-Pregl et al., 1998) with the molecular-function map, and various correlations between the map positions of 14 QTLs for tuber starch content and function-related loci were found.

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Somatic Hybridization in Potato Improvement

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Gene transfer is the basis for almost all crop improvement including potato. Conventionally, this is achieved through sexual hybridization; this rather limits the range of species from which gene flow can occur into a crop species. Wild species have contributed remarkably to the success of latter; they allowed the crops to retain their commercial status. As a result plant breeders have sought to utilize an increasing number of wild species as a source of valuable genes ranging from disease resistance to grain yield, and produce quality. But many sources of useful genes cannot be included in crop improvement programme primarily because of sexual incompatibilities. Genetic transformation, a focussed and direct gene transfer approach, require identification, isolation and cloning of the concerned genes. Further it is expensive and technically most exacting, although it may represent the ultimate strategy. However, some characters of interest may be govern by two or more and yet unknown genes; transfer of such characters through genetic transformation may pose many difficulties. Finally transfer of cytoplasmic organells, *viz.*, chloroplast and mitochondria may often be desired objectives; this, however is not possible through genetic transformation, while it can readily achieved by somatic hybridization.

Wild and cultivated species of potato have been effectively used in potato breeding but represent only a tiny fraction of the available potato biodiversity. Utilization of the wild tuber-bearing diploid species has been remained untapped potential source for transferring resistance trait into common potato (Bradshaw et al. 2006). Wild tuber-bearing *Solanum* species are widely distributed from southwestern USA to central Argentina and Chile. This extensive geographical range has resulted in types adapted to a broad range of climatic and soil conditions. In the course of evolution, these plants have also developed resistance/tolerance to different pathogens and pests. Much of this effort has involved the examination of wild species for various resistance traits related to potato. This trait is particularly attractive to breeders to widen the potato genetic base, but the barrier between the cultivated potato and the many wild species has proved a difficult task, even when unconventional crossing methods are used (Orczyk et al. 2003).

Many useful genes derives from wild sources cannot be transferred through conventional technique because of sexual incompatibilities are primarily due to difference in ploidy and endosperm balance number (EBN) (Spooner and Salas, 2006). It is extremely difficult to cross 1 EBN wild species directly with common cultivated 4 EBN potato. Limited success has been obtained by utilizing bridging species but the incompatibility of 1 EBN wild species has generally prevented the use of this particularly valuable trait. However, modern research and new techniques have made it possible to expand considerably the genetic resources available for use in breeding programs. A few methods have now become available to overcome this problem. These methods include: manipulation of ploidy and endosperm balance number (EBN), bridge crosses, mentor pollination and embryo rescue, hormone treatment, and reciprocal crosses (Jansky 2006). Somatic hybridization, which removes prezygotic and some postzygotic barriers, can likewise surmount the barrier between cultivated and wild species. Somatic hybridization can provide a means of bypassing sexual incompatibility between *Solanum* species, leading to fertile plants that can be used directly in breeding programs.

Somatic hybridization generates functional combinations of large sets of genetic material, which makes it similar to sexual hybridization. This method can also be used to overcome limitations of genetic transformation. Many of the important traits are predominantly polygenic such as late blight resistance and thus unavailable as isolated and characterized sequences that are ready for genetic transformation. Therefore, efficient methods of transformation are yet to be available for multiple genes that are expressed in a coordinated manner (Orczyk et al. 2003). On the other hand, somatic hybrids obtained directly after fusion contain all organelles from the cytoplasms of both parents. Somatic hybridization via protoplast isolation, electrofusion and

regeneration is a useful tool to transfer polygenic traits such as late blight resistance in a single step. It enables a development of tetraploid somatic hybrid between diploid wild species and dihaploid of common potato. As a result, tetraploid somatic hybrids may be utilized in conventional breeding for late blight resistance and improvement of other traits. Thus, production of somatic hybrids between tetraploid 4EBN *S. tuberosum* and diploid 1EBN wild species has been envisaged for imparting durable resistance to late blight. In consequence, aim of this somatic fusion technology is creditable to enrich the cultivated potato gene pool by incorporating genes from a new exotic wild species, in order to enhance resistance to late blight disease.

Hence, somatic hybridization is the technique enables to transfer agronomically important traits by bypassing such sexual barriers, besides the conventional and recombinant-DNA technologies approaches. Despite these crossing-barriers, many researchers have used this technique and subsequently produced somatic hybrids with cultivated potato. Production of hybrid plants through the fusion of protoplasts of two different plant species/varieties is called somatic hybridization, and such hybrids are called somatic hybrids. Therefore, somatic hybridization can be resorted to only when the following two criteria are satisfied: i) isolation of protoplast in large quantity and ii) totipotency of the isolated protoplasts.

Procedures

The protoplast fusion by electric field requires sufficient amount of suitable plant material for protoplast isolation and their culture after electrofusion for obtaining plant regeneration. The procedure involves the following stages.

- *In vitro* culture of donor plants
- Protoplast isolation from leaf meshophyll tissues
- Verification of protoplast viability and protoplast fusion
- Protoplast fusion by electric field
- Regeneration and culture fusion products
- Characterization of putative somatic hybrids

1) Prepare materials:
   - Three-week-old *in vitro*- grown microplants, raised from single nodal cutting
2) Microplant incubation condition:
   - 16 h photoperiod/40 µ mol m^{-2} s^{-1}/20ºC.
3) Pre-isolation (protoplast) incubation:
   - 48 h/dark/20ºC
4) Protoplast isolation:
   - Mince young leaf tissues (1-2 g) in a ϕ 90 mm Petri dish containing digestion solution: 10 ml digestion solution for 1 g tissue.
5) Incubation (for protoplast isolation):
   - 16 h/ dark/ 25ºC/ optional: gyratory shaking at 40-50 rpm; not exceeding 50 rpm.
6) Post-isolation handling:
   - Add 0.3 M KCl (sterile) to the digestion medium/ solution containing released protoplasts (protoplast suspension) in a 1:1 ratio (KCl: solution). For example, add 15 ml M KCl to 15 ml digestion medium/solution.
   - Filter the suspension through 40 µ nylon mesh, and collect in centrifuge tubes; 60 µ can be used but debris and/ or undigested tissues will be much.
7) Protoplast purification:
   - Centrifuge the filtrate at 50 x g (50 RCF) for 5 min.
   - Discard supernatent and then resuspend the pellets in 10 (or 9) ml of 0.6 M sucrose (sterile).

- Overlay 1 ml of 0.3 M KCl onto this protoplast suspension.
- Centrifuge at 50 x g (50 RCF) for 5 min.
- Recover the protoplast (green upper portion/live protoplast) from sucrose: KCl interface
- Dilute the recovered protoplasts with 10 ml of 0.3 M KCl.
- Centrifuge at 50 x g (50 RCF) for 5 min to form pellet of the protoplast.
- Resuspend the pellets in 1-2 ml 0.5M mannitol (sterile), centrifuge at 50 RCF) for 5 min., then retain pellets and discard supernatants
- Finally resuspend the pellets in 400-500 µl 0.5M mannitol (sterile) to a final density of $1x1^{06}$ protoplast/ml for electrofusion.

8) Electrofusion medium:

- 0.5 M mannitol (sterile)/ sterile-filtered (0.2 µ)/ pH 7-7.3/ adjust pH with 0.1 N NaOH

9) Symmetric fusion:

- 1:1 of each species

10) Electrofusion settings:

Chamber	BTX Microslide Model 453/3.2 mm gap
Alignment amplitude	16 V
Alignment time	25-30 s
Alignment field strength	50 V cm^{-1}
Electrofusion amplitude	260 V
DC pulse width	60 µs
Number of pulses	2
Electrofusion field strength	812 V cm^{-1}

11) Post-fusion culture:

- Dispense 50 µl Na-Alginate in each box of castor rack and mix well the 50 µl fusion products into it
- Add 2-3 ml Solution No 3 in each box and incubate at RT inside laminar for 30 min followed by add 2-3 ml Solution No 2 and incubate same for 1.30-2 h
- Remove Sol 2 & 3 by Pasteur pipette and add 5 ml VKMG (VKM Glucose) and tightly wrapped with parafilm.
- Incubate the castor racks under dark at 25ºC for the regeneration of calli.

Applications

Symmetric protoplast fusion approaches involving diploid *Solanum* species in combination with dihaploid *S. tuberosum* have been essentially used to develop tetraploid somatic hybrids potato having desirable introgression from wild relatives (Table 1). In the current years, application of this technology has been observed widely for the production of multiple resistant somatic hybrids. Interspecific potato somatic hybrid between commercial cultivars of potato *S. tuberosum* Agave and Delikat and wild diploid species *S. cardiophyllum* (1 EBN) has been produced for resistances to Colorado potato beetle, foliage blight and PVY (Thieme et al. 2010). In addition, somatic hybrids between a diploid potato clone DG 81-68 susceptible to *P. infestans* and a resistant diploid tuber-bearing species *Solanum* x *michoacanum* were generated (Szczerbakowa et al. 2010). Polzerová et al (2011) have developed interspecific somatic hybrids between wild diploid species *S. pinnatisectum* (1 EBN) and *S. tuberosum* for the late blight resistance in potato. Following the successful production, somatic hybrids have been applied in the potato breeding for the development of advance progenies for transferring the resistance trait. For example, Thieme et al. (2008) have developed novel somatic hybrids and their fertile BC_1 progenies having resistances to late blight, Colorado potato beetle and PVY from a diploid wild species *S. tarnii* into common potato.

Interspecific potato somatic hybrids at CPRI

Interspecific potato somatic hybrids between 1 EBN wild *Solanum* species *S. pinnatisectum* (+) dihaploid *S. tuberosum*, and ii) S. *etuberosum* (+) dihaploid *S. tuberosum* have been produced following optimized protocol of the protoplast isolation, electrofusion and regeneration of plantlets (Sarkar et al. 2011; Tiwari et al. 2010, 2011). The somatic hybrids *S. pinnatisectum* (+) *S. tuberosum* have resistance for late blight resistance, whereas *S. etuberosum* (+) *S. tuberosum* have resistance for potato virus Y. These potato somatic hybrids have been confirmed for the hybridity through molecular (RAPD and SSR markers), phenotypic assessments. Hybrids were also evaluated for the disease resistance. Ploidy level (tetraploid) of somatic hybrids has been examined through flow cytometry and guard cell count. At present, work is under progress on the development of more interspecific potato hybrids involving diverse 1 EBN wild species obtained from foreign gene banks. Molecular analyses of the potato somatic hybrids produced at CPRI, Shimla are shown in Figs. 1 and 2.

Table 1. Symmetric protoplast fusion in potato for incorporation of desirable traits from wild diploid species into *S. tuberosum*

Diploid wild species	**Trait(s) transferred**	**References**
S. acaule	PVX resistance	Yamada et al. 1997
	Bacterial ring rot resistance	Rokka et al. 2005
	Glycolkaloid composition	Kozukue et al. 1999
S. berthaultii	Salinity tolerance	Bidani et al. 2007
S. bulbocastanum	Late blight resistance	Bołtowicz et al. 2005
	Meloidogyne chitwoodi resistance	Mojtahedi et al. 1995
	Bacterial stem rot resistance	Rokka et al. 1994
S. brevidense	Tuber characteristics and insect resistance	Serraf et al. 1991
	Tuber soft rot resistance	Polgar et al. 1999
	Early blight resistance	Tek et al. 2004
S. cardiophyllum	Late blight, PVY, Colorado potato beetle resistances	Thieme et al. 2010
S. chcoense	Colorado Potato Beetle resistance	Cheng et al. 1995
S. circaefolium	Late blight and nematode resistances	Oberwalder et al. 2000
S. commersonii	Frost resistance	Nyman and Waara 1997
	Bacterial wilt resistance	Kim-Lee et al. 2005
	Tuber traits	Caruso et al. 2008
S. etuberosum	Tuber characteristics	Novy and Helgeson, 1994
	PVY resistance	Novy et al. 2007
	PLRV resistance	Novy et al. 2007
S. x michoacanum	Late blight resistance	Szczerbakowa et al. 2010
S. nigrum	Late blight resistance	Szczerbakowa et al. 2003
S. phureja	Bacterial wilt resistance	Fock et al. 2000
S. pinnatisectum	Late blight resistance	Polzerová et al 2011
S. stenotomum	Bacterial wilt resistance	Fock et al. 2001
S. tarnii	Late blight, Colorado Potato Beetle and PVY resistance	Thieme et al. 2008
S. torvum	Resistance to *Verticillium dahlia*	Jadari et al. 1992

S. tuberosum	Late blight resistance	Rasmussen et al. 1998

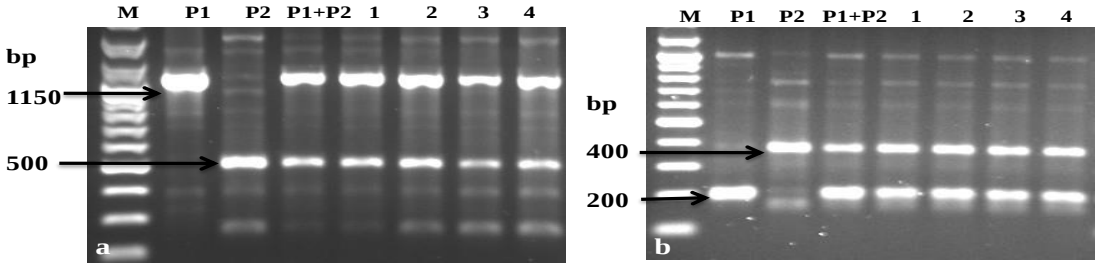

Fig. 1. Potato somatic hybrids between C-13 and *S. cardiophyllum* confirmed with possessing parental bands by a) RAPD marker OPAC06, and b) ISSR marker ISSR10. P_1= C-13, P_2 = *S. cardiophyllum*, P_1+P_2 = pooled parental DNA before PCR.

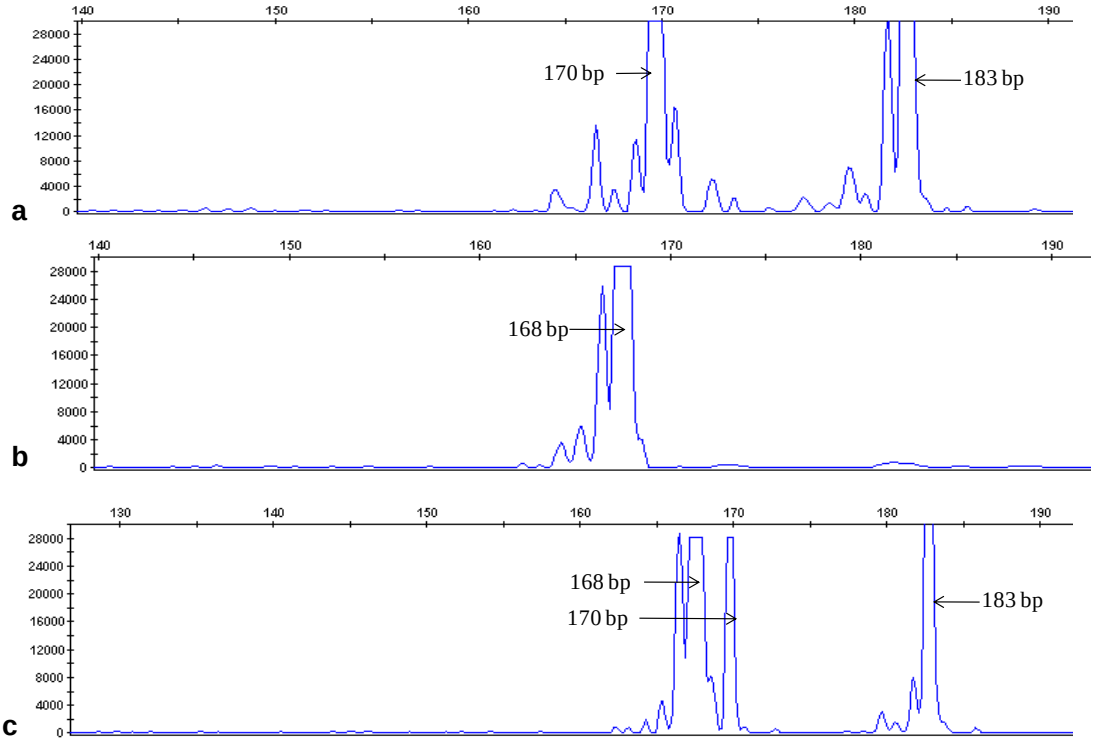

Fig. 2. SSR primer STI0012 confirmed hybridity of potato somatic hybrids C-13 (+) *S. cardiophyllum* possessing parental bands as indicated by arrow. (a) C-13, (b) *S. cardiophyllum*, and (c) somatic hybrid analyzed on the "3500 Genetic Analyzer (ABI)".

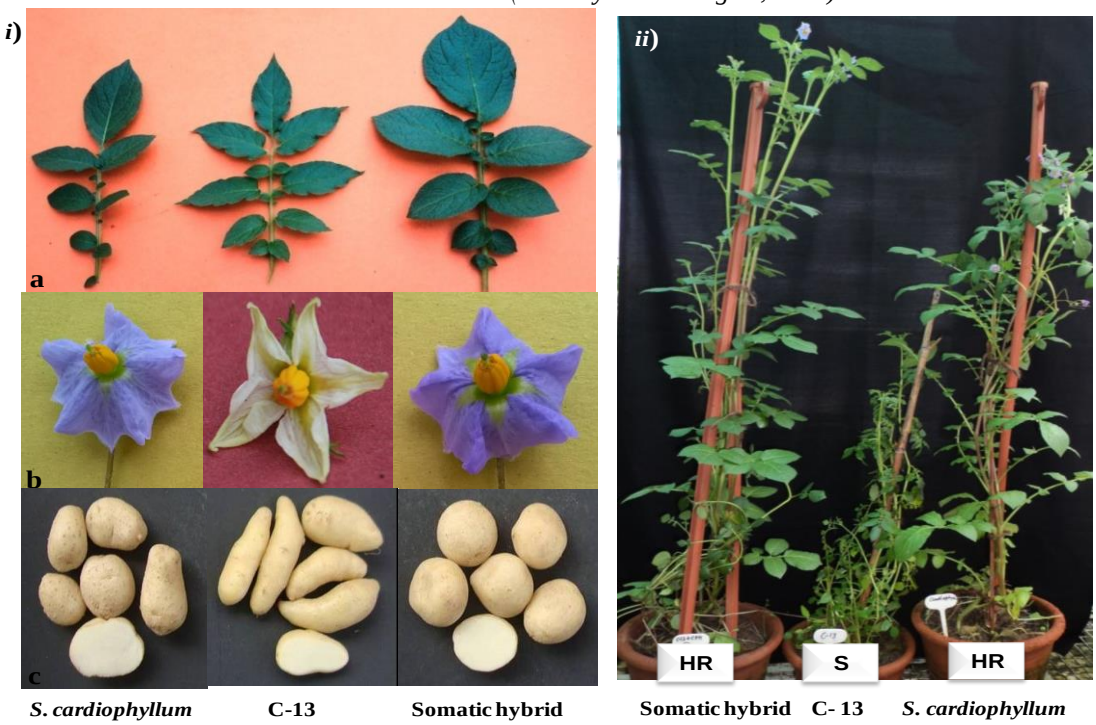

Fig. 3. i) Left panel: Somatic hybrids between C-13 and *S. cardiophyllum* confirmed by phenotypes a) leaf, b) flower and c) tuber traits possessing their parental characters; ii) Right panel: late blight resistance test of somatic hybrids confirmed by challenge inoculation of *Phytophthora infestans*. Parent C-13 was susceptible (S), whereas parent *S. cardiophyllum* and somatic hybrids were highly resistant (HR).

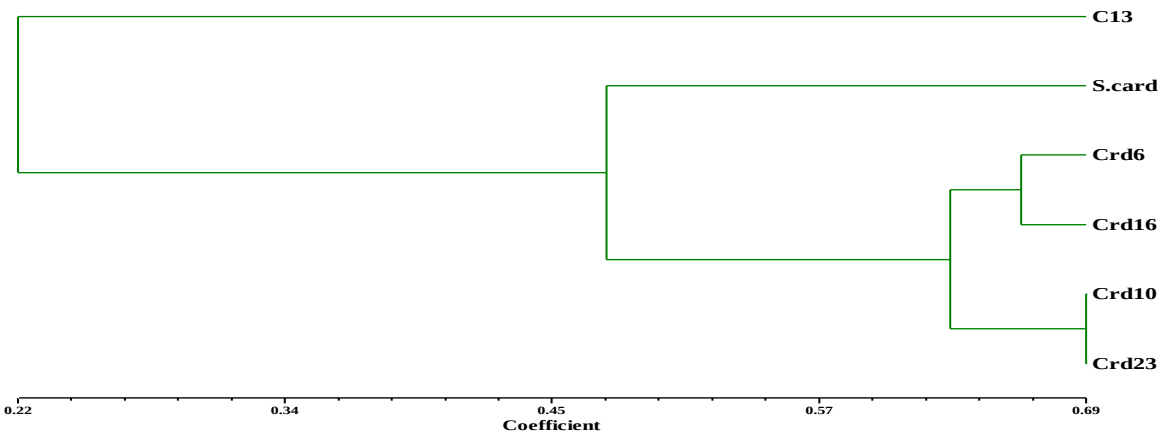

Fig. 4. Cluster analysis based on the Jaccard's coefficient of molecular profiles generated by RAPD, ISSR, SSR, AFLP and cytoplasmic markers showing genetic distinctness among somatic hybrid clones and their parents.

Advantages

- Somatic hybrids can be produced between species, which cannot be hybridized sexually.
- Somatic hybrids can be readily used in breeding programme for transfer of resistance genes
- Hybrids can be produced even between such clones, which are completely sterile.
- Cytoplasm transfer can be done in one year, while back crossing may take 5-6 years. Even where backcrossing is not applicable, cytoplasm transfer can be made using this approach.
- Mitochondria of one species can be combined with chloroplast of another species. This may be very important in some cases, and is not achievable by sexual means even between easily crossable species.

- Recombinant organelle genomes, especially of mitochondria, are generated in somatic hybrids. Some of these recombinant genomes may possess useful features.

Limitations

- Techniques for protoplast isolation, culture and fusion are very complicated.
- In many cases, chromosome elimination occurs from somatic hybrids leading to asymmetric hybrids. Such hybrids may be useful, but there is no control on chromosome elimination.
- Many somatic hybrids show genetic instability, which may be an inherent feature of some species combinations.
- Many somatic hybrids either do not regenerate or give rise to sterile regenerants. Such hybrids are useful for crop improvement. All interfamily somatic hybrids are genetically unstable and/or morphologically abnormal, while intergeneric and intertribal hybrids are genetically stable, but produce abnormal and/or sterile plants.

Conclusion

Somatic hybridization allows transfer of cytoplasmic organelle in a single generation and offer unique opportunities for combining mitochondria of one species and chloroplast of another species in a single hybrid. This capability may permit improvement of characteristics certain cytoplasmic male sterile line, which may lead to their commercial exploitation. In addition, even non-flowering and non-tuber bearing species can be utilized in breeding programme. The transfer of gene governing resistance to biotic and abiotic stresses is an important objective. In potato, this technique is already being used in commercial breeding programme of the Netherlands and Germany. In general, somatic hybrids have low pollen fertility, but they can often be used as female parents in backcrossing with one of fusion parents. It is likely that the approach of somatic hybridization will find grater applications in potato improvement for enabling transfer of useful genes from sexually incompatible species. In this context, it is important that the DNA segment carrying the desired gene from wild species is introgressed into the genome of cultivated potato and stable inherited. The possible mechanissm for such introgression are homeologous pairing leading to crossing over, and intergenomic translocation. An understanding of the gene introgression mechanism may enable their enhancement using suitable treatment; this, in turn will enhance the opportunities for utilization of somatic hybrids in potato improvement.

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Transgenics for potato improvement

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Given the significance of the potato, research on the genetic improvement of this crop is important. Potato breeders are challenged by an auto-tetraploid genome, asexual propagation, and breeding principles and practices that are quite different from those employed for the majority of diploid (or allopolyploid), seed-propagated crops and numerous market limiting traits. Moreover, the genetic base of the cultivated potato is considered to be narrow and yield stasis exists within the potato germplasm. However, the potato has an extremely rich gene pool with seven cultivated and 199 wild species. To broaden the genetic base of the cultivated potato and introduce new traits, potato species have been used as a source of new genes. Potato has always been a close companion to biotechnology. Potato, being vegetatively propagated crop, is highly amenable to asexual clonal propagation techniques *in vitro* and consequently genetic engineering. Besides, potato has the distinct advantage of possessing a commercially viable carbon sink in the form of tuber. Therefore, it has also been looked upon as a potential bioreactor for the production of novel compounds of therapeutic and industrial values. A brief account of successful application of biotechnology in potato production and improvement is discussed here.

1) *In vitro* culture

Potato is perhaps the premier example of a crop plant to which *in vitro* technology has been most extensively applied in all aspects of production, improvement and germplasm handling. This *in vitro* propagation capacity of potato has helped tremendously to overcome genetic nature of this crop imposing several limitations on seed multiplication, conservation of genetic resources and genetic improvement. The first successful establishment of tissue cultures from potato tubers was reported as early as 1951. Since then, the *in vitro* produced disease-free plants, somaclones, haploids and somatic hybrids, plant resistant to diseases and microtubers produced in test tubes have been moved from the laboratory to the field and propagated on a large scale in various countries. Nodal segment culture in which axillary buds and terminal buds grow into new plants is predominantly used for the initial *in vitro* shoot multiplication in potato. *In vitro*-derived micro-plants are used either as direct transplants in the greenhouse/field for the production of mini-tuber/tubers or as explant sources for the production of micro-tubers *in vitro*. Micropropagation revolutionizes potato seed production system by supplying disease-free transplants without any seasonal barrier. *In vitro* potato cultures have also been used to conserve and distribute valuable genetic resources, which are otherwise difficult or impossible using the conventional approaches.

2) Microtubers

Microtuber has subsequently augmented potato seed production. Micro-tubers are miniature tubers developed under tuber-inducing conditions *in vitro*. These small dormant tubers are particularly convenient for handling, storage and distribution. Unlike micro-propagated plantlets, they do not need the time-consuming hardening period in a greenhouse, and can be adapted easily to large-scale mechanized planting in the field.

The ease, with which micro-tubers can be produced, handled and exported, bolsters potato seed production schemes.

3) Potato transgenics for viral resistance

Genetic engineering by employing different strategies for virus resistance is one of the major success stories in potato transgenic biology. The best-documented approach for generating virus resistant transgenic potato is coat protein-mediated resistance, now widely effective against

PVX, PVY and PLRV. A novel strategy has been employed to control viroids, which encode none of the proteins necessary to support their own replication but rely on host components for survival. Resistance against potato spindle tuber viroid (PSTVd) was achieved by expressing a double stranded RNA-specific ribonuclease from yeast. Since the first field test of transgenic potato expressing the coat protein gene, there have been many large-scale field trials of transgenic potato, which confirmed durability of the trait. CPRI has pioneered in exploiting the all possible ways of developing viral resistant potato lines.

4) Potato transgenics for fungal disease resistance

Fungal pathogens cause several important diseases in potato. Among these, late blight (caused by *Phytophthora infestans*) was responsible for the infamous Irish potato famine of 1845 and has become a global threat to potato production, especially during the last decade because of emergence of complex races of the pathogen. Recently, a dominant resistant gene (*RB* gene) has been cloned from wild potato species *S. bulbocastenum*. Introduction of RB gene into susceptible cultivars conferred late blight resistance. Limited field trials of RB transgenic potato lines conducted by Central Potato Research Institute demonstrated durable resistance under Indian condition. Another strategy used for developing late blight resistance in potato is RNAi technology. Using Host delivered (HD) RNAi-mediated gene silencing technology successful late blight resistant transgenic potato lines have been developed by silencing the disease causing pathogen *avr* genes.

5) Potato transgenics for insect pest resistance

Potato tuber moth (*Phtorimaea operculella*) is one of the most important pests in tropical and subtropical regions. All attempts to improve potato plants for resistance to Potato tuber moth using conventional breeding methods have been failed. One of the best and common methods for biological control of the pests is the *cry1Ab* gene. This gene encoding a crystal protein that causes perturbance of the digestive apparatus of Lepidopteran insects and insect death follows. Cry1Ab protein has high mortality effects on potato tuber moth. CPRI, Shimla has developed transgenic potato lines carrying *CryIAb* gene from *Bacillus thurengenisis* against potato tuber moth (PTM). Transgenic tubers stayed free from PMT damage in both *In vitro* and field assays.

6) Potato transgenics for improved quality traits

Potato protein suffers from deficiencies in sulfur containing amino acids. Plant breeding has had limited success in improving the nutritional quality of plant proteins. Introduction of gene encoding seed albumin protein from amaranth (*AmA I*), which is rich in cysteine and methionine, has elevated the level of sulfur containing amino acids of potato proteins. CPRI, Shimla in collaboration with National Centre for Plant Genome Research (NCPGR), New Delhi has already introduced this gene into several Indian potato cultivars. This may partly help addressing protein-calorie malnutrition in the country. Transgenic potato lines to improve cold-chipping attributes by RNAi-mediated silencing of vacuolar invertase gene (*Va-INV*) and UDP Glucose Phosphatase (*UGPase*) gene are in different stages of development and evaluation.

7) Conclusions

From the 16th century Andean highlands to the 21st century cultivated potato species worldwide, biotechnology has transformed potato from test tube to the field in such a way that its production, especially in developing countries, has outpaced all other crops. These developments have far-reaching implications not only in the production and improvement of present day potato but also for the induction of genetic variability, which would enable the synthesis of novel future potatoes.

Micro-irrigation systems and water management in seed and ware potato production

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Potato (*Solanum tuberosum* L.) has a sparse and shallow root system and nearly 70% of total water is used by the crop from upper 30 cm soil layer. It requires 350-550 mm of irrigation water depending on climatic conditions, soil type, length of growing season, duration of variety, purpose of crop and irrigation methods etc. Water requirement of crop is quite high and frequent irrigations are necessary to maintain optimum moisture in the soil throughout its growth. Excess or limited availability of water, the light textured soils having low water retention capacity and shallow rooting system of the plant are some of major factors adversely affecting the crop productivity. The yield and quality of potato depends upon a proper balance between soil air and soil moisture, which can be achieved by adopting a scientific water management programme. The efficiency of irrigation water could be increased if water losses are minimized. The evaporation can be checked by the use of mulch whereas percolation losses of irrigation water, by resorting to drip and sprinkler irrigation methods. Besides, the crop should be raised with optimum levels of other production inputs as they improve water use efficiency. *Water needs of the crop constitute the amount of water required to mature the crop, encompassing consumptive water use (evapo-transpiration plus water required to build up plant tissues) and economically unavoidable water losses in the form of deep percolation and surface run off.*

Potato is cultivated by using primitive as well as advance technology. The primitive technology requires very large investment, but, only satisfactory yield can be harvested, as the crop requires a frequent and light irrigation coupled with balanced fertilization, where as, under advanced technology the sustainable yield can be achieved by applying irrigation alongwith fertilizers through drip and sprinkler methods. The technical knowledge based investment for potato production make it very suitable for self sufficiency and quality exports. The sophisticated technology viz. micro-irrigation and fertigation through drip and sprinkler methods requires heavy investment initially, however, ensure high quality produce with low production cost by increasing crop productivity per unit area & time and conserves soil fertility, environment and ground water.

1. Techniques used to find out water requirement of potato

One pre-planting irrigation is usually given for land preparation and one pre-emergence light irrigation given to ensure quick and uniform plant emergence in the field. The various approaches have been used for working out water requirement of the crop -

1.1 Time interval approach : In this approach different depths of water were applied at different intervals for scheduling of irrigation. The studies based on delta interval approach revealed that about 45-65 cm water was needed by the crop and irrigations were required to be applied at 7-10 days interval.

1.2 Soil moisture /tension approach : According to this concept, irrigations were scheduled whenever soil moisture tension increased to the defined levels and sufficient water applied at each irrigation to bring back the soil moisture in the root zone to the field capacity. Tensiometers were used to monitor change in soil moisture tension. The highest potato yields were obtained by frequent irrigations scheduled at low soil moisture of 0.2 to 0.3 atmosphere tension at 15-22 cm soil depth. With this regime, at least 63% of available soil water remained in the root zone before irrigation. This corresponded to the irrigation interval of 7-12 days.

1.3 Climatological approach : The role of climate in governing the water needs of crop was also recognized and the criteria based on evapo-transpiration and evaporation were utilized for scheduling of irrigations. Experiments designed on the basis of CPE values revealed that the potato yields in sandy soils were highest when irrigations applied at 15-20 mm CPE, on sandy loam soils at 25 mm CPE, on clay loam soils at 40 mm CPE and in loamy sand soils at 20 mm CPE. However, in another approach, irrigations applied at IW/CPE ratio of 2.0 produced yield comparable to that based on 0.25 bar soil moisture tension, but, former approach saved 12 cm water in loamy sand soils of Ludhiana.

1.4 Leaf water potential (ψ1) approach : Plant is the integrating component of soil water status and atmospheric conditions. As such water status of the plant in terms of leaf water potential may serve as a sound basis for scheduling irrigation. Work carried out on this aspect in tarai soils of Pantnagar has shown that irrigation at -2.5 bar (ψ1) gave comparable results with the approaches based on days interval, soil moisture depletion and CPE values, but more work is required on this aspect in order to draw practical conclusions.

1.5 Stress day index approach : Hukkery *et al.* advocated that SDI approach for timing of irrigation for potato by subjecting the crop at different stages of its growth to different pre-defined levels of soil moisture stress. Stress day index was formulated as : SDI = CS x SD

Where CS is the crop susceptibility factor indicating the magnitude of yield reduction due to stress at a given stage, when compared without stress. SD is the stress day factor indicating the degree of imposed stress which may be in terms of soil or plant water deficit.

SDI concept not only reveals the critical stages of crop growth but also gives quantitative information on yield reduction due to a given stress. When SDI and CS are known for different stages of growth, the SD for scheduling irrigation (SDI/CS =SD) could be worked out. However, a strong negative co-relation of the yield with the values of SDI was observed.

1.6 Critical stages of crop : Studies conducted by with-holding irrigation at one or the other growth period without giving any consideration to actual soil moisture content, have revealed controversial results. According to some reports, the crop does not tolerate any stress at any growth stage, while in others, some stages are reported to be more critical than the others. However, most of the reports, have indicated stolonization followed by tuberization as the most critical stages for potato.

1.7 Irrigation under specific situations : In North-Western parts of India , where frost is a problem, crop in such areas may have to be irrigated even on alternate days to prevent frost damage. The last irrigation to the main crop could be timed 10-12 days before the haulms die down or are killed deliberately. Early crop is harvested green and generally irrigated till the harvest, although it is desirable to withhold the last irrigation a few days before harvest particularly if the produce has to be transported to long distances. Lifting the crop when the soil is too moist impairs the transportability and keeping quality of the produce.

2. Water management in plains

About 80% of potato cultivation in India is concentrated in the north-western plains where it is grown under assured irrigation in *rabi* season (October-February). Irrigation method used in potato crop must ensure adequate and uniform application of water with minimum losses as deep percolation and runoff. The water applied at each irrigation should be just sufficient to restore the soil moisture in the plant root zone to the field capacity or to make up the soil moisture deficit. The different irrigation methods used for applying irrigation to the crop are given below -

2.1 Furrow irrigation method : Among various irrigation methods *viz*. surface, sub-surface,

drip and sprinkler methods of irrigation, the **f**urrow irrigation is the most common and popular method for irrigating the crop. The field is laid out in alternate ridges and furrows. Irrigation water applied in furrows seeps into ridges which are moistened to top by the capillary action of soil. Under mechanized cultivation, the crop is irrigated in long furrows, spaced widely at a distance of about 60 cm. In most parts of the country where mechanization is not followed, irrigation can be given in short furrows spaced at 45-50 cm apart. Whatever the form of furrow irrigation, water should never overflow the ridges as this seals the surface soil pores, thereby raises the bulk density and leads to anaerobic conditions. Such adverse effects result in seed tuber decay at planting especially in plains when soil temperatures are high. The field channels of about 70–100 cm width serve bilaterally a set of 6-8 furrows in each bed. The ridges may be wetted only upto about two-third height leaving the rest to be moistened gradually by capillary movement of water. Generally, 4-5 cm of irrigation water should be applied at each irrigation. The irrigation efficiency under furrow irrigation method is low and seldom exceeds 50%. Some water may also be lost as runoff where furrows are longer and slope is steeper than the optimum. Moreover, a lot of water percolates down in the channels, particularly when their number is large.

2.2 Micro-irrigation methods : Micro-irrigation methods improve the efficiency of irrigation water and fertilizer nutrients by increasing availability of these inputs, they also improve hydro-thermal regimes and physical conditions of the soil by maintaining the proper balance between soil air and soil water in the plant's root zone for better root growth and tuber development, which results in to higher yield and tuber numbers as well as tuber quality. With the increasing water scarcity and recurring droughts in many parts of the country, the use of drip and sprinkler irrigation have become extremely important. The water losses are minimum under drip (4-5%) and sprinkler irrigation (15-20%) against 40-50% under furrow method of irrigation. The farmers of Maharashtra, Tamil Nadu, Karnataka, Andhra Pradesh, Gujarat, Kerala, UP, Punjab and Haryana are taking steps for using these approaches for raising agricultural crops.

2.2.1 Drip or trickle irrigation method : Drip method of irrigation involves slow application of water in the plant root zone which provides scope to utilize irrigation water and nutrients efficiently. The moisture availability is always equal to the field capacity in surface soil throughout growth period. About 50% less irrigation water is required for growing potato under drip irrigation against furrow method of irrigation, it also enhances the potato production and keeps productivity at high level (20-30%) in comparison to furrow irrigation. Raised bed two or triple row planting methods are beneficial for potato cultivation with drip irrigation (Table 6). The increase in yield is contributed by an increase both in size and number of tubers. Besides, drip irrigation also provides several advantages to potato growers, including no water runoff from potato field, precise application of water, ability to apply fertilizers to the root zone, minimum leaching losses of chemicals/plant nutrients with reduced canopy moisture and risk of foliar diseases. If drip irrigation method is practiced for potato cultivation, the earthing-up could be dispensed.

2.2.2 Sprinkler irrigation method : The sprinkler irrigation system, gives uniform distribution of water without compacting the soil surface, reducing water losses in terms of deep percolation and surface run off. It could be used in special situations *viz.* in undulating topography, extremely sandy soils with very high water intake rate or fine-textured soils with very low intake rate and costly and scare water supply. Sprinkler irrigation is also beneficial in frosty nights, as a preventive measure against freezing injury to plant. The uniformity of irrigation water is assured only by overlapping pattern of sprays from different size nozzles. The nitrogen applied directly to the potato plants as foliar spray through sprinklers, helps in better root and plant growth as well as for tuber development. If the sprinkler irrigation is practiced, the closer spacing opted advantageously. The system is expected to economize on water by about 40%.

The crop yield from fields with sprinkler irrigation system is higher than those receiving under surface method of irrigation. Raised bed two or triple row planting methods are beneficial for potato cultivation under sprinkler irrigation (Table 7). However, the initial cost of the equipment consisting of pipes, risers and sprinkler heads with a pressure pump is high and technical know-how is required to install and run the system efficiently.

3. Scheduling of micro-irrigation for potato

The proper scheduling of irrigation is most important aspect for efficient utilization of available water resources at farm, particularly in water loving crop like potato. Irrigation scheduling depends on moisture retention capacity of the soil, stage of crop growth, atmospheric factors and rooting patterns of potato cultivars. One pre-planting irrigation is usually applied for land preparation and one pre-emergence light irrigation can be given to ensure quick emergence and crop establishment. Later on, the irrigations are applied to potato through drip and sprinkler irrigation methods. The water application rate/time can be worked out by using following formula-

$$\text{Application rate (mm/hour)} = \frac{\text{Discharge of irrigation water (liters/hour)}}{\text{Spacing between two drippers/sprinklers (m)} \times \text{Spacing between two rows of system (m)}}$$

Under drip method, the irrigation should be applied at alternate day on the basis of CPE (cumulative pan evaporation) for higher yields. The CPRI has done lot of work on standardization of drip and sprinkler irrigation methods. Experimental results advocated that the integral drip laterals with inline/inbuilt/flat dippers (16 mm OD/30 cm/2 lph) placed on each ridge at 60 cm distance corresponds to operate for 35-45 minutes at 125% CPE water level for each irrigation in single row planting. Whereas, for paired/triple row raised bed planting, the irrigation system should run for 1.25-1.50 hours at each irrigation depending upon the prevailing soil moisture conditions in the field (Sood and Singh, 2008 & 2014).

For sprinkler irrigation method, weekly two irrigations at 150% CPE water level are needed for sustainable crop yield. For example, sprinkler irrigation system (*e.g.* 560 lph discharge/10 m x 10 m, distance between two sprinklers and two lateral lines) corresponds to operate for 2.0-2.5 hours at each irrigation depending upon the prevailing soil moisture conditions in the field. However, the application rate/time for irrigation may vary with the specifications of the system.

4. Fertigation with micro-irrigation

The crop raised under drip irrigation/fertigation technique, one third dose of NPK fertilizers (60:27:33 kg N, P_2O_5 and K_2O/ha) through calcium ammonium nitrate or urea, single super phosphate or di-ammonium phosphate and muriate of potash, respectively should be applied as basal at planting in broad ridges/beds at about 5 cm below seed tubers. The remaining two third dose of fertilizers *i.e.*120:53:67 kg N, P_2O_5 and K_2O/ha) should be given through fertigation in eight equal splits, twice in a week starting from plant emergence to six weeks of planting (@ 15, 6.63 & 8.37 kg N, P_2O_5 and K_2O/ha per split/ fertigation through urea, di-ammonium phosphate and muriate of potash, respectively)**.** When fertilizers are used for fertigation, fill the fertilizer tank with 35 liters of water and add 15 kg N, P_2O_5 and K_2O/ha fertilizer and close the lid of fertilizer tank tightly and then run the system for at least half an hour. After fertigation, the system needs to be run for 5 minutes.

Potato grown under sprinkler irrigation/fertigation technique, one third dose of recommended nitrogen *i.e.* 60 kg N/ha with full dose of phosphorus (80 kg P_2O_5/ha) and potash (100 kg K_2O/ha) through calcium ammonium nitrate or urea, single super phosphate or di-ammonium phosphate and muriate of potash, respectively should be applied as basal at planting in the ridges/beds at about 5 cm below the seed tubers. The remaining two third nitrogen *i.e.*120 kg N/ha should be given through fertigation in eight splits, twice in a week starting from plant

emergence to six weeks of crop stage (@ 15 kg N/ha per split/fertigation through urea). When fertilizer is used for fertigation, then fill the tank with 35 liters of water and add 15 kg urea and close the lid of fertilizer tank tightly and then run the system for at least 35-45 minutes (Sood and Singh, 2008 & 2014).

5. Efficient and economic use of water

Potato is an efficient user of irrigation water, but its needs are also inflated by unproductive water losses. A number of techniques have been developed to reduce the water losses and to improve water use efficiency, as described below:

5.1 Use of mulches : The fields under potatoes have to be irrigated frequently, as mentioned earlier, and are therefore, subject to high rates of evaporation especially before full vegetative growth covering the soil surface. Reduction in evaporation during early stages of potato growth either through tillage or mulching results in increased potato yield and thereby increasing water use efficiency. Use of paddy straw mulch should be encouraged as it is reported to increase irrigation water use efficiency by 15% under Punjab conditions.

5.2 Use of anti-transpirants : Studies on use of anti-transpirant, viz. abscisic acid, have shown that it has only a transitory effect in reducing transpiration. As such this approach may not hold good in field, at least in potato.

5.3 Cultural factors : It appears quite workable to improve water use efficiency by stimulating growth through improved practices, viz. i) manuring and fertilizer use, ii) weed control, and iii) adoption of optimal cultural practices. Positive interaction of irrigation and nitrogen increased the water use efficiency. This is because the nitrogen fertilizer stimulated growth and increased yield without increasing the required quantity of irrigation water. Moreover, the crop growth with manures or fertilizers, particularly those which stimulate growth covers the ground quickly and thus reduces evaporation. The increase in evapo-transpiration or consumptive water use by the crop due to manuring and fertilizer use, may therefore, be small when compared with a large response in yield. Consequently manures and especially nitrogen fertilizers improve water use efficiency. Compared to nitrogen, water use efficiency was not affected by phosphate and potassic fertilizers. Weeds compete with crop not only for water but also for nutrient and space and, therefore, reduce the growth and crop yield, even if the water loss through weeds is replenished. The weed control by cultural or chemical methods not only improves water use efficiency but also leads to water economy.

5.4 Inter-cropping : Even with the best management practices, some water is likely to go below the root zone of the potato. Part of this water could be recovered by wheat inter-sown in furrows, soon after potatoes are earthed up. This amounts to *in-situ* recirculation of water that might have percolated down unavoidably.

5.5 Alternate furrow irrigation : Under conditions of water scarcity, 38% saving in irrigation water was achieved by applying water to alternate furrows in turns at the expense of 11% decrease in yield. This practice seems promising where irrigation water is a major limiting factor in potato production.

5.6 Stages of water stress in relation to irrigation efficiency : Knowledge of critical stages of water stress is essential for a judicious use of irrigation water. In potato, stolen initiation and early tuberization stages are the most critical. An inadequate supply of water during these stages affects the yield more than the water deficit at other stages. Therefore, plants should be irrigated at 0.3 bar tension or at 15-20 mm CPE during critical stages of growth. However, the plant could experience mild water stress (0.5 bar tension) during early and late stages of growth and this approach can be used to save irrigation water.

5.7 Use of drip and sprinkler irrigation methods : The process of irrigation consists of introducing water in to the part of soil profile, which serves as root zone for the subsequent use of the crop. Micro-irrigation is an efficient method of providing irrigation water directly in to soil. Fertigation is a new technique where nutrients are applied through drip irrigation near the root zone of the plants for providing optimum moisture and nutrients throughout the period of

crop growth, which helps in better root growth and tuber development. In sprinkler fertigation, the nitrogen is applied through sprinklers by foliar spray directly to the foliage. To increase irrigation water use efficiency, modern irrigation methods such as sprinkler and drip irrigation need to be standardized under different conditions,

6. Water management in hills

Potato is grown as a rainfed crop during summer from March/ April to Sept /October in hilly areas. The total rainfall is generally in excess of evapo-transpiration but the crop usually suffers from drought before or on set of the monsoon and from the excess moisture during the later stages of growth. The studies have shown that there is surplus of 84 cm water per annum. This much quantity of water if conserved or stored and recycled during the crop growth period by using these techniques would greatly help in mitigating the drought problem for its effective use- i) increasing soil water storage capacity; ii) conservation of soil moisture; iii) in situ water harvesting; iv) water harvesting in farm reservoirs/ponds and v). proper drainage system etc.

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Agro-techniques for processing potato production

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Potato processing offers a viable way for diversified utilization and value addition. In case of a semi-perishable commodity like potato, processing is not only a way of value addition, but it also lightens the pressure on storage, providing long-term preservation and lowering the wastage. If organized potato processing industry can be set up in the country in a big way, it will provide better prices to the farmer and uplift the economy of rural areas; will provide employment to the youth, foreign exchange can be saved on imported processed products and crop can be saved from storage losses. Out of 46.9 million tonnes potato production in India during 2016, about 3.88 million tonnes (8.6%) was utilized as raw material by the processing industry including the processing at the level of household, cottage and unorganized sector of the industry. One of the major bottle necks for growth of any agro-processing industry in any country is availability of quality raw material at reasonable prices on round the year basis. The work in this direction was started in India in late 80's and today we have five varieties for potato chips and flakes and one specific variety for making French fries. The processing purpose varieties *viz.,* Kufri Chipsona-1, Kufri Chipsona-2, Kufri Chipsona-3, Kufri Chipsona-4, Kufri Himsona and Kufri Frysona; have shown attributes of better processing shape and size, high dry matter content (21-24%) and acceptable chip/fry colour (below 3.0) together with good yield potential. In spite of good processing qualities, the proportion of processing grade tubers in the total produce has been matter of concern. Hence, the necessity of special agro-techniques was realized. The major agronomic interventions which affect grade-wise tuber distribution are time of planting, crop geometry, plant density, nutrient and water management, integrated nutrient management, source of potassium application, use of cut or whole seed tuber, depth of planting etc. The extensive experimentation was carried out on all these aspects at Central Potato Research Institute since 1996 and complete package of agro-techniques have been developed for processing varieties for the benefit of potato growers and processing industry. With the revised package of practices the percent processing grade tubers have been increased from 55-55% to 70-80%, along with saving on seed potato requirement by about 20-30% because of lower plant density and wider intra and inter-row spacing requirements of processing varieties. Agronomic studies established that the N and K requirements of processing varieties is higher by 50% than popular table purpose varieties because of significantly higher tuber dry matter and starch content. Since long term storage of potatoes meant for processing is inevitable, therefore, the effect of all the revised or developed agro-techniques on processing quality traits specially fry colour and glucose content was also evaluated in cold storage studies at 10-12^{O}C temperature for 5 months.

Selection of field: The field being used for potatoes destined for processing should be free from soil borne diseases viz., common scab, black scruff, brown rot etc. and nematodes.

Field preparation: Hot weather cultivation during May/June is highly desirable. This reduces the infestation of weeds and incidence of soil borne pathogens and pests. Before field preparation the pre planting irrigation is recommended to ensure uniform emergence. As the economical part of this crop exist in the soil hence proper field preparation is required as per soil texture.

Seed preparation and planting: Seed potato should be withdrawn from the cold store about 10 days before planting (for main crop). For early planting seed tuber should be given at least 20 days for *chitting* for higher processing grade yield. This seed should be spread in shade under diffused light and proper ventilated conditions for good sprouting. Pre sprouted tubers ensure early emergence, better stand, early tuberisation and maturity and higher total and processing grade yield. Time of planting is very vital factor for getting higher proportion of processing

grade tubers with good processing quality. For north central plains the optimum time for planting of processing cultivars is 2nd to 3rd week of October for normal planting. This ensures that processing cultivars get 110 days in the field for getting chemically mature potatoes with good yield potential.

Potato varieties released by CPRI for processing purposes

Variety	Parentage	Year	Tuber characters and use	Area of adaptation
Kufri Chipsona-1	MEX.750826 x MS/78-79	1998	Medium to large, white cream, ovoid, shallow eyes, white cream flesh, suitable for chips, French fries, flakes etc.	North Indian Plains
Kufri Chipsona-2	F-6 x QB/B-92-4	1998	Medium, white cream, round, shallow, cream flesh, suitable for chips, flakes etc.	North Indian Plains
Kufri Chipsona-3	MP/91-86 x Kufri Chipsona-2	2006	Medium, white cream, ovoid, shallow eyes, white flesh, suitable for chips, French fries, flakes etc.	North Indian Plains
Kufri Himsona	MP/92-35 x Kufri Chipsona-2	2008	Medium, white cram, round, shallow eyes, cream flesh, suitable for chips, flakes etc.	Indian Hills
Kufri Frysona	MP/92-30 x MP/90-94	2009	Medium to large, white cream, long-oblong, shallow eyes, white flesh, suitable for French fries, flakes etc.	North Indian Plains
Kufri Chipsona-4*	Atlantic x MP/92-35	2010	Medium to large, white cream, round, shallow eyes, white flesh, suitable for chips,flakes etc.	Karnataka, West Bengal and Madhya Pradesh

**Released by the Institute, notification awaited*

Crop geometry and Interculture: Potatoes meant for chipping and flakes should be planted at 67.5 cm (27 inches) row spacing; however the plant to plant spacing may vary from 20 to 35 cm depending upon the size of the seed tuber. However, for seed size tubers of (35-45 mm) the plant to planting spacing of 20 cm (8 inch) is ideal for getting higher proportion of chipping potatoes with highest net yield. However, potatoes meant for french fry should be planted at 90 × 20 cm or 67.5 ×26.5 cm (55,000 plants/ha) crop geometry. The seed tuber should be placed at 8-10 cm (3-4 inch) depth (where second earthing up is practiced) in moist zone for quick germination and to avoid greening of developing tubers. However, in conditions where second earthing up is not in practice the seed tuber should be placed at 12-15 cm (5-6 inches) in the soil to get uniform emergence and to get rid of greening. One Interculture is recommended for potato crop to increase aeration and reducing the infestation of weeds. The interculture operation should be done 20-25 days after planting when the plants are of 10-15 cm height. After doing interculture the earthing up should be completed on the same day after weeding and application of remaining N dose to have better tuber development.

Nutrient management: Among the major nutrients the role of nitrogen (N) fertilization is well documented vis-à-vis tuber growth, development, yield and quality. The inadequate N fertilization leads to poor crop growth and yield, while its excessive application leads to delayed maturity, poor tuber quality, excessive nitrate leaching and occasionally reduction in tuber yield. Tuber quality is equally vital to the processing industry, especially specific gravity, as recovery of the finished products is directly linked with this parameter. Therefore, while increasing the processing-grade tuber yield, it is essential that tuber quality does not get deteriorated. Potato being a semi-perishable commodity, its storage is inevitable to maintain the supply chain throughout the year, which requires assessment of tuber quality both at harvest and of the stored tubers. It is documented that Indian processing cultivars responded to 270 kg N ha^{-1} without deteriorating the processing quality at harvest and during 5 months after storage at 10-12 ^{o}C with the use of sprout suppressant CIPC. This dose should be applied in two equal splits, half at the

time of planting preferably through CAN or ammonium sulphate and rest half at the time of earthing up through urea. It was observed that foliar spray of urea during the crop season more specifically after 60 days resulted in deterioration of the chipping quality specially of stored potatoes. N deficiency in potatoes results in stunted growth, yellowing of the older leaves, dieback of the vine and poor yields. Excess N may delay the onset of tuber growth, increases knobby potatoes and promote excess foliage growth.

Phosphorus is essential for the early growth. Since P is immobile in the soil it can be all added as starter but it needs to be in the root zone. It is placed most efficiently as a band lateral to the seed potato during planting; this raises uptake and lowers P fixation. P deficient plants produce smaller tubers having slightly lower dry matter content and tending to be over mature at harvest. Besides tuber yield, tuber size distribution is very important to the producer and the processor, as processing of large size tuber results in higher percentage of finished product recovery and lower peeling losses. There are reports that P increases the yield of large tubers. It is also documented that P increases the dry matter content of potato as it is the constituent of potato starch. The cultivars exert considerable influence over the responses of potato to P. The varieties accounted for about 35 % variation in P dose. For Indian processing varieties the P recommendation is similar as for ware cultivars i.e. 80 kg P_2O_5/ha, and whole the amount can be applied at the time of planting. P deficiency results in leaf margins roll upward and leaves are darker green and have brown margins. Lower leaves drop. There is no negative effects directly associated with excessive P, but especially in alkaline soils, too much P can lower the uptake and use of iron and zinc.

Potassium (K) is required by plants for translocation of sugars and synthesis of starch. Potato tubers are rich in starch and therefore their requirement for potassium is relatively high; higher than any other mineral element except N. It is reported that tuber quality parameters such as dry matter percentage, specific gravity, sugar concentration, flesh colour and hollow heart disorder are affected by K fertilization. Insufficient potash may result in reduced tuber size and ultimate productivity. The potassium also influences quality parameters, and its application beyond optimum dose (depending upon soil type and crop duration) generally reduces dry matter content and specific gravity. Reducing sugars content in tubers determines the chip colour of finished product - a higher content results in darkening of finished products due to 'maillard reaction' with amino acids during frying. Therefore, for high quality processing products, reducing sugars are required to be minimum in tubers. Increased potassium doses help in decreasing the reducing sugars and lightening the chip colour. Thus potassic fertilization has a greater role to play in determining the productivity and processing qualities in potatoes. Specific gravity, in particular, is affected by the source of K fertilizer. The most widely used form of K fertilizer is potassium chloride. Generally, K application above the recommended dose decreases the dry matter with K-chloride having a higher effect than K-sulphate. The decrease in dry matter percentage was largely due to chloride ion rather than K ion. K sulphate usually results in a higher dry matter percentage of potato tubers than equivalent quantities of K in the chloride form. This could be because K-sulphate leads to faster translocation of the photosynthates from the leaves and stems to the tubers than K-chloride. Studies with Indian processing varieties indicated that K requirement of the processing cultivars is 50% higher (150 kg K_2O/ha) than the table purpose varieties. K-sulphate may be used instead of the K-chloride as it increases tuber dry matter percentage and chips recovery and decreases chip oil absorption without affected the tuber yield. It was also found the splitting of K did not benefit compared to basal dressing. K deficiency symptoms include scorching of the margins of the older leaves.

There are no blanket recommendations for potato about the application of secondary and micronutrients. It is suggested that secondary and micro nutrients should only be applied when there is deficiency identified with the help of soil test. The secondary plant nutrients (Ca, Mg and S) can be limiting on sandy soils. In Ca deficient soils the tubers are small and deformed while the foliage appears normal. It is also reported that localized Ca deficiency led to physiological disorder in tuber known as internal brown spot (IBS), which makes the produce unfit for the

processing industry. Our study established that calcium application (200 kg/ha) in two equal splits (at planting and earthing up) as gypsum helped in mitigating abiotic stress of higher temperatures to attain better tuber productivity and post harvest quality besides improving storability of the processing potatoes.

To summarize it is suggested that for growing potatoes for processing industries in north western and central plains macro-nutrient application should be made @ 270 kg N, 80 kg P_2O_5 and 150 kg K_2O per ha. This dose can also be substituted partially (25%) with organic source (FYM/vermicompost) without adversely affecting the tuber yield and processing quality of processing cultivars.

Water management: The importance of pre planting irrigation has already been highlighted. As potato is shallow rooted crop hence its requirements of irrigation water is quite high (400-600 mm). In sandy/sandy loam soils the light and frequent irrigation (8-10 days interval) is desired. The potato crop should not suffer due to water stress especially during critical stages of crop development like stolon initiation, tuberization etc. Presently modern methods of irrigation like sprinkler and drips are becoming popular as these saves about 30-50% irrigation water. It has been reported that in fertigation there is also appreciable saving of N (25%). Besides these, the yield benefits, reduced weed infestation and improved quality (higher solids, good appearance, etc.) have also been documented by using drip and sprinkler in potato crop over furrow method of irrigation.

Weed management: Weeds compete with crop for nutrients, water, sunlight and space. Due to this competition the reduction in potato yield has been reported from 10-80%. In potato crop the weed management becomes much more important because weeds serve as alternate host to potato diseases pathogens and pests. The critical period for weed control in plains is 20-40 DAP. Though many herbicides have been recommended to control weeds in potato but the most effective and popular is use of metribuzin @ 500 g/ha as pre-emergence (3-7 DAP). Soil applied herbicides require proper soil moisture for high efficacy.

Harvesting: In chipping potato crop the harvesting is recommended at chemical maturity, which has been identified at 110 days after planting for Chipsona cultivars in north central plains. The duration also ensures the higher proportion of chipping grade tubers in the total produce. At this stage cut/crack/bruised/damaged and diseased tubers should be removed to stop the spread of infection.

Curing: The basic purpose of curing is skin hardening and healing of wounds that may occur during harvesting. For curing, the healthy potatoes are heaped to a height of 1to 1.5 m with 4 to 5 width in shade and ventilated area for about 10-15 days depending upon the weather and cultivars skin thickness. The heaps should be covered with gunny bags/tirpal/patera mats etc. Heaps should not cover with polythene sheets.

Grading: It is done as per the requirement of the processing industry. Usually the potatoes of > 45 mm are taken for chipping, >75 mm by French fries and >35 mm for flakes by the industries, for higher recovery of the finished product. It can be done either by manual methods or by mechanical graders.

Potato storage: The well cured and graded tubers are packed in bags. Two kinds of bags are prevalent in India *i.e.* jute bags and synthetic leno bags. The recommended capacity for these bags is 40 kg. The potatoes meant for processing are stored at 10-12^{o}C with the use of CIPC (isopropyl N-(3-chlorophenyl) carbamate) where the accumulation of reducing sugars is minimum and chips/French fries produced are light in colour.

Impact of climate change on potato: Current scenario and adaptation strategies

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Climate change is posing serious threat to human survival on the earth in one or the other way. The disturbances in earth's climate have been greatly accelerated during the past century. It's a well-established fact that greenhouse gas (GHG) emission is responsible for climate change and is increasing rapidly at an alarming rate.

Potato is the third major food commodity of India after rice and wheat in terms of production. India has made tremendous progress in potato production and the per capita availability of potato has increased from 4.37 kg in 1950 to 21.52 kg in 2012. However, the climate change is likely to have a negative effect on potato growth in India, including productivity, production and profitability. The increase in CO_2 is expected to bring on increase in productivity of potato as reported by many workers. However, increase in temperature and atmospheric CO_2, both are interlinked and occur simultaneously and the CO_2 enrichment does not appear to compensate for the detrimental effects of higher temperature on tuber yield. Worldwide potato crop losses due to late blight have been estimated at €12 billion (Haverkort *et al*., 2009). In India, severity of late blight varies from region to region, being more severe in temperate highlands than in the sub-tropical plains with an average of 15% crop losses annually (Collins, 2000). In case of late blight,the effect of climate change on the most dreaded disease of potato in India also, could be mixed. Late blight caused by *Phytophthora infestans* is often regarded as the most important disease of potato globally. In potato cultivation, potato seed is most expensive input accounting for 40 to 50% of the production cost. Since the eastern, northeastern, Deccan and south western parts of the country are not suitable for quality seed production barring few locations, the farmers of these areas have no option but to buy the seed potato from northern India. The high hills of Himachal Pradesh, Indo-Gangetic plains of Punjab, Haryana, north-western part of Uttar Pradesh, and Bihar are suitable for nucleus and breeder seed production. However, Punjab and western Uttar Pradesh are two major states which supply quality potato seed to rest of the country. In case of climate change scenario, the temperature change is likely to affect the late blight outbreak in Punjab and western Uttar Pradesh, thus affecting the potato seed production.

Bacterial wilt is causing huge losses in warmer area of Madhya Pradesh and plateau region. Presently, in North Western Indo-gangetic region, the disease severity is very less but due to warming, the disease may enter the new areas where it is not present. The present studies were conducted to assess the yield losses due to climate change, to develop suitable adaptation strategies and to assess effect of climate change on late blight and bacterial wilt prevalence. In this paper, I'm presenting the findings of studies carried out at ICAR-CPRI, Shimla on climate change impact on potato production.

Impact on potato productivity

For impact on potato productivity, we used WOFOST (**WO**rld **FO**od **ST**udies) crop growth simulation. WOFOST, a mechanistic model which simulates the growth of a crop based upon eco-physiological processes is widely used to assess the effect of climate change on the growth and yield of many crops which includes wheat, rice, maize potato, barley, soybean, sugar beet throughout the world (Boogard *et al.,* 1998, Wolf *et al.,* 2010) and is one of three most widely used crop growth model for climate change studies (Diepen *et al.,*1987). WOFOST has been calibrated for Indian potato cultivars using the time course data on potato growth and development (Dua *et al.,*2014). Hence, WOFOST model was used for impact assessment of climate change on potato productivity and scheduling planting date and selection of suitable cultivar to minimize climate impact in Uttar Pradesh, Punjab, Madhya Pradesh, Gujarat, Bihar

and West Bengal. The simulation studies were carried out for four potato cultivars, Kufri Badshah, Kufri Bahar, Kufri Pukhraj and Kufri Jyoti. The model was run for different planting dates depending upon the locations and the cultivar selected. The simulation study was carried out for potential yields of potato cultivars for all the scenarios.IMD district normals of 1971-2000 of 61,13, 29, 13, 20 and 38 districts of Uttar Pradesh, West Bengal, Bihar, Gujarat and Madhya Pradesh respectively were used for baseline scenario (year 2000). The simulation studies were carried out for A1FI high emission scenario. For generation of scenario for 2020 and 2055, projected changes in surface air temperature for sub-regions of the Asia under SRES A1FI pathway, based on the Fourth Assessment Report (AR4) Atmosphere-Ocean General Circulation Models (AOGCMs) were added on the baseline data. Projected atmospheric CO_2 concentration based on the Bern-CC model for A1FI scenario was used for incorporating the effect of change in CO_2 concentration in WOFOST model. The figures used in this study for atmospheric CO_2 concentration were 367 ppm (for baseline), 415 ppm (for 2020) and 590 ppm (for 2055).

Punjab: The potential productivity of Kufri Badshah, Kufri Pukhraj and Kufri Jyoti was 52.6, 51.2 and 49.0 t/ha under baseline scenario. Under future climate scenario, when the effect of temperature and CO_2 fertilization was considered together, productivity of Kufri Badshah is projected to remain unchanged in 2020 (-0.08%) and will reduced by 2.62% in 2055. However, in case of Kufri Jyoti an insignificant 0.4% increase is expected in 2020 (from 48.9 to 49.0 t/ha), which will decline by 4.59% to 46.6 t/ha) in 2055 as compared to baseline yield. The maximum increase is likely in productivity of Kufri Pukhraj from 50.8 to 51.2 t/ha (0.69%) in 2020 and maximum decline (to 48.1 t/ha; 5.33%) in 2055.

Uttar Pradesh: The productivity of Kufri Badshah Kufri Bahar and Kufri Pukhraj was 46.0, 46.8 and 45.3 t/ha respectively under baseline scenario. The productivity under climate change scenario is expected to decrease by 5.5, 6.1 and 7.0 per cent respectively for Kufri Badshah, Kufri Bahar and Kufri Pukhraj during 2020 and by 9.4, 10.9, 13.4 percent respectively in 2055 as compared to the baseline scenario (Table 1).

Bihar: In the baseline scenario, the potential productivity of potato in Bihar was 40.7, 38.7 and 40.8 t/ha for Kufri Badshah, Kufri Jyoti and Kufri Pukhraj respectively. A decrease of 5.1, 6.2 and 6.9 percent in productivity was recorded during 2020 while productivity is likely to decrease by 8.7, 10.8 and 12.7% during 2055 for the three cultivars, respectively.

West Bengal: In West Bengal under the baseline scenario, the potato productivity was 38.9, 37.2 and 39.2 t/ha for Kufri Badshah, Kufri Jyoti and Kufri Pukhraj respectively. A decrease of 5.1,5.9 and 6.1 percent in productivity was recorded during 2020 while productivity decreased by 8.8, 10.5 and 12.0 percent during 2055 for the three cultivars respectively.

Gujarat: The potential productivity of Kufri Badshah, Kufri Jyoti and Kufri Pukhraj was 40.3, 36.1 and 37.5 t/ha respectively under baseline scenario 2000.The productivity under climate change scenario was found to decrease by 7.6, 9.7 and 10.9 per cent respectively for Kufri Badshah, Kufri Jyoti and Kufri Pukhraj during 2020 (Table 1). The productivity was found to increase by 19.5 per cent for Kufri Badshah while it decreased by 22.1 and 25.5 respectively for Kufri Jyoti and Kufri Pukhraj during 2055 as compared to baseline scenario.

Madhya Pradesh: Under baseline scenario of 2000, the potential productivity of Kufri Badshah, Kufri Jyoti and Kufri Pukhraj was 43.6, 40.0 and 42.2 t/ha respectively. The productivity under climate change scenario was found to decrease by 6.4, 7.3, 7.6 per cent respectively for Kufri Badshah, Kufri Jyoti and Kufri Pukhraj during 2020 and it decreased by 10.9, 12.8 and 14.3 respectively for the three cultivars during 2055 as compared to baseline scenario.

Overall, the impact analysis using WOFOST crop growth model has shown that under A1FI scenario of climate change although there will be no change in potato productivity in Punjab in 2020, in other states the productivity is likely to decline in 2020 (Uttar Pradesh - 5.5 to 7.1%, Bihar - 5.1 to 6.9%, West Bengal - 5.1 to 6.1%, Madhya Pradesh - 6.4 to 7.6% and Gujarat - 7.6 to 10.9%) and 2055 (Uttar Pradesh - 9.4 to 13.4%, Bihar - 8.7 to 12.7%, West Bengal – 8.8 to 12.0%, Madhya Pradesh – 10.9 to 14.3% and Gujarat – 19.5 to 25.2%) (Table 1). With the simple and practical adaptation measures like change in date of planting and selection of suitable variety, the decline in productivity can be brought down to 5.2, 1.9, 4.1, +1.7 and 0.8% in 2020 and 9.2, 6.6, 8.1, 3.6 and 10.4% in 2055 in Uttar Pradesh, Bihar, West Bengal, Madhya Pradesh and Gujarat, respectively.

Table 1: Change in potato yield over baseline scenario.

			Change in yield in 2020		**Change in yield in 2055**	
State	**Cultivar**	**Baseline yield (t/ha)**	**Without adaptation (t/ha)**	**With adaptation (t/ha)**	**Without adaptation (t/ha)**	**With adaptation (t/ha)**
Bihar	Kufri Badshah	40.7	-5.1	-1.91	-8.7	-6.6
	Kufri Jyoti	38.7	-6.2		-10.8	
	Kufri Pukhraj	40.8	-6.9		-12.7	
West Bengal	Kufri Badshah	38.9	-5.1	-4.1	-8.8	-8.1
	Kufri Jyoti	37.2	-5.9		-10.5	
	Kufri Pukhraj	39.2	-6.1		-12.0	
UP	Kufri Badshah	46.0	-5.5	-5.2	-9.4	-9.2
	Kufri Bahar	46.8	-6.1		-10.9	
	Kufri Pukhraj	45.3	-7.0		-13.4	
MP	Kufri Badshah	43.6	-6.4	1.7	-10.9	-3.6
	Kufri Jyoti	40	-7.3		-12.8	
	Kufri Pukhraj	42.2	-7.6		-14.3	
Gujarat	Kufri Badshah	40.3	-7.6	-0.8	-19.5	-10.4
	Kufri Jyoti	36.1	-9.7		-22.1	
	Kufri Pukhraj	37.5	-10.9		-25.2	
Punjab	Kufri Badshah	52.6				
	Kufri Jyoti	49.0				
	Kufri Pukhraj	51.2				

Impact on Late *Blight*

JHULSACAST model was used to work out the effect of climate change on potato late blight outbreak, duration of favourable period and disease severity in Uttar Pradesh, Punjab and West Bengal. The model requires hourly temperature data (°C), relative humidity (RH%) and daily rain (mm) data as input. The model was run for baseline scenario (year 2000) and future climate scenarios (years 2020 and 2055). IMD district normal of 1971-2000 were used for baseline scenario (year 2000) and for future climate scenarios of the years 2020 and 2055 A1FI scenario of temperature (SRES A1FI pathway) was used. It is not possible to predict the daily humidity in years 2020 and 2055 during cropping period, therefore, we assumed that favourable RH would start from a week of emergence of the crop in western Uttar Pradesh and Punjab respectively and proceed with 10 days intervals after the setting of required RH. Thus, the model results are the outcome of the effect of temperature input. To estimate the number of sprays required in seed crop, the daily severity values were calculated on the basis of decision support system developed for western Uttar Pradesh and Punjab (http://cpri.ernet.in). The number of sprays needed in seed

crop was calculated by dividing total accumulated severity by 180 for contact fungicides. The value of divisor 180 was derived empirically.The study was done for entire Punjab state, 23 contiguous districts of western Uttar Pradesh and 11 districts of West Bengal. The JHULSACAST model was run on available weather data for 13 districts of Punjab, 21 districts of western Uttar Pradesh and 11 districts of West Bengal.

Appearance of late blight is expected to be delayed in both 2020 and 2055 climatic scenarios in all the three states based on Jhulsacast Model. The number of days favorable for the disease will increase in Punjab. The earliest Late Blight appearance, which is 13-15 October, in 2000, may be delayed by 0-6 days in 2020 and 12-14 days in 2055. However, due to rise in temperature in future scenario, there will not be mid-season breaks in late blight favourable days. The late blight favorable days during 2000 were 105 and these increased by 30 and 35 days by 2020 and 2055 respectively. Hence more number of fungicide sprays will be required for the control of this disease. However, in the remaining two states the number of favorable days for late blight may decrease and hence less number of fungicides sprays will be required.

In western Uttar Pradesh, earliest late blight appearance during the potato crop season is predicted during 13th October to 1st November in baseline year 2000 and is expected to be delayed by 0 to 8 days in 2020 and 10-21 days in 2055. The delay in late blight appearance in 2020 and 2055 is due to expected increase in temperature by 1.08 and 2.98 ºC respectively over the baseline year 2000. In western Uttar Pradesh, potato growing season would be warmer which would decrease late blight favorable days by 7 and 27 in 2020 and 2055, respectively. The maximum number of sprays required to control late blight in potato seed crop thus would be 7.3 and 8 in future scenario (2020 and 2055) in comparison to 6.5 in baseline (2000) in Punjab. In contrast, there would be no change in number of sprays in year 2020 (over baseline year 2000) in western Uttar Pradesh, however, due to further increase in temperature in year 2055, it could be reduced by 2.

In West Bengal, earliest late blight appearance during the potato crop season was predicted during 4th to 11th October in baseline year 2000 and is expected to be delayed by 7.6 days in 2020. The delay in late blight appearance in 2020 is expected due to increase in temperature by 1.08 over the baseline scenario. However, due to delay in appearance of late blight in the season, the total late blight favorable period is likely to be reduced by 17.7 days in 2020, respectively. The number of sprays required to control late blight in potato seed crop would be 4.2 in future scenario of 2020 in comparison to 5.5.5 in baseline (2000) in West Bengal. Hence less number of fungicides sprays will be required.

Impact on survival of *R. solanacearum* in potato growing districts of plateau region

For studying the survival of *Ralstonia solanacearum* in soils of plateau region of India, results of the experiment conducted under controlled condition of various temperatures on the survival of *R. solanacearum* in soil were used to develop. A logical mathematical model which give the time (in days) in which the bacterium will not survive in the soil. The model gives the results on the basis of daily mean soil temperature. For baseline survival, IMD normal were used and for future climate, temperature rises for A1F1 scenario of the climate change for 2020 and 2055 were added on the baseline temperature. The presence of *R. solani* in the soil was presumed on 1st of January and the days it will survive in the soil of 60 potato growing districts of eight states in plateau region of India were worked out, based upon soil temperature.

Tamil Nadu and Kerala: Under the baseline climate scenario (2000), the bacterium would survive till 18th March in soils of Idukki, Kannur, Malappuram, Pathanammitha, Wayanad districts of Kerala and Dharamapuri, and Virudinagar districts of Tamil Nadu and no changes are expected in 2020. However, in 2055, the bacterium is likely to survive in soil of these districts till 03 March only. The bacterium is likely to disappear 0-05 days earlier in 2020 and 05 to 15 days in 2055.

Karnataka: The bacterium could survive in soil till 18 March in Gadag, 7 April in Dharwad, Bidar, Chitradurga, Karwar, Udupi, and 27 April in Rural and Urban Bangalore districts of Karnataka in baseline scenario. In 2020, the bacterium is likely to survival in soil till 18th march in some areas of Dharwad and Chitradurg while in rest of districts, no change is expected. The bacterium is likely to disappear 0-08 day earlier in 2020 and 11-15 days in 2055.

Andhra Pradesh, Maharashtra, Odisha and Chhatisgarh: The bacterium may survive in soil from 7 to 27 April in different districts and is likely to disappear 3-8 and 11-25 days earlier in Andhra Pradesh and Maharashtra, 3-8 and 11-20 days in Odisha and 3-5 and 11-15 days in Jharkhand in 2020 and 2055, respectively.

Jharkhand: Under the baseline (2000), the bacterium could survive in soil maximum till 27 April in Gumla and 17 May in Ranchi and Singhbhum. No change is expected in Gumla in 2020, however, in Ranchi and Singhbhum, the survival is likely to be up to 6th May only.

Madhya Pradesh and Gujarat: The bacterium may survive in soil from 27 April to 17 May in different plateau districts of Madhya Pradesh and Gujarat in 2000. The bacterium is likely to disappear 3-8 days earlier in 2020 and 5-15 days in 2055 in Madhya Pradesh and 3-5 days earlier in 2020 and 11-26 days in 2055 in Gujarat.

Rajasthan: Under the baseline climate scenario (2000), the bacterium survived in soil till 17 May. The period is likely to reduce by 0-5 days in 2020 and 5-15 days in 2055.

Adaptation and Input Strategies

The various adaptation strategies to combat the impact of climate change on potato productivity may include breeding short duration and heat tolerant cultivars, developing potato cultivars that tuberises at higher night temperatures. Mining for biodiversity to heat tolerance should be given priority. Breeding drought and salinity tolerant cultivars would be effective to face the future challenges of climate change. Use of wind breaks around fields and crop residue mulches for some period after planting, using drip and sprinkler irrigation in place of furrow and basin methods and altering cultural management in potato based cropping systems are few examples of agronomic management practices to reduce the impact of climate change. Besides, conservation tillage and on farm crop residue management are required to increase input use efficiency. Advance planning for possible relocation and identifying new areas for potato cultivation is needed. Improvement and augmentation of cold storage facilities and air conditioned transportation from producing to consumption centers will be required for storage and transportation of this semi-perishable commodity. Strengthening education, research and development in warm climate production technology for ware and seed potato crop is also required to meet the production targets in future climates.

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Practices for Quality Seed Potato Production

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Potato being a vegetatively propagated crop, the perpetuation of viral, mycoplasmal and other soil borne pathogens brings down the quality of seed stock and results in poor yields in subsequent multiplication of such seeds. Keeping in view the problems of hills grown seed and the limited area available for healthy seed production in hills, efforts were made to produce healthy seed in plains. Surveys were conducted in important potato growing areas of the country by CPRI from 1952 onwards. During the survey a remarkable consistency in the aphid build up was found. In north-western and central Indo-Gangetic plains, the aphid population remained very low during October to December months. Taking full advantage of very low aphid population coupled with the use of improved cultural practices; it was made possible to produce healthy seed in plains also and the technique is known as "Seed Plot Technique".

During the last about six decades, seed production has shown spectacular progress in technology development and production strategies. The improvement in quality and quantity is commendable. At present 94% of the total quantity of seed is produced in eight states of sub-tropical plains and remaining 6% in hilly states. India is the only leading country in Asia which has developed scientific seed production technology for sub-tropics by taking advantage of low aphid period and absence of soil borne diseases and insect-pest.

Basic requirement for seed production

Basic requirements like suitable areas, soils, fertilizers, manures and suitable varieties etc. play a vital role in a successful seed production programme. A quality seed can be produced in areas and fields which are free from serious soil borne pathogens and pests. High hills above 7000' msl are suitable for nucleus and breeders seed potato production. Indo-Gangetic plains of Punjab, Haryana, North-Western parts of Uttar Pradesh, Madhya Pradesh and Bihar are suitable for seed production under low aphid period from October to March and are the primary source of high quality seed. Parts of North-western Uttar Pradesh, Madhya Pradesh, Bihar and entire West Bengal, Odisha, Rajasthan and Gujarat are only secondary source of seed production due to brown rot and aphids. There are two seasons for seed production *i.e.* summer and autumn. In summer, seed is produced under long days and rain fed condition in high hills from January to October whereas, in autumn, the seed is produced in sub-tropical plains under short days and irrigated conditions from October to March. The optimum temperature for foliar growth is 18-22^{o}C and 10-16^{o}C is good for tuberization.

Seed Plot Technique (SPT)

The technique, of growing seed potato crop during low aphid period with healthy seed from October to January/ February coupled with the use of insecticides, roguing and dehaulming from the last week of December up to second week of January, was developed by the CPRI, Shimla in 1959 and is called as "Seed Plot Technique". Quality seed production was possible under this technique in sub- tropical plains by advancing the date of planting from December end to first week of October.

Characteristic features of SPT

- There should be a low aphid or aphid free period of 75 days after the planting of crop.
- Adopt 2-3 years crop rotation to take care of soil borne pathogens.
- Seed crop should be grown in isolation of 25 meters from ware crop.

- Seed should be procured from reliable sources and must be free from viruses and soil born pathogens. Cold stored seed of right physiological age should be used.
- Crop should be planted 10th October in Punjab, 25th October in Haryana, Rajasthan, Western Uttar Pradesh and 5th November in Eastern Uttar Pradesh, Bihar, West Bengal, Odisha.
- Systemic insecticide such as Thimet 10G to be applied in split doses of 10 kg/ha at the time of planting and earthing up against sucking insects.
- Pre-sprouted seed with multiple sprouts may be used, which ensures quick, uniform and early germination.
- Inspect the seed crop thrice at 45, 60 & 75 days during growing season to remove off type of diseased plants.
- Spray the crop with mancozeb @ 2.5 kg/ha at 10 days intervals from 3rd week of November and spray Curzate M-8 @ 3.0 kg/ha or Ridomil Gold @0.25% as and when late blight is observed.
- Spray the crop with Rogor (1.25 L/ha) or Imidacloprid 17.8%SL (0.4L/ha) alternatively at 15 days interval from 1st week of December to control the insect vectors.
- Irrigation should be with hold in 3rd week of December i.e. 7-10 days before haulms killing in north-western plains and 1st week of January in north-eastern plains.
- Haulms killing to be done in the end of December in Punjab, Haryana, Western Uttar Pradesh; by 10th January in Central Uttar Pradesh, Madhya Pradesh and by 15th January in eastern Uttar Pradesh, Bihar, West Bengal and Odisha.
- Harvest the crop, 15-20 days after haulms killing when the fields are in workable condition and tuber skin is hardened. Cure the crop in heaps in a cool shady place for about 15-20 days.
- Treat the produce with commercial grade Boric Acid 3% solution for 20 minutes to prevent surface borne pathogens. Dry in shade and fill in bags, sealed and labeled properly and cold store.

Selection of field: For raising a potato seed crop, soil should be free from perennial weeds and soil-borne pathogens such as scabs, brown rot, wart and black scurf and pests like cut worm (*Agrotis ipsilon*). For minimizing the perpetuation of soil-borne diseases, it is desirable to adopt 2-3 years crop rotations preferably with cereals. As far as possible, the seed crop should be grown in a field where potato has not been grown for last two years. A well-drained, light textured sandy loam soil with neutral to slightly alkaline soil pH is preferred.

Hot weather cultivation: In the Indo- Gangetic plains, opening the soil by deep tillage and keeping it exposed to extreme high temperatures during hot summers reduces the incidence of soil-borne diseases and controls weeds and cutworms. Deep ploughing the field in April end and keeping it open in May and June with one or two more ploughings serve the purpose of hot weather cultivation.

Green manuring: Raising and ploughing under the 45-55 days old green manuring crops of sunhemp (*Crotalaria juncea*) or dhaincha (*Sesbania aculeate*) during rainy season at least one month before potato planting in the plains and growing of French bean or other leguminous crops in the hills as green manure is beneficial in reducing pest and disease incidence. Green manuring improves the soil fertility and water holding capacity to benefit growth and yield of potato.

Tillage: Potato seed production demands minimal mechanical interference in the standing crop to check spread of viruses through physical contact. Therefore, field should be cleaned of stubble and perennial weeds by adequate tillage operation before planting. If the field is relatively free from weeds, minimum tillage can be practiced to save on the fuel, time and money to reduce cost of cultivation. Minimum tillage combined with chemical weed control is best

suited for seed production as it ensures minimum interference in standing crop. 2-3 ploughings followed by plankings make the soil loose and friable suited for potato planting.

Isolation of seed plots: The seed crop should be separated from crops meant for ware purpose by a distance of at least 5 meters to avoid mixture and spread of viral diseases. Isolation is also required between different varieties of the seed crop.

Varieties: Zone-specific varieties and crop period for seed production are as under:

Zones	Crop Period	Varieties
North-Western hills	Summer (15th April –October)	Kufri Jyoti, K. Himalini, K. Girdhari, K. Kanchan, K. Chandramukhi,
North-Western plains	Autumn (1st week October – Feb./March)	Kufri Jyoti, K. Chandramukhi, K. Badshah, K. Pukhraj, K. Surya, K. Khyati, K. Satluj
North Central plains (MP)	Autumn (3rd week October – Feb./March)	Kufri Jyoti, K. Chandramukhi, K. Sindhuri, K. Pukhraj, K. Lauvkar, K. Chipsona I , III & IV, K. Surya, K. Lalima
North Central plains (UP)	Autumn (2nd week October – Feb./March)	K. Bahar, K. Sadabahar, K. Anand, K. Surya, K. Chipsona I, III & IV, K. Frysona, K. Gaurav, K. Badshah,
North-Eastern plains	Autumn (4th week October – Feb./March)	K. Jyoti, K. Chandramukhi, K. Kanchan, K. Sindhuri, K. Lalima, K. Arun, K. Ashoka, K. Pukhraj, K. Surya, K. Chipsona I , III & IV

Seed source: For on farm multiplication of quality seed by the farmers, it is essential to obtain healthy, disease free, true-to-the-type and treated seed from reliable source, preferably from a government agency. The foundation or certified seed tubers should be used to start with and stocks should be replaced every 3-4 years.

Pre-sprouting: The pre-sprouting treatment of the seed tubers before planting ensures multiple, stout and healthy sprouts, which helps in quick emergence, uniform stand and early maturity of the crop. It also improves yield, number of tubers and proportion of seed-sized tubers in the produce. For pre-sprouting, withdraw the seed tubers from cold store 10 days before planting. Keep it in pre-cooling chamber of cold store for 24 hours.

Whereas, in the hills, take out the tubers from country stores during first fortnight of March. Spread the seed tuber in thin layer preferably on cemented or pucca floor in a ventilated room under diffused light. If tubers are already over-sprouted due to malfunctioning of cold store or any other reason remove such sprouts. It helps in checking apical dominance. Sort out the blind, hairy sprouted, rotten and diseased tubers. Transfer the well-sprouted tubers in the trays to the field for planting. While transferring the sprouted tubers to the trays and at planting, care should be taken to avoid damage to the sprouts.

Planting method: Use of cut tubers is prohibited and only whole tubers are used for seed crop. In the plains inter-row spacing for planting seed crop manually, bullock and tractor drawn implements may be kept at 40-45, 50-55 and 60cm, respectively. Planting of seed-sized tubers of 35-50g at 60cm inter and 20cm intra-row spacing is best for seed potato production. Plant the large size tubers by increasing the intra-row spacing from 20 to 30 cm depending upon the size of seed tubers. Small-sized tubers are planted at reduced intra-row spacing. The approximate intra-row spacing for <25, 25-50, 50-100 and >100g seed size is 15, 20, 25 and 30 cm with inter-

row spacing of 60cm, respectively. Seed tubers are placed at a depth of 5-7 cm from top of the ridges made manually or by tractor drawn implements.

Manure and fertilizers: Application of well rotten farmyard manure (FYM) @ 20-25 t/ha in absence of green manuring is beneficial for seed crop. It improves soil physical condition, soil fertility and water holding capacity of the soil. The FYM should be incorporated into the soil 20-25 days before planting. Nitrogen needs of seed crop are 25-30% lower than the ware crop. Excess N increases the yield of undesirable extra-large size tubers and produces dark green foliage masking the symptoms of viral and mycoplasmas diseases and detection of infected plants becomes difficult during roguing. In the plains, the crop requires a basal dose of 75kg N, 60-80kg P_2O_5 and 100-120 kgK_2O/ha at planting, whereas, 100 Kg each of N, P_2O_5 and K_2O is the recommended basal dose for the hills. Top dressing of 75 kg N/ha at earthing up in plains and 20 kg N/ha in the hills given through urea is adequate. The basal dose of N can also be applied through urea by incorporating it into the soil at least 48 hours before planting during the field preparation. It comes over the constraints in using costly nitrogenous fertilizers of ammonium sulphate (AS) and calcium ammonium nitrate (CAN).

Intercultural operations and weed control: The objectives of intercultural operations in potato are weed control, earthing up for firming up the ridges to prevent exposure of growing tubers and for application of split dose of Nitrogen and Thimet insecticide. However, operations involving human, animal and implement movement in standing seed crop should be minimal to prevent transmission of plant viruses through physical contact. It would be better if these operations are completed by 20-25 days after planting when plants attain the height of about 10-15 cm and foliage cover is still small. The split dose of Nitrogen and Thimet should be applied at hoeing and earthing up about 5cm away from the plants. The hoeing must not be delayed beyond 30 days in plains and 45 days in the hills after planting to avoid damage to the plant roots, foliage and stolons, which may adversely affect the number of tubers resulting in reduction in yield. Pre-emergence weedicides like Metribuzin @ 0.75 kg/ha, Oxyfluorfen @ 0.15 kg/ha, Linuron @ 0.5 kg/ha, Alachlor @ 1.5kg/ha and Isoproturon @ 0.75 kg/ha applied 2-3 days after planting are effective. Pre-emergence herbicides are most effective when applied in moist soil. Therefore, if soil is dry apply herbicides after first irrigation as soon as it is possible to enter the field. In case pre-emergence herbicides are not used, spray paraquat @ 0.5 kg/ha at about 5-10% plant emergence of potato provided sufficient weeds have appeared, as it kills only emerged weeds. Chemical weed control eliminates manual hoeing. Thus, full earthing up at planting combined with chemical weed control effectively minimizes undesirable physical intervention in standing seed crop.

Water management: Pre-sowing irrigation/ rains before land preparation is beneficial for early and uniform emergence. If pre-sowing irrigation is omitted at the time of field preparation, irrigate the crop immediately after planting. First irrigation following planting should be light to minimize damage to the newly formed ridges. Heavy irrigation before emergence leads to anaerobic conditions resulting in rottage of seed tubers, gappy emergence and reduced tuber yield. Second irrigation is given a week after first irrigation. Subsequently, irrigate the crop at 7-10 days interval depending upon the soil and weather conditions. Avoid flooding over the ridges and irrigate only up to 2/3 height of the ridges. As far as possible, irrigate during morning and evening hours. In a normal seed crop, 6-8 irrigations are required. Light and frequent irrigations are much better than heavy irrigations given less frequently. Excess moisture makes lenticels prominent due to rupturing and seed tuber quality is impaired. It also promotes certain diseases. Stop irrigation at about 10 days before dehaulming in light soil and 15 days in heavy soils. Moisture stress restricts re-growths after dehaulming and hastens curing of peel of seed tubers.

Plant protection: Additional plant protection measures against aphid and other vector transmitting viral diseases are required in the seed crop. Application of granular systemic insecticide, Thimet 10G @ 20 kg/ha at the time of earthing up takes care of jassids, leafhoppers and white flies at early stages of growth up to 30-35 days. After appearance of aphids, two sprays of imidacloprid 17.8% S.L. @ 0.003% may be repeated at an interval of 12-15 days depending on duration of the crop and level of infestation. Drenching of the ridges with chlorpyriphos 20EC @ 2.5litre /ha effectively controls cutworms attack during the early stages of the crop. In white grub prone areas, Chlorpyriphos 20EC @ 2.5 litre /ha should be applied either after mixing with sand or can be sprayed on the ridges before the final earthing up.

For control of early and late blight, one prophylactic spray of Mancozeb @ 0.2% is given. It may be repeated at an interval of 7-14 days depending upon the weather condition. It will also take care of other foliar diseases like phoma blight, etc. In case of persistent and severe attack of late blight, spray of systemic fungicides like curzate M-8 @ 0.3% or Ridomil Gold @ 0.25% may be given and repeat the spray after 7-10 days if required.

Inspection and roguing: Inspection of the seed crop to remove or rogue out the off type and diseased plants showing mosaic, mottling, veinal necrosis, crinkling and rolling of leaves, marginal flavescence and purple top roll symptoms is essential. The first roguing is done 35-40 days after planting and the second is done 20 days after the first roguing while the third roguing is done 5-7 days before dehaulming. At each roguing, make it sure to remove the tuber and tuberlets of rogued plants.

Dehaulming: Removal of haulms of the seed crop is essential by 5-25 January in the plains before aphids (*M. persicae* and others) population reaches the critical level of 20 aphids/100 compound leaves. Cut the haulms by 15th August in the hills. Dehaulming is done by manually cutting with the sickle close to the ground or by spraying non-selective herbicide Paraquat @ 0.5kg/ha. In the plains, if haulms are removed manually, it is preferred to keep the vines/haulms on the ridges to protect exposed tubers from high temperature and direct sunlight. Re-growths of leaves if any are also cut after a week of dehaulming, because the tender and succulent leaves are more attractive to aphid vector.

Harvesting and curing: Start digging 10-15 days after dehaulming when peel is firm to withstand handling operations. In the Indo-Gangetic plains potato digging beyond February promotes rottage due to soft rot and charcoal rot. Digging may be done either manually by spades or by mechanical potato digger. Exercise care to avoid bruising of tubers during harvesting, handling and transportation. After harvesting, keep potato tubers in heaps on raised beds for about 15 days for hardening of peel and shedding of adhered soil from tubers surface. Heaps of about 1.5m high and 3.5m broad at the base and variable length as needed are convenient, effective and economical. Cover the heaps with paddy straw or tarpaulins.

Grading and seed treatment: Proper size grading of tubers in the produce of seed crop is beneficial. It also helps in controlling the seed rate effectively by adjustment of spacing according to tuber size. Before grading, surface dry the produce and sorts out all cut, cracked and rotten tubers. Seed tubers are usually graded into four grades, *viz.* extra small (<25g), small (25-50g), medium (50-100g) and large (>100g). Seed tubers should be dip-treated with boric acid (3%) solution against tuber borne diseases of common scab and black scurf for 25-30 minutes. The fresh boric acid solution can be used for 20 times for treatment, provided tubers were washed clean with water. Dip treatment with organo-mercurial compound, Emisan 6 @ 0.25% for 20 min is also effective, but is considered hazardous and may be avoided. The treated tubers should be thoroughly dried in shade before bagging and storage.

Packing and storage: Keep the seed tubers in 50 kg gunny bags and store in cold store (4°C) in the plains latest by the end of February. The produce in the high hills can be stored in country stores by the end of February. The treated seed bags should be properly sealed and labeled "Poisonous" to avoid human consumption mistakenly.

Suggested Readings

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Quality parameters of processing potato

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Potato is one of the agricultural commodities, which is processed in several processed products and these products are popular almost throughout the world. The products like chips, French fries, flakes etc are available in any corner of the world besides several indigenous products in different geographical regions. In India be it north or south; east or west you will find several recipes based on potato. However, to make some of the processed products like chips, French fries or flakes potato tuber needs to have certain minimum quality attributes to make the products of superior quality and for better economic returns to the processor. Physical traits of tuber, desirable for making different products, are shape, size, depth of eyes, thickness of skin, external and internal defects and flesh colour. For chips making tuber should be round to oval shape while for French fries oblong tubers are desirable. The eyes should be shallow and peel thickness less to minimize the peeling losses during processing. Potatoes must be free from the defects like cuts, cracks, hollow heart, internal brown spots and greening. Similarly shrivelled tubers are also not desirable for processing as peeling losses are higher and product texture is poor.

The major biochemical traits of tuber considered important for any processing genotype are starch, reducing sugars (mainly glucose & fructose), sucrose, free amino acids, phenolic compounds and glycoalkaloids. The starch content has major bearing upon the dry matter or solids content of tuber and which is very important with respect to product recovery, oil content and shelf life of the finished product besides the taste and texture. Starch or dry matter content is mainly determined by the genotype, but is also strongly affected by the growing environment and cultural practices. In India several indigenous varieties have been developed with high dry matter content (> 20%) (Kufri Chipsona-1, Kufri Chipsona-2, Kufri Chipsona-3, Kufri Himsona and Kufri Frysona) and suitable areas have been identified to get better dry matter content values. The Malwa region of Madhya Pradesh and Gujarat state have been shown to produce potatoes of superior quality with respect to chips and French fry making. The distribution of starch or total dry matter content within the potato tuber is also very important especially for making French fries of uniform quality.

Next to dry matter or starch content, the reducing sugars are very important traits with respect to product colour, taste and acrylamide content. Higher content of reducing sugars result in brown colour and bitter taste in the fried potato products besides development of potentially toxic acrylamide formation through 'Maillard reaction' Again genotype has a strong influence on the tuber sugar content and is also affected by growing environments, cultural practices and tuber maturity. Distribution of reducing sugars within the tuber is also important to get uniform product quality. The Indian processing varieties possess <0.05% reducing sugars at harvest in fully mature tubers. The tubers produced in Madhya Pradesh and Gujarat region have relatively lower values of reducing sugars. The role of reducing sugars along with free amino acids, especially asparagines, has become more important as their higher levels are reported to result in the formation of potentially carcinogenic acrylamides in the fried finished products. Thus besides lower reducing sugar content, research efforts are also needed to be directed towards quantifying the levels of asparagines content, if required.

Sucrose levels along with the reducing sugars content determine, to a large extent, the potential free sugars development during storage. Lower sucrose levels at harvest are desirable for long term storage of processing genotypes. However, sugar development during storage, either cold induced or senescence induced, is a very complex phenomenon involving several biochemical traits and metabolic processes, but genotype specific storage conditions management can result in maintenance of lower reducing sugar levels for longer periods. To avoid enzymatic browning, lower values of phenolic contents are required and this traits is especially more important for making dehydrated products/chips. The processing purpose

genotypes should have very low values of glycoalkaloids, as they impart bitter taste to product and are also potentially toxic from health point of view.

In conclusion processing genotypes should be a balanced combination of tuber physical and biochemical traits to yield products of superior quality at fresh harvest and after long term storage.

Table 1: Quality requirements for various processing products of potato

Parameter	**Chips**	**French fries**	**Flakes**
Tuber shape	Round to oval	Oblong to long	Round to oval
Tuber size	45-80 mm	> 75 mm	>35 mm
Eyes	Shallow	Shallow	Shallow
Specific gravity	> 1.080	> 1.080	> 1.080
Dry matter (%)	> 20 %	> 20 %	> 20 %
Reducing sugars (mg/100 g f wt)	< 100	< 150	< 150

Table 2: Sugar content of processing varieties at different locations in India

Location	Reducing sugars (mg/100 g fresh wt)					Sucrose (mg/100 g fresh wt)				
	Kufri Chipsona-1	Kufri Chipsona-2	Kufri Chipsona-3	Atlantic	$LSD_{0.05}$	Kufri Chipsona-1	Kufri Chipsona-2	Kufri Chipsona-3	Atlantic	$LSD_{0.05}$
Agra	17.0	28.2	38.0	47.8	7.7	127	161	99.4	166	18.4
Deesa	24.3	31.5	23.5	23.1	7.2	233	260	218	180	10.9
Durgapura	10.7	53.1	9.3	8.5	1.8	243	249	207	168	12.9
Faizabad	16.5	22.4	12.4	10.4	3.1	161	183	130	199	11.7
Indore	11.4	36.7	10.3	23.5	3.8	126	156	135	154	16.4
Jalandhar	174.0	42.2	27.1	91.4	7.0	329	195	166	127	12.5
Jorhat	59.7	62.2	91.7	64.1	7.9	166	196	165	159	18.2
Kota	19.5	30.3	26.7	18.9	4.6	147	168	195	146	19.3
Kufri	43.8	98.2	16.2	15.6	2.0	163	193	164	173	21.9
Ladol	19.2	21.5	14.6	9.6	2.7	214	248	231	176	17.8
Modipuram	24.3	27.8	20.5	36.0	3.9	173	198	157	184	16.7
Ooty	27.5	23.9	32.9	8.9	2.3	162	110	163	126	16.4
Raipur	19.1	18.7	20.0	18.8	3.9	182	158	101	98.1	19.5

Post Harvest Management of Potatoes

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Potato contains approximately 80% water and is a semi-perishable commodity. About 90% of the potatoes produced in our country are grown in the Indo-Gangetic plains and are harvested during January to March, the beginning of the long hot summer. This makes it very difficult to store the harvested potatoes, as under these conditions potatoes cannot be stored without refrigeration for more than 3-4 months after harvest because of enormous losses due to rottage, shrinkage, sprouting and attack of different microbes and pests. According to an estimate, a higher proportion of potatoes (16%) in the country is wasted as post-harvest losses (PHLs) than that used as seed (8.5%) or processing (7.5%). This is an unfortunate situation that nearly 1 million t in excess to the total potato production in Canada during the triennium ending 2010 (FAOSTAT) itself was wasted in India. Losses occur at every stage of the supply chain. These losses result due to lack of proper storage facilities, absence of proper handling, transportation, pre- and post-harvest treatment. Besides, the attack of pathogens at conducive temperatures and environment increase the losses tremendously and therefore, proper handling, desired treatments and safe post-harvest environment is required to reduce the losses in potatoes after harvest.

Post-harvest losses

Hot summer temperatures, lack of state of the art cold storage facilities and massive transportation of potatoes from northern to southern states are the causes of the high wastage of potato in absolute terms. Various factors which play key role in post-harvest loss of potatoes are being discussed in subsequent heads.

Pathogenic Factors

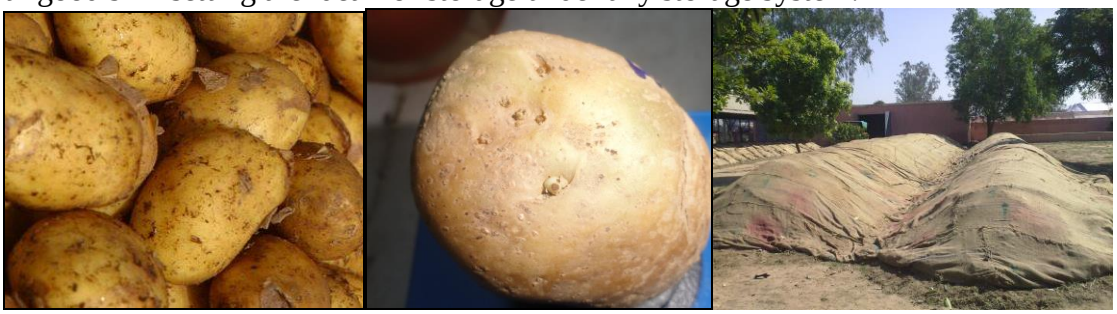
Losses in potatoes caused by pathogens are greater than the losses due to physiological causes. Physical damage to tubers during harvesting and handling aggravates the attack of bacteria and fungi leading to quantitative loss. Generally, the more common storage diseases caused by fungi are late blight, dry rot and pink rot. The most severe bacterial disease that causes rotting is soft rot. When infection occurs in the field, rotting begins in the field and continues during storage. When infection occurs after harvesting, it is generally through mechanical injury as in the case of dry rot. High humidity and condensation of water on tuber surface can lead to infection by soft rot. Among the insect pests, tuber moth causes maximum damage during storage and is common in potatoes stored under higher temperatures, as is the case with non-refrigerated storage.

Fungal diseases: Among the diseases of fungal origin, the most important ones causing pre and post-harvest losses are the late blight and the dry rot. **Late Blight** is caused by *Phytophthora infestans* and the disease affects all plant parts, viz., leaves, stems and tubers. Tubers develop reddish brown, shallow to deep, dry rot lesions. The affected tuber flesh becomes 'caramalised' with a sugary texture. Tubers carrying the pathogen are the real carriers and serve as the source of the disease in the subsequent season. When the storage conditions are good, the rot remains dry and doesn't extend. However, when the storage conditions are faulty, the dead tissues open a route to the secondary soft rot. The **Dry Rot** caused by *Fusarium sp.* on the other hand appears during storage. The skin wrinkles forming irregular concentric rings, and in general tuber tissue decay forms pockets of pink, red or bluish colour, which contain spore masses in the mycelium. The disease incidence can be reduced with proper curing and avoiding bruising during harvest and transport.

Bacterial diseases: Among the main diseases of bacterial origin, Bacterial wilt (*Ralstonia solanacearum*) and soft rot (*Erwinia carotovora var. carotovora* and *Erwinia carotovora var. atroséptica*) are the major threats. **Brown rot** is a destructive disease to the plant and causes rotting of the tubers in transit or storage. The **Soft Rot** causes high amount of losses particularly during storage and every year about 2% losses have been estimated due to rots. Excessive moisture, high temperature, excess nitrogen, tuber injuries and poor ventilation during storage are the important factors helping the disease development and spread. Initially a small area of tuber tissue around lenticel or stolon attachment point becomes water soaked and soft. Under low humidity, the initial soft rot lesions become dark and sunken, while under high humidity, the lesion may enlarge and spread to larger area. Tubers in advanced stages of decay are usually invaded by other organisms and the decaying tissue becomes slimy with foul smell and brown liquid ooze. At harvest, many small rotted tubers with intact skin can be seen. Among the control measures, avoiding excess irrigation and nitrogen, providing proper drainage prevents the spread of the disease. Harvesting the crop before soil temperature rises above 28°C is recommended. The crop should be harvested only when the tuber skin is fully cured. Care should be taken to avoid injury to tubers and bruised injured tubers are sorted out. Treating the tubers with 3% boric acid for 30 min and drying them under shade minimizes infection during storage.

***Potato Tuber Moth (PTM)*: PTM** *(Pthorimea operculella)* in larvae state damages foliage mining between the inferior epidermis and superior of the leaves. It is also introduced in the tuber mining burrows and filling them with their excrements. The initial invasion happens in the field and continues in storage. The tunnels formed in the tubers make them unfit for any consumption and in conducive conditions the rottage by secondary pathogens is aggravated. Therefore, in the areas of PTM infestation in traditional and room tempearature storages proper care like removal of infested tubers, restriction of PTM entry into storage and use of dried leaves of some herbs (e.g. Lantana) in storage is recommended.

Losses due to Pre- Storage Factors: The quality during potato storage depends on the quality of tubers reaching the store and its management starts from the production stage itself. Cultural practices affect the quality of tuber, since potatoes grown in improperly prepared fields are mechanically damaged at harvesting and have reduced storability. Adequate pest and disease management is also necessary for producing potatoes with good keeping quality. Hence, management of pre-storage factors that affect the keeping quality of potato is prime step in good storage management and about 3-8% losses have been attributed to these factors. The potatoes need to be harvested in dry weather and irrigation has to be stopped two weeks before dehaulming. Harvesting is done 10-15 days after haulm cutting for facilitating skin setting. Besides this, curing /suberization (the process by which wounds are healed in potatoes under optimum conditions of <25ºC temperature and 95% relative humidity) is also important. Potatoes harvested under wet soil conditions must be dried before storage because even little moisture on the surface of the tubers could lead to infection and rotting during the storage. Only mature tubers with good skin setting are ideal for storage under any storage system.



Uncured and a cured potato tuber and curing in field

Losses at Harvesting and Handling: The mechanical damage during harvest is responsible for loss initiation in potatoes in form of cuts and bruises hence care should be taken to ensure that cuts and bruises are minimized and the exposure to sun should be avoided. Exposure from sun heat causes excessive rotting and damages can take place as a result of direct contact with the sun at the time of harvest. This may also occur before harvesting when the plant is dead or cut. Potatoes harvested in hot weather rot more than those removed in more temperate conditions. When air temperature is 32°C or higher, it is not advisable to dry potatoes and optimum temperature of air for drying has been recommended as 26.5°C or less. Proper care at the time of harvesting, curing of potatoes in temporary heaps and careful handling may help in reducing the losses to a great extent.

Losses during Storage: Losses during storage are affected by factors like physiological tuber condition, mechanical damage suffered during harvest and handling, as well as by storage conditions. Mechanical damage (cuts and bruises) facilitates invasion and development of microorganisms that cause illnesses and rotting. It is appreciated that weight loss shows a lineal relationship with regard to nature or magnitude of physical damage. On the other hand, losses by rottage increase exponentially with regard to magnitude of physical damage. In general, it is necessary to reduce tubers physical damage to minimize losses during storage.

Losses during Transport, Marketing and Distribution: Losses to potato crop during transport, marketing and distribution have been estimated to be about 3-8%. It is recommended that transport of potatoes should be completed rapidly to avoid sun damage. Transferring tubers in sacks generate a larger percentage of damages unless the handling is done very carefully. Thus crop handling inside the field or transport to exterior with trucks or trailers has great importance. There are mechanical damages to potatoes before leaving the field. These damages become more evident later during storage. To avoid mechanical damages in all the operations, it is necessary to convince the personnel to utilize proper handling like: Potatoes should be placed inside containers and not thrown from distance, truck drivers should not stand on potato sacks but on the platform of the truck and full sacks of potatoes should be placed in position and not thrown at truck loading and discharging. Use of soft linings is recommended in trailers and trucks that transport potatoes. A straw bed should be used in trucks, or pads can be made with sewn sacks half filled with straw and laying potato bags on these shall significantly reduce the bruising. It is also necessary to securely tie the load to avoid movement of sacks, resulting in bruising. Another point is that potatoes should be handled the absolute minimum number of times possible. All labour involved with potato handling should be supervised carefully to guarantee an appropriate operation.

Storage Practices to Minimize Losses: In India, potatoes are generally stored in cold stores and used for both seed and table purposes. Seed potatoes are best stored in cold store maintained at 2-4°C and at about 95% relative humidity. But cold stored potatoes are not suitable for table and processing purposes. Potatoes stored in cold store accumulated sugars and become sweet in taste and are therefore, less suitable for consumption. Because of high accumulation of reducing sugars, these potatoes produce dark coloured chips which are unacceptable both colour-wise and taste-wise. Thus, the storage requirements of potatoes should be in accordance with the purpose for which potatoes are stored. It is suggested that cold stores maintained at 2-4°C should be used exclusively for the storage of seed potatoes only. Table and processing potatoes should be stored at 10-12°C, after treating the potatoes with a sprout suppressant for long-term storage. For short-term storage of table and processing potatoes, non-refrigerated storage methods like heaps can be used profitably. Handling of potatoes with due care is expected to reduce the post-harvest losses.

***Storage of Seed**:* Seed potatoes are best stored at 2-4°C and 90-95% RH in cold stores, because at this temperature, sprouting does not occur and weight loss is minimum. The farmers have to pay the rent for storing potatoes in these cold stores and they take out these potatoes at the time of planting in the next potato season. The only problem regarding the storage of seed potatoes in cold stores is the uneven distribution of these cold stores over locations and non-regulatory storage charges.

A traditional cold storage and stack of potatoes inside the store

Storage of Table and Processing Potatoes: The potatoes to be used for table and processing purposes have different storage requirement than the seed potatoes. Essentially storage for table and processing potatoes may be divided into two categories viz. Non-refrigerated storage systems and refrigerated storage systems as discussed below.

Non-refrigerated storage systems for table and processing potatoes: These are the storage systems where no artificial refrigeration is used. These storage methods can be used to store potatoes for 3-4 months (i.e. up to June) after harvest in February/March. However, once the monsoon begins, potatoes cannot be stored in a non-refrigerated store because it works only under dry conditions. Short-term storage of potatoes for 3-4 months is good enough because it helps avoid distress sale immediately after harvest and the farmer can get better returns by selling the potatoes in May or June, when potato prices start rising. It is relatively easy to store potatoes under non-refrigerated conditions when they are dormant. Indian potato varieties have a dormancy period of 6-8 weeks. Once the dormancy period is over, sprouting begins. Weight loss in potatoes during storage is mainly due to water loss from the tubers and the weight loss is much more in sprouted tubers. The extent of rotting is high under non-refrigerated storage systems as the temperature and humidity conditions are favourable for aggravating the infection.

A modified potato heap

Refrigerated storage for table and processing potatoes: Fresh potatoes are available for consumption only for a few months, after the harvest. For a major part of the year, potatoes from cold stores are consumed. Though cold storage facility was developed primarily for the storage of seed potatoes, they have been used for the storage of table potatoes as well. But, 2-4°C is not

the ideal storage temperature for table and processing potatoes as at this temperature, potatoes become sweet due to sugar accumulation and are not preferred for consumption. Further, cold stored potatoes do not keep well once they are taken out of the store. Therefore, storage of table and processing potatoes is suggested at 10-12°C. This method of potato storage is comparatively new to our country, but a good number of cold stores (approximately 700) are now storing potatoes by this technology and these potatoes are being marketed as low sugar potatoes (table) and depending on the variety stored, potatoes are suitable for processing as well up to about 6-7 months. However, at this storage temperature, potatoes sprout. Therefore, it is necessary to treat the stored potatoes with a sprout suppressant like CIPC (Isopropyl N-(3-chlorophenyl) carbamate). The treated potatoes are safe for consumption after 3-4 weeks of treatment.

A commercial cold store using 10-12°C storage technology, potatoes and chips

Electron Microscopic in Potato Virus Diagnostics

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Electron microscope is one of the most powerful scientific tools for carrying out detail structural studies of biological materials. In general, the purpose of any microscope is to form magnified image of a specimen so as to observe its maximum structural details (resolution). Resolution is a measure of capability of an image - forming system to produce separate images of adjacent objects. Historically, electrons have been discovered by J.J. Thomson in 1897, and revealed to possess wave like motion by Lou's de Broglie, in 1924. The electromagnetic lens that converged a beam of moving electrons was designed by H. Busch in 1926, leading to the first design of an EM by M. Knoll and E. Ruska in 1932. Electron Microscope were developed due to limitations of Light Microscopes, which are limited by the physics of light to 500x or 1000x magnification and a resolution of 0.2 micrometers. In the early 1930s this theoretical limit had been reached and there was a scientific desire to see the fine details of the interior structures of organic cells. This required 10,000X plus magnification, which was just not possible using light microscope. An EM, in which the image is formed by electron transmitted through a specimen, is known as Transmission Electron microscope (TEM). An EM uses a beam of fast moving electron for the formation of a magnified image of the specimen.

Principle

In TEM, electron beam is used as a source of illumination where extremely small negatively charged electrons given off by a heated tungsten filament can be accelerated by high voltage to produce a coherent electron beam and can be focused by an electric field. The electric field of a doughnut-shaped electromagnet surrounding the electron beam acts just like the glass lens, which focuses the light beam on the specimen. A similar electromagnet is used as the objective lens to form a highly magnified image of the specimen; and one or two electromagnetic lenses further magnify and project the image onto a fluorescent viewing screen or light sensitive sensor such as a CCD (charge-coupled device) camera. The image detected by the CCD may be displayed in real time on a computer monitor. Both particles and ultrathin sections are held in the electron beam on carbon-coated grids. These grids are thin enough to be transparent to the electrons. The grid is held in a movable holder for observation in the TEM.

Viruses infect plants cause significant economic losses in potato production. These viruses have to be reliably detected since the concentration of the viruses would be very low in infected plant tissues. Therefore, there is a need to develop an accurate, sensitive and reliable detection technique for detection of viruses in suspected samples. In general viral diseases can be detected or diagnosed by mosaic patterns on leaves, stunting of the plant, leaf malformations, and tuber malformations. To be specific, different techniques such as electron microscopy (EM), Immuno sorbent electron microscopy (ISEM), serology (ELISA), nucleic acid based hybridization and polymerase chain reaction (PCR) methods are used to detect the viruses. Almost all the above diagnostic techniques like biological, serological and molecular gives an indirect evidence of the causal agent (etiology) whereas, Transmission Electron Microscope (TEM) gives a direct access to see the causal agent. TEM is one of the most powerful scientific tools for carrying out detail structural studies of biological materials.

Methods of detection

In TEM studies, negative staining made it easy for detection of viruses from liquid samples. This led to the widespread application of TEM in the field of basics of virology and rapid diagnosis of viruses (Brenner and Horne, 1959). Negative staining also provides morphological information about symmetry and capsomer arrangement of the viruses (Philippe, 2008) and hence provides a rapid and accurate identification of viral diseases (Wild 2008; Bernd

and Gunther 2009). Due to the advancement of the science, electron-opaque gold nano particles labelled immunoglobulin (IgG) complexes were used successfully for electron microscopic detection of plant viruses at the ultra-structural level.

Immune-gold electron microscopic technique is also known as GLAD i.e., gold-labelled antibody decoration which has been coined by Pares and Whitecross (1982). Lin and Langenberg (1982) used colloidal gold-labelled IgG for localization of Barley stripe mosaic virus (BSMV) in ultrathin sections of wheat cells. Later on Lin (1984) described the use of gold-labelled IgG complexes for rapid and highly specific detection of Barley stripe mosaic virus (BSMV) in wheat, Tobacco mosaic virus (TMV) in tobacco, Wheat streak mosaic virus (WSMV) in wheat, Cowpea mosaic virus (CPMV) in cowpea, Brome mosaic virus (BMV) in barley, Potato leafroll virus (PLRV) in potato and Barley yellow dwarf virus (BYDV) in oats by simple leaf dip method. Similarly Raigond et al. (2013 and 2015) developed immno gold electron microscopic technique for clear delection of potato viruses in suspected potato leaf samples (Fig).

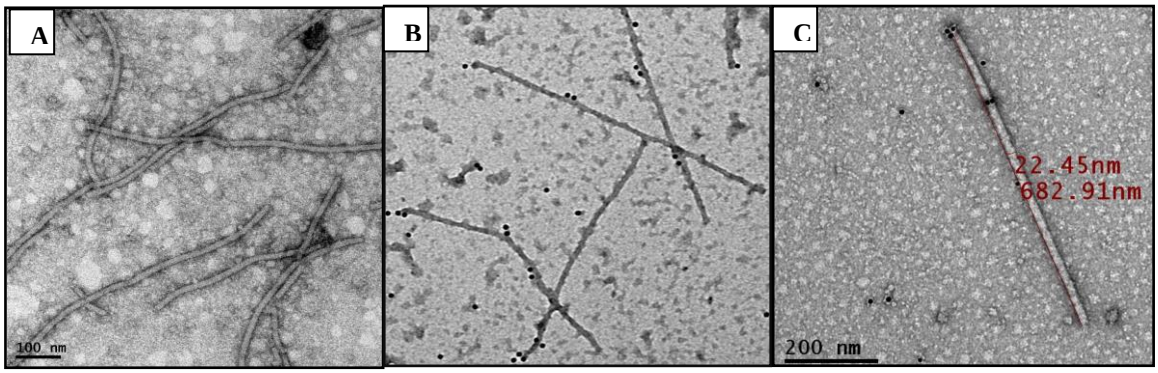

Fig : Electron microscopic detection of *Potato virus A* by direct leaf dip method (A) and immune-gold electron microscopic based detection of *Potato virus M* (B & C).

Here visualization of the antigen-antibody reaction is achieved by using colloidal gold (CG) labelled antibodies. Therefore, this technique is more sensitive in comparison with classical direct electron microscopy and immune electron microscopy. At the same time it has the advantage of being highly specific and can detect the viruses under low concentration in infected tissues. Negative staining also provides morphological information about symmetry and capsomer arrangement of the viruses and hence provides a rapid and accurate identification of viral diseases (Berned and Gunther 2009.)

Conclusion

TEM is a useful technology which enables rapid and clear identification of the plant viruses. Leaf-dip and the immune-gold electron microscopic technique were found to be one of the best techniques, as it is coupled with serological reactions which can be visualized under TEM. Therefore, this technique can be used for rapid and specific detection of viruses in plants.

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Production of disease free planting material through hi-tech seed production

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Potato is one of the world's most important non-cereal high yielding horticultural food crop, which is native of Peru-Bolivia in the Andes (South America) and seems to have been introduced in India from Europe by Portugeese in the early 17th century. India has taken a giant leap in terms of potato area (1.96 million ha) and production (45.3 million tones) as compared to 1949-50, the year of establishment of CPRI, when the total production was 1.54 million tones from an area of 0.234 million ha. Currently the national average productivity of potato is 22.76 tones/ha. As of now, country is well placed to meet the emerging challenges for diversifying the potato production and stabilizing its market. At present the country has an area of approximately 1.96 million ha under potato and requires about 4.9 million tones of quality seed at the uniform seed rate of 2.5 t/ha. However, Central Potato Research Institute is producing only about 3500 tones of breeder seed per annum. In potato cultivation, potato seed is most expensive input accounting for 40 to 50 percent of the production cost. Moreover, a high rate of degeneration causes the seed to deteriorate after a few multiplications. Potato seed tuber is vegetatively propagated and most essential input in successful potato cultivation and accounts for about 50% of the total cost of cultivation. Degeneration of potato seed stocks due to virus diseases is most limiting factor in potato seed production. Therefore, seed stocks should be free from viral and other seed-borne diseases. To maintain the higher productivity, it is essential to have a scientifically sound seed production system through which high degree of health standard of the seed crop is maintained

A well organized scientific strategy of breeder seed production was envisaged in 1962-63 through clonal selection, tuber indexing and stage-wise field multiplication of healthy indexed tubers in subsequent four generations. Indexing of tubers against contagious and insect transmitted viruses is done by ELISA against PVX, PVS, PVM, PVA, PVY and PLRV. Crop inspection, roguing of diseased plants and immunodiagnosis are the regular features of the programme to improve the seed quality. The breeder seed produced by CPRI is supplied to various state Govt. organizations for further multiplication in three more cycles' viz., Foundation-1, Foundation-2 and Certified seed under strict health standards. However, the current status of breeder seed multiplication by the state government is not as per the desired seed multiplication chain. There is huge shortage of certified seed in the country. The conventional system has limitations like i) low rate of multiplication ii) requires more number of disease free propagules in the initial stage iii) development of 100% healthy seed stock from infected material is slow and time taking iv) progressive accumulation of degenerative viral diseases is there in each field exposure and v) several field multiplications of initial disease-free material (7 years). The only way-out to overcome the above said limitations is augmentation of seed production through Hi-tech system to improve the quality and to reduce the field exposure. Potato has readily responded to the totipotent nature of plant tissues in micropropagation and it has become easy to export/import disease free planting material without any risk of importation of deadly diseases and the exchange of plant material.

Needs of Hi-tech seed potato production

- Tropical countries or for those countries which do not have isolated and virus free potato growing areas.
- Those countries which want early certification for seed production.
- Countries having explosive increase in new potato growing areas.

- ❖ Early supply of pre nucleus/nucleus seed to commercial growers by reducing the field exposure time.
- ❖ Improved tuber quality.
- ❖ Reducing the load of degenerative diseases.
- ❖ Utilize the resources and trained manpower year the round.
- ❖ Taking critical decision like choice of propagules, tuber inducing agent, environment, medium components and economics of scale.
- ❖ Vertical growth and reduce pressure on land.

Seed production through hi-tech system has been started by Central Potato Research Institute Shimla in the recent past. Under this system, there are three different sub-systems:

i). Microplant based seed production system
ii). Microtuber based seed production system
iii). Aeroponic based seed production system.

Under hi-tech seed production system, nucleus planting material will be produced in the laboratory under controlled condition. The virus free plants will be used as mother plant for micropropagation. The microplants/microtubers will be planted in net-house conditions for production of mini-tubers (G-0). The minitubers produced in generation-0 will be multiplied in generation-I at a spacing of 60 x 15 cm. The produce of generation-I is further multiplied in generation-II. The produce of stage IV and generation-II will be called as breeder seed and supplied to public and private organization for further multiplication in three clonal cycles viz. Foundation-1, Foundation-2, Certified Seed. The adoption of hi-tech seed production technologies developed by the Institute has led to opening of more than 20 tissue culture labs throughout the country. Several private seed companies such as M/s Reliance Life Sciences, Navi Mumbai; Cadila Pharmaceuiticals Ltd., Ahmedabad; KF Bioplants, Pvt. Ltd., Pune/Banglore; Transgene Bioplants Pvt. Ltd., Chandigarh; Phulwari Bio-Tech Ltd., Chandigarh; Gufic Bioscinece Ltd., Mumbai; Chamal Agritech Ltd., Chandigarh; Elegant Flower Company Pvt. Ltd., Kolkata; Dayal Biotech Pvt. Ltd., Meerut; Merino Industries Ltd., Hapur; Kalindi Agro Biotech Ltd., Gurgaon; Rose-N-Shine Biotech (P) Ltd., Pune; Hindustan Bioenergy Ltd., Lucknow; Vasantdada Sugar Institute, Pune; Technico Agri Sciences Limited, Chandigarh; Sangha Seeds, Jalandhar; Hindustan Bioenergy Ltd., Lucknow; Pallishree Limited, Kolkata; Pallishree Limited, Kolkata; Devleela Biotech, Raipur; Neva Plantations Pvt. Ltd., Kangra; Krishiraj Tissue Culture Nursery, Jalna; Palm Grove Nurseries, Bangalore; Mahindra Subhlabh Services Ltd., Mohali; Dawar Agritech, Kurukshetra; Bhatti Tissue Tech, Jalandhar are taking virus free in-vitro plantlets from CPRI for further multiplication in their seed production programme.

Micropropagation of disease free mother plant: Soon after varietal release, 10-20 healthy uniform tubers are selected and planted under controlled conditions in the pots in poly/net house for indexing against the viruses. The ideal temperature for plant growth as well as virus multiplication should be 20-25^{0}C. The plants are tested by ELISA for virus freedom after 6 to 7 weeks of planting or 6 to 8 leaf stage. The infected plants with viruses during ELISA testing should be destroyed and only the healthy plants should be retained for further testing by polymerase chain reaction (PCR) for virus freedom. The infected plants obtained during PCR testing are removed. Finally healthy plants obtained during series of testing will be used as mother plant for micropropagation.

Development of healthy mother plants from virus infected plant: Sometimes we may not be getting even a single plant completely free from viruses after releasing of the variety. In such situation, meristem tip culture coupled with thermotherapy has become a powerful and

successful tool for virus elimination from infected plants and has been successfully applied in potato for development of virus-free plants.

The plants are tested against potato viruses and viroids like PVX, PVS, PVA, PVY, PVM, PLRV, PALCV and PSTVd through ELISA, EM and PCR. In case, no plant is found free from the virus infection then the plants that are infected with minimum number of viruses are selected for meristem tip culture. Using nodal/sprout cuttings, the *in vitro* stocks of selected plants are developed and further sub-cultured in Ribavarin (20 ppm) modified MS media for chemotherapy. This culture is then given thermotherapy at 37^0C and 16 h photo period (120-200 µmol/m^2/s^{-1}) in the culture room for nearly 20 days. Using stereomicroscope, the apical/axillary meristem (0.2 to 0.3 mm) is excised from *in vitro* plants aseptically with the help of sterile scalpel, needle and blade. The excised meristem is grown in the test tubes containing MS medium with growth regulators and incubated in the culture tubes at 25ºC and 16 h photo period (120-200 µmol/m^2/s^{-1}) in the culture room until the meristem germinates. The meri-clones are then sub-cultured through nodal cutting after it attains a height of 4-5 cm and the pedigree is maintained. The fully grown mericlones should be tested against potato viruses like PVX, PVS, PVA, PVY, PVM, PLRV, PALCV and PSTVd through ELISA, EM and PCR. The virus-free cultures should be sub-cultured once in every 3-4 weeks so as to get more number of virus-free microplants. The microplants should be hardened for 2 to 3 weeks in the poly/net house before planting in the pots filled with peat moss under mist in poly house. The plants are further tested against all above said potato viruses through ELISA, EM and PCR after 40-45 days of planting. Remove the infected plants obtained during testing and retain only the healthy plants. Finally healthy plants obtained during series of testing will be used as mother plant for micropropagation.

Microplant based seed production system: Three to four weeks old helthy microplants are transferred to protrays filled with sterilized peat moss. The microplants can be planted in portray with root or without root (cuttings). For planting with root, the media sticking to the root should be properly washed off. After transplanting, drenching is done with the mancozeb (0.25%) solution. The portrays are then transferred to the growth chambers and kept in dark for 48 h subsequently in 16 h photoperiod for 2-3 days. Once the plantlets are established in portrays (4-5 days), these portrays are transferred to hardening chamber and kept at 27ºC for 10-15 days. The hardened plantlets should be removed from portrays along with peat moss and transplanted on nursery beds in mixture of soil, sand and FYM (2:1:1) in rows at 30 x 10 cm spacing under insect proof net house condition. 5% of the plants are tested by ELISA. Rogue out all virus infected plants, off-type plants, abnormal and stunted observed during inspection**.** Allow the microplant crop to mature and harvest the minitubers. Each microplant shall yield 6-8 minitubers. Seed crop should be harvested 15 to 20 days after haulms cutting when the tuber skin is hardened. The seed tubers thus produced are minitubers. Curing is done by keeping the seed tubers in heap for about 15 to 20 days in a cool shady place. After curing, the seed tuber should be graded into >3 g and treat with 3 per cent boric acid solution for 10-15 minutes to prevent surface borne pathogen inoculum. Minitubers harvested from microplants (Generation-0) are called as nucleus seed. Store the minitubers in country store in hills while cold store at 3-4°C in the plains. Minitubers weighing >3 g will be planted in Generation-l in the field during next season. Whereas, <3 g minitubers may be recycled once again in Generation-0 under controlled poly/net house conditions if the crop meets the G-0 criteria, the produce can be used for raising G-1 crop in the field.

Microtuber based seed production system: The microplants are tested for virus freedom before initiating microtuber production. The virus-free stock plants are mass multiplied through nodal cuttings on semisolid MS medium in culture tubes (25 x 150 mm) following the standard procedure upto 10 cycles. 3-4 weeks old explants are transferred into 250 ml conical flasks or

culture bottles containing 25-35 ml liquid MS medium without agar. The culture tubes are incubated at 25^0C and 16 h photo period (120-200 µmol/m^2/s^{-1}) in the culture room. After 3-4 weeks of incubation, the unutilized liquid propagation medium is decant from the conical flask/culture bottle under aseptic conditions and 30 ml of microtuber induction medium is poured into it. The microtuber induction medium is based on MS basal media supplemented with 10 mgl^{-1} N^6-benzyladenine (BAP) and 80 gl^{-1} sucrose/commercial sugar. After adding induction medium, the cultures are incubated under complete dark condition at 15°C for 60 to 90 days depending on the genotype. Microtubers develop epigeally at the apical as well as axillary buds of the shoots. In general, 15 to 20 microtubers weighing 50-300 mg are produced in each flask/culture bottles. Before harvesting, greening of the microtubers is done in the culture room by incubating microtuber induced cultures under 16 h photoperiod (approximately 30 µmol m^{-2} s^{-1}light intensity) at 22-24°C for 10 to 15 days. Then carefully remove the cultures along with microtubers from conical flasks or culture bottles and manually harvest the green microtubers. Avoid damaging the microtubers, especially the thin periderm during harvest. The harvested microtubers are then washed and treated with 0.25% mancozeb for 10 minutes, and allowed to dry in the dark at 20^0C for 2 days. Grading of microtubers in <4m, 4-6mm and >6 mm should be done while packing. Pack the treated microtubers in perforated polythene covers and store in a refrigerator for 4-5 months until planting. Take out the microtubers from the refrigerator after about one month before planting for breaking the dormancy.

Aeroponic seed production system: The conventional system is quite effective but it has low multiplication rate and higher field exposure increases the risk of viral infection. Production of potato through aeroponics promotes availability of healthy seed potatoes. In addition, aeroponics allows easy identification and roguing of diseased plants. Furthermore, potato seed produced through this method could enjoy accelerated growth due to improved aeration of the roots and optimal nutrient uptake obtained from an atomized nutrient solution. Keeping this in view, tissue culture based system of quality seed production was integrated with breeder seed production programme. The conventional way of producing potato minitubers through micro propagation is to multiply *in vitro* material in insect proof net houses. The conventional method uses substrate made of soil and mixture of various components. This method usually produces 10-12 minitubers per plant depending on cultivar. The aeroponic system offers the potential to increase production in terms of number of minitubers per plant from three to four times. Aeroponics is the process of growing plants in an air mist environment without the use of soil or an aggregate medium. Since water is used in aeroponics to transmit nutrients to the plants. In aeroponics, plants growth is facilitated by suspending them in air, in an enclosed environment, and providing necessary nutrients by spraying on roots. The nutrient solution is continuously re-circulated through the system and monitored for pH and EC and amended whenever necessary. There is a tremendous scope to increase healthy seed production vertically by adopting aeroponic technology of seed system where increase in multiplication rate from 5:1 to 50:1 can be achieved. We do not need any excess area for aeroponic based healthy seed production. Only one percent of conventional water usage is required which is basically recycled water. The top portion of the plant is exposed to the open air and a light source. The aeroponic seed production system has very high productivity. It prevents exposure to unfavourable soil conditions and the minitubers harvested from this system will be free from all soil-borne pathogens. Desired size of minitubers can be harvested sequentially and this could reduce the cost of minituber production.

Various systems adopted for the production of pre-basic seeds of potato, the hi-tech system appears to be the best in many respects. Considering the potential benefits of the system such as rapid production of seed, spacious, healthy and clean material, good nutrient monitoring system, improvement of growth and survival rate of plantlets, constant air circulation and ecologically friendly, this system has a potential of revolutionizing potato seed production industry.

Fig: Hi-tech seed production system

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Potato Nematode Management

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Nematodes are microscopic roundworms that live in many habitats. At least 2500 species of plant-parasitic nematodes have been described, characterized by the presence of a stylet, which is used for penetration of host plant tissue. They mostly attack on roots and underground parts of plants, but some are able to feed on leaves and flowers. Generally, nematode infestations in plants are not easily recognized since the external foliage symptoms are often mistaken to nutrient deficiency symptoms. These nematodes are also enhance the severity of other diseases caused by fungi, bacteria, several other nematodes act as carriers of viral diseases and form disease complexes (Swarup and Seshadri, 1987). The earliest record of parasitic nematode infestation in potato crop was of the cyst nematode recorded in the year 1881 by Julius Kuhn from Germany. This cyst nematode was established as one of the major plant protection globally accounting for an average tuber yield loss of about nine per cent, which is about 43 million tons (Krishna prasad, 2006). In India, the root knot nematode infection in potato tubers was first noticed by Dr. Thirumalachar in 1951. Later, the report of cyst nematode occurrence in potato crop from the Nilgiri hills in South India by Dr. Jones in 1961 activated the nematological research not only in potato but also in other crops. About 90 species of nematodes belonging to 38 genera have been reported to be associated with potatoes in India alone. But among these, the potato cyst nematodes (PCN) and root-knot nematodes have been reported as the major pests of potato.

1. Potato Cyst Nematodes

Potato cyst nematodes *viz.*, *Globodera rostochiensis* (Woll) and *G. pallida* (Stone), also popularly called the Golden nematodes, are one of the dangerous pests hindering the sustainable production of potato in any countries including India (Seshadri and Sivkumar, 1962). Their small size, intimate association with their host and adaptation for long term survival in the soil in the absence of a suitable host present them challenging to the farmers, scientists and policy makers. Potato cyst nematodes are one of the important biotic constraints in the sustainable production of potato in many countries worldwide including India. They are subjected to stringent quarantine and/or regulatory procedures, wherever they occur.

Origin and distribution

Potato cyst nematodes originated in the Andean mountains of South America along with its principal host, potato. They were then introduced into Europe in the 1850's and later spread throughout the world through the introduction of improved varieties. Presently, Potato cyst nematodes have been reported from 65 countries with *Globodera rostochiensis* in all countries and *G. pallida* in 47 countries. In India, Dr. F. G. W. Jones first detected the nematode in 1961 from a field in Vijayanagaram farm in Ootacamund, The Nilgiris district, Tamil Nadu at an elevation of 2125 m above MSL. Later on, their occurrence was also reported from Kodaikanal hills. Realizing the potential danger by this nematode to potato cultivation, 'Golden Nematode Scheme' jointly financed by ICAR and the Government of Tamil Nadu was launched in Ootacamund in October, 1963 and the Destructive Insect Pest Act, 1919 was suitably amended by the Tamil Nadu Government in 1971 to impose domestic quarantine and ensure strict checking of seed potato for marketing from infested field of Ootacamund. Hence in India, Potato cyst nematodes are mainly restricted to the Nilgiris and Kodaikanal hills of Tamil Nadu. However the presence of PCN in Karnataka and Kerala, though in very low intensities, necessitates the strengthening of domestic quarantine to prevent its further spread to new areas.

Species and pathotypes: There are two species of PCN *viz., Globodera rostochiensis* (Wollenweber, 1923) and *G. pallida* (Stone, 1973). The differential host reactions of PCN populations from Nilgiris and Kodaikanal hills revealed that the pathotypes Ro1 of *G. rostochiensis* and Pa2 of *G. pallida* are the most prevalent forms. The other prevalent pathotypes are Ro2 and Ro5 of the former and Pa1 and Pa3 of the latter.

Host range: Potato cyst nematodes are confined to the family Solanaceae with potato, tomato and brinjal as the most preferred hosts.

Means of dispersal: The major routes of dissemination of PCN is through the contaminated seed potatoes, movement of farm workers, livestocks and farm implements from infested land, irrigation water, high winds, soil in gunny bags used for transportation, etc. Ensuring strict hygiene practices will help minimize PCN spread.

Yield loss: Worldwide PCN alone causes estimated losses up to 30% in potato crop yield. The tuber yield loss estimates vary from 5 to 80 per cent depending on the initial inoculum level. The economic threshold level for crop loss due to PCN is usually around 20 eggs per g of soil, which may vary with the environmental interactions and host tolerance.

Symptoms: At low PCN population densities, most plants can tolerate nematode invasion and respond by developing more lateral roots as wound response, without affecting their growth and yield. However, as the degree of invasion increases, the plant is unable to compensate and ultimately exhibits a range of symptoms. The typical above ground symptoms of PCN can be divided into two phases. In the first half of the growing season, PCN cause reduced photosynthetic rate, production of fewer stems and smaller, thicker leaves and overall stunting of the haulms. In the second half of growing season, leaves senesce earlier, water and nutrient uptake are reduced causing incipient wilting during hotter days and the number of the tubers and dry matter production are reduced. The above ground symptom first appears in the field as a small patch of stunted plants with pale yellow coloured leaves (Plate 1). These patches increase in size gradually year after year and cover the entire field if potato is continuously grown without any protective measures. Close examination of the roots of infected plants reveal the presence of small pinhead sized, white or yellow female nematodes sticking to the roots (Plate 2).

Biology: The hatching of cysts is stimulated by the chemical substances called hatching factors present in the potato root diffusates (PRD). There are at least 25 hatching factors (Byrne *et al*., 1996) responsible for hatching of both the species of potato cyst nematode. Maximum activity of PRD is reached three weeks after plant emergence and most actively from the root tips. Devine *et al.* (1966) identified two hatching factors for *G. rostochiensis* as the potato glycoalkaloids, solanine and α-chaconine. Blaauw *et al.* (2001) identified the compound Solanoeclepin A, which occurs naturally in potatoes, as one of the agents involved in hatching of cysts and determined its structure. Hatching depends on host root diffusate, physical conditions before and during juvenile emergence and hereditary preconditions of the cyst. The second stage juvenile (J2) coming out of the cysts moves actively in soil and invade the roots by rupturing with its stylet. It enters through the epidermal cell walls and eventually settles with its head towards the stele and feeds on cells in pericycle, cortex or endodermis by forming a feeding tube. This induces enlargement of root cells and breakdown of their walls to form a large 'syncytium' or 'transfer cell' with dense granular cytoplasm that provides nourishment for nematode development. The nematode molts and remains in the syncytium until its development is complete. The sex of the nematode is determined during J3 stage. The females become sedentary, swollen and remain attached to the roots and posterior part of the body comes out by rupturing the root cells. Males retain their thread shape and come out of the roots to locate and mate the females. The immature

females of *G. rostochiensis* are golden yellow in colour while *G. pallida* are white or cream in colour. After the female dies, the body wall thickens to form a hard brown cyst that is resistant to adverse weather conditions. Each cyst contains 200-500 eggs and are easily dislodged in soil at harvest. The cysts can survive in soil for 20-30 years even in the absence of a suitable host crop. The life cycle is completed in 35-40 days and generally, one generation is completed in one year. However, there are evidences of second generation of *G. rostochiensis* being completed because of its shorter dormancy (45 to 60 days) and long crop duration (120 days) and *G. pallida* has a dormancy of 60 to 75 days. Once J2 have developed inside cysts, they enter an extreme form of dormancy, known as 'diapause' and they cannot be stimulated to hatch until the diapause has ended. Only 60 to 80 per cent of juveniles can be stimulated to hatch in the presence of root diffusates and it never reaches 100 per cent. This is a part of the survival strategy of PCN and some juveniles will remain dormant for several years before hatching.

Management: The potato cyst nematodes arc the most successful plant parasi tic nematodes exhibiting a highly specialized survival mechanism. They have a limited host range in family Solanaceac. It is an accepted fact that once they have established themselves in a given locality, they can not be completely eradicated and thus they have to be managed by adopting several plant protection strategies. Once established, PCN are very difficult to be eradicated from infested fields. Despite massive chemical control measures taken up to eradicate PCN soon after its appearance in the Nilgiris, the nematode remains a serious endemic pest of potato in this region due to intensive cultivation of potato and favourable climatic conditions. PCN have to be managed by a combination of preventive and management methods as follows.

(a) Preventive methods:

Preventive measures include

• Ensuring strict international quarantine regulations to prevent introduction of new pathotypes into areas which are now free of these pathotypes.

• Implementing strict domestic quarantine procedures to prevent further spread of this pest to new areas that are free from this pest.

• Adopting good sanitation at field level to prevent the spread of nematodes from infested areas through the farm implements, irrigation water, gunny bags, etc.

(b) Integrated management

As no single method of control is fully effective in giving desirable level of nematode suppression, an integrated nematode management package incorporating judicious blend of various management options such as chemical, biological and cultural methods is being advocated in the Nilgiris to bring down the PCN population to levels that permit profitable cultivation of potato.

Chemical control: Chemical control of PCN using nematicides is an effective and reliable method of bringing down the nematode population quickly. Currently, application of Carbofuran 3G at 2 kg a.i./ha at the time of planting is being recommended as a part of package of practices for potato in the Nilgiris to bring down the PCN population. However, it is not that much effective to control the PCN population. Therefore, new soil fumigant, Basamid 90 G was evaluated in different doses in field conditions. Before applying Basamid 90 G, made the soil surface compact, irrigated with ten to twenty liters of water per sq. metre. Afterwards applied soil fumigant, Basamid 90 G. Covered the treated area with a tarp for 10-12 days and sealed the edges with soil. This method allows the effective gases to act more efficiently on cyst of PCN. After this removed the tarp, soil samples collected and counted cyst in all the treatments. In Basamid 90 G@ 50 g/m^2 recorded less nematode multiplication (Rf = 0.87) followed by Basamid 90 G @ 40 g/m^2 (Rf = 1.05) as compared to Carbofuran 3G @ 65 kg/ha (Rf = 1.17) and untreated control (Rf = 1.34).

Resistant varieties: Growing resistant varieties is an effective, economical and eco-friendly method for the management of PCN as it requires no additional expenditure, labour and technical skills. Concerted breeding efforts at ICAR- Central Potato Research Station, Muthorai released 'Kufri Swarna and Kufri Neelema' high yielding varieties with combined resistance to local pathotypes of PCN and late blight disease in the Nilgiris. one advance hybrids OS/01-497 with combined resistance to PCN and late blight has been recommended for its release in the 34th Group Meeting of Potato Workers of All India Co-ordinated Research Project (Potato) at ICAR-CPRI, Shimla, 2016.

Crop rotation: Since the host range of PCN is restricted to few plants in Solanaceae, crop rotation with non- solanaceous crops is widely recommended for management of PCN. A three to four year rotation with crops like radish, cabbage, cauliflower, turnip, garlic, carrot, green manure crop like lupin etc. brings down the cyst population by more than 50 per cent. Growing potato in summer and rotational crop in autumn and spring was found to be successful in managing PCN in the Nilgiris.

Intercropping: Intercropping with French Beans (75:50) was profitable as it recorded higher potato equivalent yield and was effective in bringing down the cyst population.

Integrated management practices: For eradication of PCN integration of trap crop (45 days), soil solarization (4 weeks), furadon (3G @ 2kg a.i./ha) application followed by organic amendment and bio- control agent recorded the maximum yield and minimum reproduction factor in the resistant variety Kufri Neelima. Soil solarisation (4 weeks) combined with neem cake (5t/ha) and *Trichoderma viride* (5kg/ha) recorded less nematode population.

Biological control and organic amendments: Recent studies revealed that application of biological control agents *viz., Pseudomonas fluorescens* and *Paecilomyces lilacinus* and organic amendments like neem cake blended with *Trichoderma viride* show promise in suppressing PCN population. Efforts are being made to incorporate these eco-friendly components into the integrated PCN management package.

2. Root Knot Nematodes

The root knot nematodes (*Meloidogyne* spp.) are amongst the most economically important nematode pests of potato. They are prevalent all over the world and can cause significant crop loss in both warm and cool climatic conditions, depending on their species. They have a wide host range and many important cereals, pulses, vegetables, fruits and ornamental crops are good hosts including potato. In India, the root knot nematode infection in potato tubers was first noticed by Dr. Thirumalachar in 1951. Later on, it was reported from almost all potato growing regions of our country.

Species: Although many species of Meloidogyne are known to infect potato, only four species are reported from India *viz., M. incognita* (Kofoid and White, 1919); *M. javanica* (Treub, 1889); *M. hapla* (Chitwood, 1949) and *M. arenaria* (Neal, 1889).

Distribution: Root knot nematodes are recorded from all potato growing countries of the world. In India, the dominant root knot species affecting potato both in hills and plains has been *M. incognita* while *M. javanica* infestation is in mid hills and plains of northern India. *M. hapla* is confined to hilly tracts of Uttar Pradesh, Himachal Pradesh, Jammu and Kashmir and Tamil Nadu. *M. arenaria* is reported form the plains of Uttar Pradesh.

Host range: Root knot nematodes have a very wide host range and have been found to infect more than 2000 plants worldwide that includes major field and horticultural crops. This makes

them very difficult to control because they can always survive and reproduce on other host crops including weeds.

Yield loss: Upon severe infection, the root knot nematodes drastically affected the potato yield which recorded even lesser than the quantity planted in Himachal Pradesh and Karnataka, during 1960s. An initial inoculum of 200 juveniles per 100 ml soil resulted in an overall yield reduction of 40 per cent with 100 per cent tuber infestation. Besides the direct yield loss, root knot nematodes also cause indirect damage in potato in the form of blemishes and deformations in tubers which make it unmarketable. Thus the nematodes cause both quantitative and qualitative loss in potato.

Interaction with other micro-organisms: Root knot nematode infection is found to predispose potato plants for early blight fungus *Alternaria solani* and brown rot bacterium *Pseudomonas solanacearum*. It is suspected that the nematode favours the spread of brown rot disease in Himachal Pradesh, Uttar Pradesh and Karnataka.

Life cycle: The vermiform second stage juveniles hatch out from the egg masses, penetrate the young potato roots and starts feeding. This leads to the formation of specialized, enlarged cells called 'giant cells' which provide nourishment to the nematodes throughout its development. Hence knots or galls appear in the roots because of the specific host parasite interaction. The second stage juveniles (J2) undergo molting and pass through J3 and J4 stages and finally become adult females or males. The adult females are sedentary and pear shaped while males are migratory and vermiform or thread shaped. Males move out of the root to locate and mate the females. The females lay about 300 to 400 eggs in gelatinous matrix, usually adhering to the root galls. These eggs readily hatch and infect fresh roots. At the time of tuber formation, the juveniles usually enter the tubers since the root system would have started decaying. Life cycle is completed in 25-30 days during summer and 65-100 days in winter. The eggs and juveniles survived for more than 100 days in summer months in Shimla hills. In the hilly regions, two generations are completed by the time of tuberization and hence tuber infestation is invariably observed even under low initial inoculum levels, whereas, in plains, tuber infestation could be low because of the shorter crop duration and preference of the fresh roots by the newly hatched juveniles.

Symptoms: The above ground symptoms due to root knot nematode infection include stunting and yellowing of plants with chlorotic leaves due to the hindrance in water and nutrient uptake in roots by nematode infection. In roots, the characteristic swellings called 'galls' are formed (Plate 3). The galls on the root are small and often unnoticed but the warty 'pimple-like' blemishes on the tubers due to nematode infection reduce the commercial value and keeping quality of potato (Plate 4). Brown spots are evident in the flesh of the cut tubers due to the presence of nematodes (Plate 5).

Management: Because of the wide host range and semi-endoparasitic adaptation of the root knot nematodes, complete eradication is not possible from an infected area, if once established. The problem may be managed by adopting the following strategies under our conditions.

Cultural practice: Avoiding seed tubers from infected areas and planting of nematode-free tubers. Deep ploughing and sun drying during summer months exposes the infective juvenile stages of the nematode to direct sun, thereby killing them. Adopting good sanitation and keeping the field free of weeds which otherwise would serve as alternate hosts for nematodes. Crop rotation with non-host crops like maize or wheat helps in minimizing the nematode damage. Early planting of spring crop in first week of January and late planting of autumn crop in second and third week of October reduce nematode infection in potato due to the lower temperature

prevailing during crop period. Growing trap crops such as marigold, *Tagetes patula* in alternate rows with potato reduce nematode
population in soil.

Chemical control: Application of carbofuran at 1-2 kg a.i./ha reduce nematode infestation and increase yield. The efficiency of these pesticides increase when applied in two split doses once at planting and then at the time of earthing up. This helps keep juveniles of RKN at less than the threshold level of 10 to 20 larvae /100 g of soil sample.

Breeding for resistance: The post-inoculation development and reproduction of root knot nematode was hindered in Kufri Dewa. The availability of resistant materials needs to be exploited further to breed nematode resistant variety as this is the only economic means to manage nematode under field conditions.

Integrated approach: Since a single method of control is uneconomical and not adequate for better nematode management, a judicious blend of the above methods is always advisable for achieving higher crop production.

Fig: PCN on potato root-ball; PCN infected potato field; Root knot nematode infected potato roots, Root knot nematode infected potato tubers showing warty pimples, Brown spots indicating the presence of root knot nematodes inside the tuber

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Nutrient Management in Potato Production

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Potato produces higher dry matter production per unit area and time as compared to other field crops. Being a very intensive crop it requires higher rates of nutrients For profitable production besides other management factors nutrient management is most important. Sufficient supply of nutrients must be ensured for optimum potato growth and there are 14 soil-derived elements or nutrients considered to be essential for growth of plants. Potato plant has a shallow and sparse root system which is not able to explore enough volume of soil to meet the entire nutrient demand from the soil. So the crop often responds well to external source of nutrients. Moreover, most of potato growing area lies in the plains and plateau region and the soils of these regions invariably are low in organic carbon and available nitrogen, low to medium in P and medium to high in K. Secondary and micronutrients are also reported to be deficient from some intensively cropped areas. In absence of very site specific recommendations arbitrary use of nutrients has resulted in nutrient imbalances and under utilization of applied nutrients. Poor nutrient use efficiency not only leads to wasteful expenditure, it also causes environmental pollution. Therefore, nutrient application should be made on the basis of plant demand to ensure high fertilizer use efficiency so that the amount of unutilized fertilizers is reduced to environmentally acceptable levels. Plant demand is a function of growth rate, growth stage, climatic conditions, and cultivar. Since the amount of fertilizer applied to a potato crop should depend on the supplying power of the soil, the potential for nutrient losses, and the growth potential of the cultivar (Dean, 1994) the amount of nutrients required by a potato crop are related to and should be based on a realistic yield potential for the selected cultivar and soil.

Pre-season soil tests provide critical information to determine the amount of residual nutrients available. In-season soil analysis is an additional tool to monitor nutrient availability and complement petiole analysis. In-season soil tests can predict potentially limiting soil nutrients (primarily nitrogen) which can be adjusted prior to significant drop in petiole concentrations (Lang and Stevens, 1997). Dry matter and specific gravity as these parameters has direct influence on recovery and quality of processed product hence nutrient management for production of processing potatoes is little different than ware potato. It is designed to produces maximum processing grade tubers, having high dry matter and specific gravity (Table 1). Ware or seed crop may be harvested little early prematurely considering other factors, the processing potatoes must be harvested at its full maturity normally at more than 100 or 110 days. Therefore, the nutrient requirements of these cultivars are accordingly more.

Soil and tissue testing: Based on a soil test value, the amount of fertilizer recommended is the amount needed to produce optimum yield of the current crop. But these recommendations do not contain any specific provision for the amount of a nutrient removed with the crop or any additional amount of nutrient to build up the soil's ability to supply the nutrients for future crops. Thus, these recommendations will need to be modified and fine-tuned to fit each potato management unit to optimize yield, quality, and environmental protection at each location.

Tissue analysis has been used for many years as an additional nutrient management tool to: 1) diagnose a nutrient deficiency or toxicity, 2) to help predict the need for additional nutrients (primarily nitrogen), and 3) monitor the effectiveness of a fertilizer program. The basis behind tissue analysis is that maximum yield and quality

are associated with an optimum range of nutrient in the tissue sampled. If the level of nutrient falls outside this range then, then corrective measures should be taken. The most common tissue used for nutrient analysis in potato is the petiole (leaf stem and midrib) of the fourth leaf from the shoot tip. It is critical that this tissue stage is collected because younger or older tissue will have different nutrient concentrations and can lead to erroneous interpretations. For sampling, approximately 30 to 40 leaves should be collected and the leaflets stripped off and discarded. Petioles are then sent to a laboratory for analysis. Most diagnostic criteria for tissue analysis are based on a sample taken during the tuber bulking stage. Samples taken too early in the season or soon after a fertilizer application may not accurately reflect the true nutritional status of the plant if uptake of applied fertilizer by roots has not occurred. For irrigated potatoes, tissue analysis should begin about one week after final hilling and at least four days after a fertigation. The information on nutrient deficiency symptoms, suitable source, dose, time and method of fertilizer/manure application to efficiently manage nutrient is provided here. The recommendations provided here are will have to be modified and fine tuned to meet the needs of each farm.

Primary Nutrients:

Nitrogen is is the most yield limiting nutrient in majority of the potato growing soils. required in large amounts to maintain optimum shoot and tuber growth**.** Nitrogen has a great influence on crop growth, tuber yield and its quality. The potato plants having sufficient N have vigorous growth, increased leaf area index produce large tuber size as well as numbers. Variety, yield potential or goal, growing season, soil organic matter content, and previous crop are the important factors to consider while deciding on the rate of N to apply. If manure is used, then an estimate of N availability from the manure should be incorporated into the overall N applied. In general, early maturing varieties and those grown for early markets require less N than late maturing varieties. Too much N will delay tuber initiation and maturity leading to excessive vine growth at the expense of tuber growth thus resulting in low yield. Its deficiency leads pale yellow foliage in case of acute deficiency and these symptoms first appear at lower leaves and later on whole plant gives yellowish look leading to premature dropping of lower leaves thereby decreasing photosynthetically active leaf number. Low or high soil pH, sandy or light soils, low organic matter and drought conditions, high rainfall (leaching) or heavy irrigation, addition or high levels of non-decomposed organic matter/manure (eg straw) makes the N deficiency worse. Sulphur deficiency, root parasites (eg PCN), deep planting of tubers, water shortage or heavy metal toxicity can cause similar symptoms. A mature ware crop of potato yielding 25-30 t/ha tuber remove 120-140 kg N/ha and for processing cultivars it is supposed to be still higher. Therefore, application of nitrogen in form of fertilizers and manures becomes indispensable to meet N needs of the crop. Organic residues from previous crops in the rotation or from cover crops planted prior to potatoes influence the availability of nitrogen during the growing season. Possible microbial tie-up of nitrogen must be accounted for in early-season nitrogen management.

Nitrogen requirement depends on variety, purpose (ware or seed potato) and the type (ware or processing). Experiments with processing potatoes have shown that N requirement of these cultivars are about 150% of those of ware potatoes and for Kufri Chipsona-1 and Kufri Chipsona-2 the optimum dose to get high production of processing grade tuber was 270 q/ha in sandy loam soils of the west central plain. The economic potato response for ware crop in alluvial soils of Indo- Gangetic plains in the country has been noticed up to 180 kg N /ha. Some of the varieties like Kufri Anand and Kufri Sutlej may respond up to 240 kg N/ha. In recent years with newer cultivar the optimum dose of nitrogen was in the range of 190-240 kg/ha at Patna At

Ranchi response of nitrogen was observed up to 240 kg/ha, whereas, under river bed cultivation in Dessa (Gujarat), crop has respond up to 600 kg N/ha. Source, time and method of application are important for improving the N use efiiciency**. S**ufficient N is needed in early stages to build up crop canopy and to enhance leaf area thus giving longer period for tuber development during tuber bulking phase which is 40-60 days in plains.In general, application of N in two split dose *i.e.* half at planting time and rest at time of earthing up produces higher yields and results in higher N recovery than applying entire dose at planting. For convenience in use it is recommended that half of N be applied at planting through calcium ammonium nitrate and remaining at earthing up at about 25-30 days after planting through urea.

Among different sources, ammonium sulphate (21% N) is the most preferred one due to S contained in it, followed by calcium ammonium nitrate (25% N). However, urea (46% N) being cost effective, is most commonly used. High dose of urea has been found to have adverse effect on plant emergence by increasing the osmotic pressure particularly at higher doses. Therefore, care must be taken to avoid direct contact of urea with seed tubers at the time of planting. Plant takes nitrogen in NH_4^+ and NO_3^- form but majority of the nitrogen in soil is in complex organic combination so its availability depends on the rate of mineralization of organic matter which is often too low to meet the potato requirement. Further organic residues high in carbon also remove available nitrogen from the soil solution during the microbial decomposition which will be released after the death of the microbes.

It is important to place the fertilizer in the vicinity of roots for better interception. The uptake and efficacy of N fertilizer depend upon the method of application. Potato having sparse root system, The placement of N fertilizer in side bands (10 cm deep and 5 cm away from seed tubers) at planting has been found to be beneficial to broadcasting and furrow placement with respect to tuber yield and nutrient recovery.

Phosphorus: Phosphorus plays a critical role in root development and overall plant health, which is directly related to yield. It is the constituent of ATP , the storehouse of the energy. It Increases number of tubers, produces tubers of uniform size, boosts bulking and increases the yield of tubers and hastens maurity. Application of P at optimum rates increases tuber starch and vitamin C content but higher levels adversely affects protein content. In the event of P deficiency, the growth of plant is retarded, particularly during early growth stages. The plants are stunted with thin and dull, dark green leaves without luster. The leaves are curled with purple pigmentation sometime with marginal scorching.. Its deficiency is observed in acidic or very alkaline (calcareaus) soils, soils having low organic matter and in cold or wet soils.

A healthy crop of potato removes about 25-30 kg P_2O_5 /ha. Being a shallow rooted crop the fertilizer use efficiency for P is 10-15% and its application needs vary with the agro-climatic region, variety, crop sequence taken and soil type. The optimum dose for P varies from 60 to 100 kg P_2O_5/ha, for plains. The most common P fertilizers used in potato cultivation are single super phosphate and DAP. Farmyard manure is another important source of P, which over the long run increases the available P status of soils. Recently a high buildup in available soil P has been observed in many potato growing areas and hence its applications should be based on soil/plant tests to avoid wasteful expenditure. The proper placement of the fertilizer has a great influence on the P and its application at planting time in furrows is better than its application above or below seed tubers or as broadcasting. Potato plant needs most of P at early stages of development thus its application at planting is considered more useful. However, once phosphorus levels optimum (upto 20 ppm) to adequately support plant health, large increases in phosphorus application rate to support increased yields are unnecessary.

Many farmers apply pre-plant phosphorus even when soil analysis concentrations exceeds the upper optimum limit (20 ppm) with the belief that a positive growth response and unutilized P will be used by next crop. Heavy phosphorus application does increase the risk of phosphorus moving off fields into surface water, with potential negative environmental impact.

Potassium is the third yield limiting nutrient element in potato production. It increases impart resistance against drought, frost and diseases. Its application activates number of enzymes involved in photosynthesis, carbohydrate metabolism and proteins and assists in the translocation of carbohydrates from leaves to tubers. It also increases the yield by increasing the number and yield of large sized tubers, therefore, its management has special significance in processing potato production. Application of SOP produces tubers with higher dry matter, starch and Vitamin C content. The chip quality of potato tubers is also affected by K source and application of K through MOP has been reported to decrease enzymatic discoloration and phenol content thereby reducing the browning of potato chips.

In the event of K deficiency edges and tips of the lower leaves become yellow and later necrotic. The yellowing starts from the margins and eventually spreads between the veins as the deficiency progresses. Leave margins curl up, often wither and collapse prematurely. Potatoes require high levels of potassium in concentrations which are comparable to or greater than nitrogen. Potassium is taken up from the soil solution as the potassium ion (K+) which is replenished predominately from the exchange sites on soil colloids. Therefore, soil extracted K+ (reported in ppm) provides an index of soil potassium supplying ability. Chances of response to applied potassium are less if the extractable/available k in soil is more than 105 ppm in light textured soils of plains. Its deficiency is frequently observed in sandy or light soils , under drought conditions and in areas of high rainfall or heavy irrigation.

The use efficiency for K it ranges between 50-60 per cent and the recommended fertliser dose for K varies from 100-150, kg K_2O/ha, respectively, for plains. In high crop intensity area, application of K on removal basis is needed rather than its application on response basis. In processing potatoes, due to their large tuber size and higher dry matter, requirement K is normally more than ware crop and for Kufri Chipsona-1 and Kufri Chipsona-2 it recommended to apply 150 kg K_2O/ha as basal application. Among potassium fertilizers potassium chloride (MOP) and potassium sulphate (SOP) have been studied in depth and potassium sulphate has been found to be the best in term of its beneficial effect on tuber quality viz., dry matter, ascorbic acid and sugar content but due to its high cost, it has not found much use and MOP is most commonly used. The proper placement of the fertilizer has a great influence on the P and K use efficiency in this crop. Its furrow placement has been more economical than broadcasting or band placement. Like P, application of K at planting time in furrows is better than its application above or below seed tubers or as broadcasting. Although there are reports of increased disease resistance with the use of potassium chloride, high rates of potassium chloride have also been related to significant reductions in potato tuber quality. Applying a major portion of total season potassium fertilizer prior to planting has been found effective in obtaining maximum yields. However, banding potassium fertilizer materials beside the seed piece at planting has the potential to elevate salt levels in the area in which sprouting of the seed piece occurs, causing detrimental results in root development. Split application is more beneficial in light textured soils.

Secondary nutrients -Calcium (Ca), magnesium (Mg), and sulfur (S) in most of the Indian soils contain sufficient amounts of these secondary nutrients to meet the requirements of potato crop where sufficient organic matter is applied. However, acid

sandy soils low in organic matter and receiving high rainfall may require addition of these nutrients.

Calcium plays an important role in maintaining tuber quality in storage and reducing internal tuber disorders. Calcium deficiency may occur sometimes on high testing Ca soils and can result in tuber storage and internal breakdown problems due to water or temperature stress. Under such conditions addition of calcium can be applied if the potatoes are to be stored and storage problems have been encountered previously. Calcium sulfate (gypsum) and calcium nitrate can be used as Ca sources. Gypsum can be applied at or before planting and if calcium nitrate is to be used it should be incorporated into the rootzone soil after emergence. Soil application of $CaCl_2$ at 20 kg/ha was more effective than foliar application of $CaCl_2$ (0.01%) in increasing the potato yield mainly due to increase in yield of large and medium sized tubers. Its application also reduces the severity of soft rot during storage and results in emergence of healthier sprouts. Although the relationship between calcium and tuber quality has received significant interest, research has not established a direct relationship A possible link between low calcium availability to tubers and the severity of internal brown spot (IBS) has been found in some studies. Under some field conditions IBS has been decreased by calcium fertilization. Although calcium and magnesium are essential plant nutrients for plant growth they are seldom limiting in central Washington soils. Calcium is immobile in plant tissues. For translocation of Ca into the tubers during bulking, calcium must be taken up by the stolons and/or stolon roots.

Magnesium plays a central role in photosynthesis, as it is present in the centre of each chlorophyll molecule. It is also involved in various key steps of sugar and protein. Its deficiency symptoms appear first at lower leaves. The older leaves will develop pale interveinal chlorotic patches in a herringbone pattern along the main vein. Chlorotic patches become brown as the deficiency continues. The leaf margin often remains green and may curl up and become brittle. . Magnesium deficiency can be a problem in soils where high rates of potassium fertilizer have been used. High rates of potassium fertilizers may cause magnesium deficiency where available Mg is less, Response to applied Mg may be expected in soils having less than 50 mg/kg available 9extractable) Mg . Magnesium sulfate or potassium-magnesium sulfate are the most common Mg sources available.

Sulphur is recognized as fourth major nutrient after N, P and K and in view of its role in improving crop quality. It is required in many metabolic activities and its deficiency is similar to N in many ways. In case of deficiency, the pale yellowing of leaves starts from upper leaves. Water shortage or heavy metal toxicity can cause similar symptoms Severe deficiency of S results in retarded plant growth, reddening of stems and curling of leaves inward. Sulfur requirements can often be met from soil organic matter breakdown. Rainwater and irrigation water contain some sulfate and can also provide a significant proportion of the sulfur needed for growth. Ammonium sulfate, SSP, potassium sulfate and fertilizers if used for N, P and K sources will supply sufficient sulfur to meet the crop requirements. Elemental sulfur is not an immediately available form and must be oxidized by soil bacteria to sulfate before it is can be used by the plants. Its oxidation will have an acidifying effect on the soil. Potato cannot utilize soluble S from the deeper layers in soil profile thus the soils sufficient in available S for cereals crops are likely to be deficient in S for potato crop and light textured soils low in organic matter may require S from external source. The major sulphur carriers are elemental S (85-100% S) ammonium sulphate (23.7% S), single super phosphate (12.0%S), potassium sulphate (18.0%S) and gypsum (13-15%S). Potato response to applied S depends upon the S status of the soils. Sharma et al. (2011) reported that the quality and yield of potato was influenced by sulphur dose

from an experiment conducted at Malwa agro-climatic zone of Madhya Pradesh. They found that highest tuber yield size of tuber, dry matter production was recorded at 45 kg S/ha. Kumar et al. (2008) reported from an experiment conducted at Udaipur, Rajasthan, that application of 60 kg S/ha proved beneficial in increasing growth, yield attributes, yield and uptake of nutrients in potato crop.

Micro-nutrients

Even though, micronutrient elements are needed only in traces, many soils may not supply them in sufficient quantity for healthy growth and optimum yield of potato. Further the use of high analysis pure NPK fertilizers and high yielding potato varieties with increased nutrient demands, the decreased use os lity of farmyard manure and recycling of residue, have resulted in micronutrient deficiencies particulary B and Zn in many soils. Zinc, iron, boron and molybdenum have been reported to increase the tuber number of medium and large grade. Zinc, copper, manganese, boron and molybdenum have been shown to increase ascorbic acid content of tubers. Zinc fertilization reduced the content of compounds which is implicated in enzymatic discoloration Iron is and is essential for photosynthesis and plant metabolism. Thus important for early leaf development, strong growth and crop productivity. Manganese deficiency results in poor skin finish of the tubers . Tubers are more easily damaged during lifting and storage. . Boron is associated with improved crop development. Improved tuber quality, reduced incidence of internal internal browning. Molybdenum is involved in nitrogen metabolism, pigment and chlorophyll synthesis. Beneficial for growth and yield.

Micronutrients play an important role in suppressing plant diseases and improving the resistance of plants.. The effect of micronutrients on reducing the severity of diseases can be attributed to the involvement in physiology and biochemistry of the plant, as many of the essential micronutrients are involved in many processes that can affect the response of plants to pathogens (Marschner, 1995). Micronutrients can also affect disease resistance indirectly, as nutrient-deficient plants not only exhibit an impaired defense response, but often may also become more suitable for feeding as many metabolites such as reducing sugars and amino acids leak outside the plant cell. Micronutrients play an important role in plant metabolism by affecting the phenolics and lignin content and also membrane stability and can affect resistance indirectly, as in deficient plants they become more suitable feeding substrate.

Deficiency symptoms:

Zinc: Its deficiency in potato, often known as fern leaf or little leaf, appears on young developing leaves. Younger leaves show inter-veinal chlorosis and necrosis which occurs in irregular patches. Whitish spots develop within the brown necrotic tissue. Symptoms may also start on older leaves. Deficient plants show severe stunting and bronzing or yellowing of the foliage, usually around the leaf margins, starting from the tips. Youngest leaves are cupped upwards and rolled to such an extent that the terminal growth resembles that of ferns. Leaves of affected plants are smaller and their upper internodes are shorter. Its deficiency is mainly observed in organic soils, high pH soils, soils receiving high phosphorus application and under cold wet conditions.

Iron: Essential for photosynthesis and plant metabolism. Thus important for early leaf development, strong growth and crop productivity. Its deficiency appears initially as a yellowing (intercostal chlorosis) of young leaves near the growing point of the plant. Deficiency is marked by pale green and yellow color of the youngest leaves, while the veins remain darker green. Sometimes the youngest leaves contain no chlorophyll at all and are white in color then. Netted green veining occurs when traces of iron are absorbed and translocated along the veins for chlorophyll formation. With time, the leaves become light yellow to nearly white. During the deficiency stage, blade tips

remain green for longer time. Iron deficiency mainly occurs in calcareous soils and high pH soils, in soils having high copper, manganese or zinc soils, in other soils with a due to the low availability of Fe. In specific clay soils Fe becomes fixated and deficiency is the consequence.

Manganese: Its deficiency results in poor skin finish of the tubers. The first sign of its deficiency is yellowing and slight cupping of younger leaves. Pinkish colour develops at the base of younger chlorotic leaves and relatively old leaves show dark to black spots. With increased deficiency, dark to black spotting develops between the veins with a large increase in spotting, appearing along larger veins and the mid rib. These symptoms, darkening and cupping, increase in severity with time. During mild deficiency, upper parts of the plants become somewhat chlorotic but do not develop dead spots.

Copper: Normally potatoes are not sensitive to copper deficiency. Symptoms are seldom visible in the field**.**. Copper deficiency causes permanent wilting of plants. Under acute deficiency young leaves roll in. Leaf tips and margins may dried off without preceding chlorosis. .

Boron deficiency causes the formation of a bushy plant with droopy leaves. The young leaves of B deficiency plants are thickened, crinkled and bordered by light brown tissue, which extends to the intercostal areas. The growing points and the shoot tips die off. In severe cases, the leaf margins are cupped upward. Its deficiency, like calcium deficiency, affects the growing points. Immature central leaves become deformed and the growing point dies. In case of mild boron deficiency, slight upward curling of the margins of the older leaves is visible. Gupta (1979) reported that B deficiency in potato results in the death of growing points, with short internodes giving the plant a bushy appearance. Leaves thicken and margins roll upward, a symptom similar to that at potato leaf rot virus. High levels of nitrogen, calcium, cold wet weather and periods of drought may lead to boron deficiency.

Molybdenum deficiency symptoms are expressed as marked chlorosis and reduced growth and yield. Young leaves become uniformly yellow green. Yellowing of leaf blades is similar to nitrogen or sulfur. Deficiency may be observed in soils having low levels of soil organic matter.

Methods of micronutrient application: There are three main approaches to tackle micronutrient disorders in potato: application of a micronutrient carrier to the soil, foliar application of micronutrients to each crop and treating mother seed tubers with micronutrient sources. Micronutrients are phytotoxic and foliar spray during dry spell (during the time of bright sunshine and low humidity) should be avoided between 11 a.m. and 3 p.m. to prevent scorching of leaves. The doses of different micronutrients for optimum potato plant growth and development are presented in table 3. Generally, most of them are taken up during the early growth stages thus, it favours early fertilization with micronutrients. Sulphate salts of different micronutrients are the cheaper and most commonly used sources.

Organic manures: Well-decomposed farmyard manure (FYM) and compost made from animal excreta and litter, are bulky in nature and supply small amounts of plant nutrients are classified as bulky organic manures. They are generally applied @ 15-30t/ha depending upon availability. These organic materials contain enough of secondary and micronutrients, besides primary nutrients, that contribute significantly to increased crop yields, soil fertility and physical condition. Soil's physical condition is improved through increased water infiltration, water holding capacity, aeration and permeability, soil aggregation, rooting depth, decreased soil crusting, bulk density, run off and erosion.

Green Manuring: Due to decreased use farmyard manure more interest for use of manuring crop os growing among the farmers. Green manurin improves the fertility and productivity of soil by adding organic matter as well as additional nitrogen, particularly if it is a legume crop. A leguminous crop producing 10-20 tons of green manure per hectare adds about 60-100 Kg/ha of nitrogen when ploughed under. The increase in tuber yield after green manuring may be to the tune of the order of 30-50 percent in soils where organic carbon content is low.

Table 1. Quality requirements of potato for chip processing

Tuber shape	Tuber size (mm)	Eyes	Sp. gravity	Dry matter (%)	Starch, (%)	RS, (%) d.w basis	Texture
Round to oval	40-60	Shallow	1.085	22-25	15-18	1.25	Firm to mealy

Table 2. Critical levels of soil available P and K for potato

Soil nutrient status	Soil test values (ppm)	
	Olsen's P	Ammonium acetate-K
Low	< 10	< 105
Medium	10 – 20	105 - 150
High	> 20	> 150

Table 3. Critical limits and doses of micronutrient application for correction of their deficiency in potato

Micronutrient	Source	Critical limits in Soil (ppm)	Soil application (kg/ha)	Spray application (g/100 lit. water)	Tuber soaking treatment (g/100 lit. water)
Zinc	Zinc sulphate	0.75	25	200	50
Iron	Ferrous sulphate	6.60	50	300	75
Manganese	Manganese sulphate	2.00	25	200	50
Copper	Copper sulphate	0.32	25	200	50
Molybdenum	Ammonium molybdate	0.20	2	100	20
Boron	Sodium borate	0.50	2	100	20

Viral diseases and their management (including diagnostics) in potato production

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The potato (*Solanum tuberosum* L.) is an important crop worldwide. Potato production is not an easy task, as potatoes are affected by many kinds of viruses and their various strains belonging to different taxonomic groups. Viruses contribute to "running out", or degeneration, of seed stocks. Viral diseases are prevalent throughout the India but are most severe in North-eastern plains and plateaux where population of aphid vectors is high throughout the crop season. Tomato leaf curl New Delhi virus (ToLCNDV), potato leaf roll virus (PLRV) and potato virus Y (PVY) are the most important viruses in India. Viruses once introduced may persist and spread in agricultural environments and under favourable conditions they may multiply rapidly and cause significant yield loss and economic damage. Therefore, sustainable potato production is possible only if the diseases are kept under check, especially in sub tropics where the weather is highly conducive for common viral diseases.

Major Viruses and Viroid of Potato

Apical Leaf Curl Disease of Potato: Apical leaf curl disease has recently emerged as one of the most devastating viral disease of potato in India. It was first observed during 1999 in northern India and the association of a begomovirus with this disease was confirmed through electron microscopy in the year 2001. Further the virus was confirmed as a strain variant of *Tomato leaf curl New Delhi virus* (ToLCNDV) on the basis of nucleotide sequence of genomic components (Usharani *et al*., 2003). The incidence of apical leaf curl disease has been observed to be higher in early planted crop when temperature is high in October, than in November planted potato crop. Recently, 40-70 per cent infection was recorded in Indo-Gangetic plains of India. There are even reports of up to 100 per cent infection causing heavy yield losses. The disease incidence has positive correlation with whitefly population and whitefly infestation period.

Causal virus: It is caused by a strain of *Tomato leafcurl New Delhi virus* (ToLCNDV) belonging to the genus, *Begomovirus* and family, *Geminiviridae*. It is a bipartite geminivirus consisting of two circular ssDNA molecules (DNA A & DNA B) of ~2.7 kb encapsidated in geminate particles. The DNA A and DNA B components of ToLCNDV-potato share >90 % and 86.6- 91.7 % identities, repectively with ToLCNDV infecting cucurbitaceous crops, tomato and okra (Jeevalatha *et al*., 2012).

Symptoms: Infected potato plant show curling of apical leaves, crinkling and a clear mosaic symptom, later entire plant appears bushy and stunted due to reduced internodal distance. The primary infection in the field appears within about 40-45 days from the date of planting.

Fig. 1: Symptoms of apical leaf curl disease on potato plants

Transmission: Under field conditions the virus is transmitted by whitefly (*Bemisia tabaci*) in a persistent manner. Once an adult has acquired the virus by feeding, it retains the virus for long period. The females are more efficient in transmitting the virus than the males. It is also transmitted through seed tubers from one generation to the next generation.

Disease cycle: Under field conditions, the whiteflies acquire the virus and transmit from infected plant to healthy potato plants. Once the plant is infected, the infection is systemic in a given plant and therefore the tubers are also be infected (serves as primary source of infection) with the virus. If this infected tuber is planted then it spreads the virus into the upcoming seedling/plant and again the secondary spread is by whitefly as mentioned earlier. Therefore, whiteflies play a vital role in secondary spread of the virus. This entire cycle repeats till proper management practices are adopted.

Management strategies

Diseases caused by viruses cannot be easily controlled by chemicals alone. Therefore, it has to be managed by adopting the following measure on co-operative basis and in an integrated approach:

Cultural Management

- Use virus-free seed tubers for planting.
- Place yellow trap (15X30cm^2) just above the canopy height @ 60 traps/ha at equidistance from each other.
- Prompt destruction of crop residues after crop maturity/harvest.
- Uproot and burn (roguing) the plants showing virus symptoms immediately.
- Manage the weeds at regular interval and avoid/remove the reservoir plants like okra, brinjal and datura.

Host Plant Resistance

- Kufri Bahar is tolerant to ToLCNDV-potato

Chemical Control

- Seed treatment with imidacloprid (200SL) @ 0.04% (4ml/10lit) for 10 minutes before planting.
- First spray with imidacloprid (200SL) @ 0.03% (3ml/10lit) at the time of emergence of crop.
- Second spray with thiamethoxam (25WG) @0.05% after 15 days of crop emergence.

Potato Leafroll: Potato leafroll is one of the most prevalent viral diseases of potato in India. All Indian potato varieties are susceptible to this virus. Infected plants produce only a few, small to medium tubers. Yield loss normally ranges from 20-50% in India but in extreme cases may be as high as 50-80 %. However, it is lower in autumn season (7-16 %) than in spring season (39-60%).

Causal Virus: Potato leafroll virus (PLRV) (Syn. = phloem necrosis virus) is the type species of the genus *Polerovirus*, in the family *Luteoviridae*. PLRV has small, isometric virions (23-25 nm dia) which are primarily confined to the phloem of the infected plants. At genome level, Indian isolates are closer to European and Canadian isolates (95.8 to 98.6 %) than to an Australian isolate (92.9 % to 93.4 % similarity). In nature, potato is the principal host of PLRV yet several plant species, mostly from *Solanaceae*, are known as hosts *viz*., *Datura stramonium*, *Physalis floridana, Solanum villosum*. It can also infect some non-solanaceous plants like *Amaranthus caudatus, Gomphrena globosa etc*. (Loebenstein *et al*., 2001).

Symptoms: Depending upon the age of infection, the infected plants show two type of symptoms *viz*., primary or secondary. The primary symptoms are confined to top young leaves, which usually stand upright, roll and turn slightly pale in certain cultivars. Most varieties, however, develop reddish/pink colour in top leaves starting at the margins, sometimes accompanied with slight rolling of the leaflets. Secondary symptoms develop when the plants are grown from infected seed tubers. Such symptoms are rather prominent in older leaves, *i.e.* absent or less pronounced on younger top leaves. Infected plants have characteristic pale, dwarfed, and upright appearance with rolling of lower leaves that turn yellow, brittle and are leathery in texture. In some cultivars, a reddish or purple discolouration develops on the margins and underside of the leaves. Singh *et al*. (1982) reported occurrence of three groups of PLRV *viz*., mild, moderate and severe strain in India based on their symptom severity on the test plant, *Physalis floridana*.

In storage, the tubers from the infected plants, in certain varieties develop phloem necrosis, but most Indian varieties do not develop this necrosis. Often non-viral false or pseudo "leafroll" symptoms are also seen in the field, induced either by purple top roll (phytoplasma), cutworm, canker, black scurf (fungi) or calcium deficiency, water logging, *etc*. Some varieties also have the characteristic of foliage showing rolling/cupping towards maturity.

Fig. 3: Primary leaf roll symptoms on infected potato plants

Fig. 4: Leaf roll symptoms due to secondary infection (seed borne) of

Disease Cycle and Transmission: The virus is tuber borne and transmitted efficiently by aphid in a persistent manner (circulative type i.e. aphids normally need longer feeding periods on diseased and healthy plants). Primary infections are normally caused by the viruliferous aphids coming either from distant or nearby fields and/or diseased volunteer plants arising from the infected tubers.The green peach aphid (*Myzus persicae*) is the most efficient and important vector of PLRV and also *Aphis gossypii. Macrosiphum euphorbiae* transmits potato strains less efficiently. *M. nicotianae* was found to be an efficient experimental vector. PLRV is not transmitted by mechanical inoculation seed or pollen but it is transmitted experimentally by grafting.

Management

- Use of virus free seed tubers.
- Multiply virus free seed only in aphid free areas/periods coupled with good sanitation practices, roguing of infected plants and dehaulming the crop as soon as the vector populations exceeds critical limit of 20 aphids/ 100 compound leaves.
- Application of suitable contact/systemic insecticides to control aphid vectors in field and also in stores.
- Tubers can be freed from PLRV infections by storing tubers in ordinary stores in summer months at 29 to 35° C. Two hr daily heat treatment of tubers for 6 weeks at 40°C inactivates PLRV. Hot water tuber treatment also (50 to 55°C for 15-20 minutes) has also been found effective in eradicating the virus. In tissue culture, thermotherapy helps to eliminate PLRV from meristomatic tissue of plant.

Potato Mosaics

Viral infection cause enormous economic losses particularly in the areas of tropics and semi tropics which provide ideal conditions for the perpetuation of viruses and their vectors. Any

attempt to minimize the losses caused by these diseases must always be, preceded by a correct and precise detection and diagnosis of the causal agent. Nevertheless, it is very difficult to diagnose the plant virus infections as the symptoms may vary depending upon the plant variety involved, the environmental conditions and strain of the virus. Sometimes different viruses can cause similar symptoms in the same plant species and sometimes the disease could result from the synergistic effect of infection caused by two or more different viruses. The most wide spread viruses causing mosaics in potatoes are usually PVY occurring worldwide. In combination with *Potato virus X*, it causes an even more destructive disease known as rugose mosaic. These viruses found wherever potatoes are grown.

Causal Agent: In general majority of the potato viruses like PVY, PVX, PVA, PVM, PVS, PAMV and PSTVd (viroid) causes mosaics, mottling/crinkle, necrosis, etc., either individually or in different combinations. Whereas, *Potato leafroll virus* causes upward rolling and *Tomato leaf curl virus* causes curling of young leaves. These viruses generally infect potato and few other hosts from the family of *Solanaceae* like pepper *(Capsicum* spp.*),* tobacco *(Nicotiana* spp.*),* tomato *(Lycopersicon esculentum)* and other families like *Chenopodiaceae, Leguminosae, Compositae* and *Amaranthaceae.*

Symptoms: Mosaics are the most common symptoms expressed by plant viruses infecting potato plants. Overlapping symptoms are invoked by different viruses individually and/or in different combinations, such as typical greening or yellows, mild or severe/rugose mosaics, faint mottle, chlorosis of plants and discolouration or distortion of foliage. Rugosity of leaves is accompanied by interveinal chlorosis and/or interveinal puckering. Besides, there can be necrosis of the veins (lower side), petioles and even inward curling of lamina which may lead to premature defoliation. Mosaics and mottle are prominent in cool and dull weathers.

Latent or Faint Mosaics

Potato Virus X (PVX): It is latent in many of the cultivars whereas the severe strains can cause mosaic symptoms and the appearance of mosaics is limited by the veins. Bright day light generally affects visual symptoms, which can be observed under low light or placing a white card below the leaf.

Potato Virus S (PVS): Usually latent or very mild or barely perceptible mottle and faint vein banding. Some cultivars develop mild rugosity while few strains invoke bronzing of leaves and in few cultivars tiny necrotic spots can also be seen.

Mild Mosaics

Potato Virus A (PVA): Slight and transient mosaics that are particularly visible in cloudy weather. Mosaic faint mottling, slight crinkling of leaves and inter-veinal and veinal chlorosis. These symptoms can be reinforced by placing a sheet of white paper under the leaf.

Potato Virus M (PVM): Spoon-shaped leaves corresponding to a soft curling of the leaves (while the potato leaf roll virus produces "cracking" of the roll). Called leaf rolling mosaic or para crinkle. It occurs on developed plants, preferentially on the topmost leaves.

Potato Spindle Tuber Viroid (PSTVd): The symptoms of PSTVd are very obscure and unusual. Generally the plants are slightly stunted, erect, often with curling leaves darker green than healthy. Eyes are numerous in number with distinct eye brows. Tubers are cylindrically longer than normal with tapered ends with cracks upon infection with severe strain of PSTVd.

Severe Mosaics and Complex Diseases

Potato Virus Y (PVY): Generally the symptoms vary with the strains of the virus or variety of potato i.e., plants will be stunted with mild or severe mosaic, veinal necrosis. Ordinary strain (PVY^{O}) and stipple-streak strain (PVY^{C}) causes necrosis, yellowing of leaflets and premature leaf drops. PVY^{NTN} strains cause mild mosaic and clearly visible raised necrotic ring spots on the tuber surface which later become sunken and skin cracking.

Fig. 5: Potato plants showing symptoms of PVY

Crinkle (PVX+PVA): Infection of PVX and PVA may lead to heavy blotching of distorted, erect leaves with wavy margins, severe stunting, mottling, necrotic spotting and streaking of leaflets.

Rugose (PVX+PVY): The primary cause of rugose mosaic is due to the infection by PVY but in combination with PVX may result in severe rugose symptoms, stunted growth and leaf drop or curling with veinal necrosis in lower leaves and severe mosaic in upper ones.

Rosette (PVX+PVS+PVM or PVX+PVA+PVY+PVM): Severe stunting of the infected plants with single or a few stems per hill, puckered, roughened leaflets with inward and yielding a few small tubers.

Disease Cycle and Transmission: Tubers are the main source of infection and all mosaic evoking viruses are tuber perpetuated. Highly contagious viruses and viroid are readily transmitted mechanically by leaf, root or tuber contact of diseased and healthy plants, though the viroid also readily spreads through pollen and true seed. Contagious viruses are also transmitted by the cutting knife, as well as cultivating and spraying equipments. The other viruses are transmitted mainly by aphid species like *Myzus persicae* and *Aphis gossypii*.

Management Strategies

Diseases (mosaics) caused by viruses can be managed by adopting an integrated management as follows:

1) To avoid the spread of contagious viruses/viroid, observe strict sanitation in the field and also in stores, right from harvest to planting.
2) Disinfect all field equipments by dipping in or washing them either with 3% trisodium phosphate or calcium hypochloride (1%) solution.
3) Essentially use disease-free seed stocks from approved or reliable sources.
4) Rogue out the diseased plants carefully along with their tubers and dispose them away from the field at regular intervals during the growing season.

5) Dehaulming the crop before the aphids cross critical level to enforce rigid control of the insect vector and
6) Seed treatment with imidacloprid (200SL) @ 0.04% (4ml/10lit) for 10 minutes before planting.
7) First spray with imidacloprid (200SL) @ 0.03% (3ml/10lit) at the time of emergence of crop.
8) Second spray with thiamethoxam (25WG) @0.05% after 15 days of crop emergence.
9) Combine thermo-and chemo-therapy followed by apical meristem culture for eliminating the viruses from seed tubers to produce pre-nucleus/mother seed stocks.

Persistent and Non-persistent Viruses: Potato viruses are transmitted by aphids in two basic ways. The virus is either non-persistent (stylet-borne) or persistent and circulative (they are ingested and persist in the aphid throughout its life). The most common persistent and circulative virus affecting potatoes in India is potato leaf roll (PLRV). Common non-persistent or stylet-borne viruses are PVY, PVA, PVS and PVM. Because the aphid has to feed for more than several minutes to transmit persistent-circulative viruses, control by insecticides is highly effective, whereas with a stylet-borne virus the virus is transmitted as soon as the aphid stylet penetrates the epidermal cells of the leaf- too soon for any insecticide to kill the aphid and prevent virus transmission.

Integrated management of potato viruses: No single approach as outlined above will yield desirable result. A combination of them or most of them will be the only lasting solotion. Schedule of integrated control of potato viruses include: inspection of seed production areas and rejection of fields with mosaic incidence higher than the prescribed level, killing of vines of seed crop at the recommended date or earlier and not to allow re-growth of the vines, destroy volunteer potato plants and weeds in and around the seed crop, monitor the population of vectors and application of insecticides to keep the aphid vectors below the critical level, use of properly disinfected tools, maintaining proper isolation of the seed crop from virus sources, use of the best quality certified seed tubers for planting, avoiding use of cut tubers as seed for seed crop, planting seed crop at a specified period to avoid exposure of the crop to the vectors, minimising chances of virus spread through farm machinery and stop irrigation 10-15 days before harvest to allow skin curing.

Diagnostics: Effective management of potato viral diseases depends essentially on their rapid detection, accurate identification and sieving them out through indexing. diagnostics can be employed for varying purpose which may include:

1. To determine the presence and quantity of the virus in potato crop in order to take plant protection measures.
2. To determine the extent of viral incidence and consequent yield loss.
3. To certify seed potato planting materials for plant quarantine and certification programs.
4. To assess the effectiveness of application of cultural, physical, chemical, or biological methods of containing the viruses.
5. To assess viral infection in plant materials in breeding programs.
6. To detect and identify new viruses rapidly to prevent further spread.
7. To study taxonomic and evolutionary relationships of viral pathogens.
8. To resolve the components of complex diseases incited by two or more viruses.
9. To study pathogenesis and gene functions.

Diagnostic techniques for viruses fall into two broad categories: biological properties related to the interaction of the virus with its host and/or vector (e.g. symptomatology and transmission tests) and intrinsic properties of the virus itself (coat protein and nucleic acid). These diagnostic methods provides greater flexibility, increased sensitivity, and specificity for rapid diagnosis of virus diseases in disease surveys, epidemiological studies, plant quarantine, seed certification, and breeding programs.

Biological indexing, symptomatology and physical properties: Initially biological assays were developed which are still in use, since they are simple, require minimal knowledge of the pathogen and are polyvalent. Furthermore, biological indexing is still the only method available to detect uncharacterized but graft transmissible agents. Its sensitivity is considered to be very high due to the viral multiplication in the host plant used as indicator.The symptoms on the potato plants commonly are used to characterize a disease having viral aetiology and for rouging of diseased plants in an attempt to control the disease. Visual inspection is relatively easy when symptoms are clearly characteristic of a specific disease.

Electron microscopy: Electron microscopy (EM) provides very useful information on the morphology of the virus particles and is commonly used to examine viruses in crude extracts from infected plants. Examination of negatively stained virions by electron microscopy reveals flexuous rod-shaped particles with no obvious terminal structures in PVY and PVA.

Serology based techniques: Serology involves use of specific antibodies to detect their respective antigens in test specimen. Antibodies are composed of immunoglobulin (Ig) proteins produced in the body of an animal in response to the presence of antigens. Each antibody is specific to a particular antigen and will bind to it. Serological or immunological methods are generally specific, sensitive enough and have been used successfully for a number of years for the detection of potato viruses. However, there are some limitations to the use of antibodies in potato virus diagnosis. Firstly, the nature of the cross reactions between heterologous antibody-antigen complexes are not well understood. Secondly, diagnosis is based on only part of the organism's structure such as the coat protein of a virus which represents only a small proportion of the information about the virus. Thirdly, serology is only useful when the antiserum has been prepared or when an antigen is available for producing an antiserum. Although antibodies production may take several weeks, they are suitable for both laboratory and field conditions and can identify strains within species. However, different diagnostic protocols based on antibody-antigen are routinely being used for detection of major potato viruses and they have been briefly discussed below.

Immunosorbent electron microscopy: The Immuno sorbent electron microscopy (ISEM) for detection and identification of potato viruses by combining electron microscopy and serology are highly sensitive. PLRV is a small, phloem-restricted virus occurring in very low concentration and poses problem in detection with conventional electron microscopic detection. Optimum parameters were determined for the reliable and sensitive immune electron microscopic diagnosis of PLRV along with other important potato viruses. PLRV was best detected when the virus and its antibodies interacted in liquid phase followed by trapping on the grid coated with protein A and homologous antibodies.

Precipitation, gel diffusion and agglutination tests: Precipitin tests (either in liquid medium or in agar/agarose) rely on the formation of visible precipitate when adequate quantities of virus and specific antibodies are in contact with each other. These tests are routinely used by some investigators, but agglutination and double diffusion tests are more commonly used. In double diffusion tests, the antibodies and antigen diffuse through a gel matrix and a visible precipitin line is formed where the two diffusing reactants meet in the gel. The double diffusion method can be used to distinguish related, but distinct, strains of a virus or even different but serologically related viruses. In an agglutination test, the antibody is coated on the surface of an inert carrier particle (e.g., red blood cells), and a positive antigen–antibody reaction results in clumping/agglutination of the carrier particles which can be observed by the naked eye or under a microscope. Agglutination tests are more sensitive than other precipitin tests and can be carried out with lower concentrations of reactants than are necessary for precipitation tests.

Enzyme-linked Immunosorbent Assay (ELISA): The ELISA has significantly increased the ability to detect and study potato viruses, and is currently the most widely used method for the detection of potato viruses due to its simplicity, adaptability, rapidity, sensitivity and accuracy. The double antibody sandwich (DAS-ELISA) test on a solid phase (usually plastic) is most commonly used. Virus is first selectively trapped by a specific antibody adsorbed on a solid

surface, a specific enzyme-labeled antibody (conjugate) is added to the immobilized virus, and the reaction is measured visually or spectrophotometrically, after adding a suitable enzyme substrate.

Dot blot assay: In immune blots or dot-blots assays, antibodies or virus particles bound to nitrocellulose membrane filters are used. Dot blot ELISA tends to be rapid, easy to perform and conservative of reagents and often more sensitive than ELISA carried out in a microtiter plate. Immunoblot assays use the same reagents used in microtiter plate ELISAs, except that the substrate produces an insoluble product which precipitates onto the membrane. Positive reactions can be determined visually. Assays in which antibodies or antigens are bound to nitrocellulose or nylon membranes have been used to detect PVS, PYX , PVY and PLRV.

Tissue blotting, tissue squashes and southern blotting: In this technique blots are made by pressing the freshly cut tissue surface gently but firmly on a nitrocellulose membrane. Antigens in tissue blots are detected by enzyme labeled probes and this technique has been used to detect PVX and PVY from tubers in the field. Southern blotting combines transfer of electrophoretically separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridization. In this method total DNA is isolated from infected plant and transferred on membrane followed by its hybridization with radiolabeled probe which is generally made from partial or full length viral nucleic acid. Southern blotting method has been used for quantitative determination of many begomoviruses like ToLCNDV.

Lateral flow immune assay kits (LFIA): It is based on the interaction between the target virus and immunoreagents (antibodies and their conjugates with colored colloidal particles) applied on the membrane carriers (test strips). When the strip is dipped into the sample being analysed, the sample liquid flows through the membrane and triggers immunochemical interactions resulting in visible coloration in test and reference lines. LFIA kits for the detection of five viruses viz., PVX, PVA, PVS, PVM and PVY either individually or in combination of two viruses have been developed.

Nucleic acid based techniques: Nucleic acid based techniques of potato virus diagnosis are well described and the sensitivity of the methods exceeds that of serology and have the advantage of targeting the genome of the virus. Some of the disadvantages of nucleic acid based techniques are the equipment, facilities, expertise and the time required to determine the optimum conditions for a particular application of the technique. Further, while several serological tests can be conducted in the field for rapid diagnoses, nucleic acid based methods are still carried out in laboratories. However, several nucleic acid based methods are very robust, accurate and higly sensitive in detection of potato viruses and have been discussed below in brief.

Conventional PCR or RT-PCR: RT-PCR protocols have been standardized for detection of major potato viruses that can detect very low level of virus inoculum. Several variations of RT-PCR like Immuno capture PCR (IC-PCR), Print-capture PCR (PC-PCR), nested PCR, multiplex RT-PCR and multiplex nested RT-PCR have been standardized for detection of potato viruses to differentiate strainal variation of a particular virus. Multiplex RT-PCR is a time- and reagent-saving amplification technique in which multiple primer sets are used to amplify multiple specific targets simultaneously in the same sample. IC-RT-PCR, DB-RT-PCR and PC-RT-PCR assays were successfully used for the detection of PVY virions and PC-PCR for the detection of ToLCNDV-potato.

Real-time PCR: Real time RT-PCR protocols have been developed for potato viruses in several laboratories. TaqMan® duplex RT-PCR have been used for the detection of TRV, PMTV, PLRV and PVY. Agindotan *et al*. (2007) reported an assay where four common potato-infecting viruses, *Potato leafroll virus*, *Potato virus A*, *Potato virus X* and *Potato virus Y*, were detected simultaneously from total RNA and saps of dormant potato tubers in a quadruplex real-time RT-PCR. A single tube real time RT-PCR was reported by Mortimer-Jones *et al*. (2009) to detect single infections of PLRV, PVX, PVS and TSWV simultaneously in a single assay from bulked samples equivalent to 300 dormant tubers. Multiplex real-time RT-PCR has also been used to detect single and mixed infections of PLRV and PVY in seed potatoes using molecular beacons.

Rolling circle amplification (RCA): RCA is an isothermal method for reliable diagnosis of geminiviruses and presumably all viruses with small single-stranded circular DNA genomes. This technique has been found useful for characterizing viral DNA components of several geminiviruses from experimental and natural host plant sources. RCA amplified viral DNA can be characterized by restriction fragment length polymorphism (RFLP) analysis and directly sequenced up to 900 bases in a single run, circumventing cloning and plasmid purification. RCA is better, easier and cheaper than polymerase chain reaction (PCR) or antibody-based detection of geminiviruses. RCA in combination with PCR assay increases the sensitivity and specificity of the assay.

Nucleic acid spot hybridization: Nucleic acid hybridization techniques have been standardized for detection of many potato viruses. Nucleic acid hybridization of DNA or RNA probes has the advantage of being able to detect the nucleic acid of the virus in both forms, single-stranded and double-stranded. cRNA probes can be labelled with isotopes or nonradioactive probes. cRNA probes are preferable to cDNA probes for detecting RNA viruses. An RNA extraction from infected tissue is blotted onto a membrane and the probe hybridized to it and detected. Both radioactive probes (^{32}P labelled) and non radioactive probes (fluorescent probes) were used for the detection of PSTVd.

Microarrays and macroarrays: Microarrays are high-density arrays with spot sizes smaller than 150 microns. A typical microarray slide can contain up to 30,000 spots. Macroarrays are generally membrane-based with spot sizes of greater than 300 microns. Arrays printed with probes corresponding to a large number of virus species can be utilized to simultaneously detect all those viruses within the tissue of an infected host. Agindotan and Perry (2007) reported a macroarray sytem using 70-mer oligonucleotide probes immobilized on nylon membrane for the detection of CMV, PVY and PLRV. It is convenient, cost-effective macroarray and microtube hybridization (MTH) system in which cDNA probes immobilized on nylon membrane, target viruses were amplified and labelled with biotin and then hybridization was carried in hybridization oven and colorimetrically detected using nitro blue tetrazolium (NBT)/ bromo-4-chloro-3-indolyl phosphate (BCIP) reagent. Bystricka *et al*. (2005) described a microchip using short synthetic single-stranded oligomers (40 nt) instead of PCR products as capture probes for detection of PVA, PVS, PVM, PVX, PVY and PLRV, in both single and mixed infections. Sip *et al*. (2010) reported oligonucleotide microarray for the detection of mixed infections of PVA, PVS^{O}, PVM, PVX, PVY^{O}, PVY^{N}, PVY^{NTN} and PLRV.

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Application of proteomics and metabolomics in crop improvement

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Availability of genome sequences at large scale is proving to be vital for gaining better insights into the genome structure and organization of different forms of life, including plants. However, real challenge still remains in deciphering the function of the encoded proteins, their interaction with other proteins or biomolecules through which these contribute to the functioning of the organism as an entity and at systems level. A systems-level understanding is essentially required if we intend to fully capitalize on plants to address global challenges such as food, and fuel shortages, changing global climate etc. The first step in a systems approach is to identify all of the system components (e.g., genome, transcripts, proteins, metabolites). Because information regarding the function of the system as a whole is desired, all of the measurements and experimental observations are most informative when conducted on a global scale at high spatial and temporal resolution. This approach has given rise to new field known as Systems biology. Systems biology is the study of the interactions and interplay between all properties of biological systems. It involves integrating the existing knowledge about biological components, building a model of the system as a whole, and extracting unifying organizational principles that explain the form and function of living organisms. This lecture will briefly describe the two important components of systems biology. These are: proteomics and metabolomics.

Proteomics

Proteome refers to the protein complement of a particular biological system at any given point of time. The term "proteome" was first used by Wilkins et al. (1995) to describe the protein complement to the genome. Though proteomics is theoretically the analysis of the full protein population of complex biological systems, the available technologies are not capable of fulfilling this description experimentally. Conceptual progress in proteomics has made it possible to analyze entire proteomes at previously unprecedented density and accuracy. Various steps involved in proteomics studies are described below.

Methodologies for plant proteomics:

1. Extraction of proteins:

Sample preparation procedures for proteomic analysis require the reliable and consistent extraction of proteins from tissue samples. Plant tissues usually contain high levels of proteases and secondary metabolites which cause problems with protein extraction and proteomic procedures. The existence of secondary metabolites displays species/tissue- specificity and varies with age or developmental stage. Secondary metabolites can severely affect the performance of protein extraction and separation. For example, phenolics can build irreversible complexes with proteins, and the oxidation of phenolics by phenoloxidases and peroxidases can cause streaking on 2-DE gels. Pigments, polysaccharides, and lipids can also cause severe disturbances in 2-DE gels. For total protein extraction, an ideal protocol should reproducibly capture all the protein species in a proteome with low contamination of other molecules. The extraction procedure must be optimized for plant species, tissue, or cell compartment. The three main extraction protocols commonly employed for plant proteomics are (1) TCA/acetone precipitation, (II) phenol extraction and (III) TCA/acetone/phenol extraction.

TCA/acetone extraction: The method is based on protein denaturizing under acidic and/or hydrophobic conditions that help to concentrate proteins and remove contaminants. TCA/acetone extraction involves the cleanup of tissue powder with 10% TCA/acetone, which is usually more effective than either TCA or acetone alone. Plant tissues are rich in proteases, which cause the proteolytic degradation of proteins and the loss of high molecular weight proteins. However,

homogenizing plant tissue in TCA/acetone almost immediately inactivates proteases and precipitates proteins. Also, TCA/acetone extraction is valuable for removal of interfering compounds and the enrichment of very alkaline proteins such as ribosomal proteins from total cell lysates. **Limitation:** The resulting pellets from TCA/acetone precipitation may be difficult to dissolve.

Phenol-based extraction: In this method, plant tissue powder is extracted in the extraction buffer/buffered phenol (pH 8.0) and followed by methanol (or acetone) precipitation of phenol phase. Phenol dissolves proteins (including membrane proteins) and lipids leaving water-soluble substances (polysaccharides, nucleic acids, etc.) in the aqueous phase, thus proteins in phenol phase are purified and concentrated simultaneously by subsequent methanol (or acetone) precipitation. In the case of tissues containing high levels of phenolic compounds pulverizing plant tissues with PVPP can help to remove phenolic compounds. The important points for successful use of phenol extraction are (i) keeping samples at low temperature during the first extraction step and (ii) careful recovery of the phenolic phase after centrifugation. Phenol can minimize protein degradation resulting from endogenous proteolytic activity. Many studies showed that phenol extraction gave satisfactory results in recalcitrant plant tissues rich in components which inhibit electrophoresis. Overall, phenol protocol is more efficient than TCA/acetone precipitation. **Limitation**: Toxicity nature of phenol and more time-taking.

TCA/acetone precipitation/phenol extraction: This is integrated phenol-based extraction with TCA/acetone cleanup steps prior to protein extraction. The protocol holds the merits of both TCA/acetone precipitation (for removal of phenolics, lipids, and pigments) and phenol extraction (for removal of phenolics, lipids and pigments, polysaccharides, nucleic acids, and salts). In the TCA/acetone/phenol extraction protocol, tissue powder purged by TCA/acetone is used as the starting material in phenol extraction of proteins. The protocol has been proved to be very effective to handle with recalcitrant tissues and provides enhanced 2-DE-based proteomic analysis of plant tissues. Although the TCA/acetone/phenol extraction is time consuming and laborious compared to both the TCA/acetone precipitation and the phenol-based extraction, it has the potential to generate high-quality protein samples for some kinds of recalcitrant plant tissues.
Limitations: Possible protein loss due to multiple cleanup steps.

2. Separation and detection of proteins:

There are two preferred methods for separation of complex protein or peptide samples: (1) two-dimensional (2-D) gel electrophoresis; and (2) liquid chromatography (LC).

Two dimensional gel electrophoresis

Majority of proteome studies undertaken on plant samples have used two dimensional gel electrophoresis (2D-GE) for separation of proteins. This is mainly because of advances in isoelectric focusing (IEF) which now routinely utilize immobilized pH gradient (IPG) strips in the first dimension. This has significantly enhanced the ease and reproducibility of 2D-GE for proteomic analysis. The basic procedures and techniques for undertaking 2D-GE are available in many biochemistry books/ manuals. After the IEF has completed, strips are equilibrated in an SDS loading buffer and placed on top of a precast acrylamide gel. Once the sample has successfully been arrayed in the second dimension acrylamide gel, sample analysis requires a successful digestion, and peptide extraction to be undertaken. The basic procedure has been widely reported and used with many minor modifications. One of the underlying objectives of 2D-PAGE is the reproducibility and consistency of protein extractions. Many attempts have been made to improve extraction reliability and performance utilizing a variety of detergents, chaotropes and mechanical disruption techniques. Such optimization is often required due to the nature of different samples and the lack of completer solublization in non-denaturing homogenization buffers.

After 2D-GE the gels need to be stained for detecting proteins. Although several methods for protein detection have been reported, silver and Coomassie Brilliant Blue (CBB) staining methods are still the preferred methods for in-gel protein detection. Silver staining is ~ 10-fold

more sensitive than CBB, with a detection range of 0.1–1 ng. However, silver staining has problems such as inferior reproducibility, poor linear dynamic range, and non-quantitative negative-staining of some modified proteins, all of which complicate downstream quantitation and spot matching. Broad dynamic range fluorescent protein stains including SyproRuby™, Deep Purple™, and ruthenium II, which have detection sensitivities around 10–20 ng are promising, but expensive, alternatives to Coomassie and silver staining as general protein stains.

3. Identification of proteins:

Protein identification using mass spectrometry

Mass spectrometry is the most preferred technique employed for identification of proteins. The development and commercialization of two different "soft" ionization approaches, electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) enabled large macromolecules such as proteins to be analyzed either in flowing, liquid solution or in a dry, crystalline state, respectively. The importance of these developments was appreciated by the scientific community and in 2002 led to John Fenn and Koichi Tanaka sharing the Nobel prize in Chemistry. The utilization of MS for the identification of proteins has become the primary technique for large scale protein analysis in recent years. The choice of sample delivery methods and subsequent analysis are dictated by availability of hardware, costs and experimental objectives. As stated above the two most popular sample delivery devices currently being used in proteomics are MALDI and ESI. These sources are connected to various types of mass spectrometers, which further influence the type of data and quality produced. Both delivery methods share similarities as they rely on the processing of an isolated sample with cleavage agent such as trypsin and the resulting peptides are introduced into the mass spectrometer as peptide ions in the gas phase.

Matrix assisted laser desorption ionization

The most widely used method of sample delivery and analysis uses the MALDI source attached to time of-flight mass spectrometer (MALDI-TOF). This combination has been successfully employed in the proteomic identification of proteins. This method of analysis is best suited for gel-separated proteins producing samples of relatively low complexity. This approach uses the absolute mass of each peptide to produce a peptide mass fingerprint (PMF) of the protein, which is then used to search a database of theoretical peptide masses. The analysis of samples by MALDI-TOF to produce PMF data is relatively high-throughput technique but produces data of poorer quality when compared to MS/MS –type analysis. This has a direct impact on pattern-matching confidence scores and creates more ambiguity when interrogating a large database for a match. The delivery process relies on aromatic acid matrices (e.g. dihydroxybenzoic acid or hydroxycinnamic acid) mixed with a digested sample. The matrix transforms the energy from LAZER to the sample leading to a release of peptides from the matrix. The LASER desorbs the peptides from a solid dried sample into the gas phase for analysis by mass spectrometer. No amino acid sequence information is obtained by basic MALDI-TOF analysis. Matching of data is based on the similarity of the masses to the predicted patterns from an *in silico* digestion of the sequence database. In most MALDI-TOF instruments there are now techniques that provide some sequence information by a post-source decay (PSD) process. However, PSD will generally only provide sequence tags for three to four amino acids from 10-15 amino acids peptide. While these can be useful and provide an increase in matching confidences, they do not offer the same matching capabilities as authentic MS/MS spectra from a tandem mass spectrometer.

More recently, MS/MS capabilities have been made available with the coupling of the MALDI source to tandem mass spectrometers allowing full-peptide fragmentation. The MALDI source has commonly been attached to a tandem mass spectrometer with a TOF component due to synergistic reasons involving timed points of ionization and the pulsing nature of the TOF (e.g. TOF-TOF and Quadrupole-TOF (Q-TOF), but more recently has also been coupled to Ion trap (IT) mass spectrometers. This arrangement of MS components provides the advantages of sample throughput and high-confidence matching of MS/MS spectra into a single system. Moreover, these systems now have capacity to deal with complex lysates with the development

of LC-MALDI applications allowing chromatographic separations to be directly spotted onto MALDI plates. One of the considerable advantages of this technique is the ability of lockdown samples providing the capacity to return to a plate for a subsequent detailed analysis of a particular spot of interest. The LC-MADLI setup appears to be providing an excellent means of obtaining proteomic depth and protein coverage when compared to other methods. These systems thus provide significant advances in the large-scale analysis of complex systems with unambiguous matching capabilities, high-throughput applications and the capability to analyse complex samples.

Electrospray ionization

The ESI source is commonly used to analyse complex mixture and is capable of interfacing with a wide range of mass spectrometers that usually provide MS/MS capabilities. ESI is an excellent method for the ionization of a wide range of polar molecules and relies on the sample of interest being dissolved in a solvent and delivered to the source in a thin capillary. A high voltage is to the tip of the capillary, producing a strong electric current which causes the emerging sample to be dispersed into an aerosol of highly charged droplets. These droplets are vaporised by an inert gas such as nitrogen-releasing charged species for analysis by the mass spectrometer. Whatever the arrangement of MS components that is utilized, this approach relies on the fragmentation of each peptide in some sort of collision cell within the mass spectrometer yielding a fragmentation spectra or MS/MS spectra. Each MS/MS spectrum can then be interpreted individually to suggest a probable amino acid sequence (de novo sequencing) or can be used to search a database containing theoretical MS/MS spectra to suggest both an amino acid sequence and probable identity of the original protein. The analysis of sample using ESI can readily be achieved manually using a syringe apparatus often provided with the system, but by far the most convenient and sensitive procedures involve a high-performance liquid chromatography (HPLC) connected to the ionization source. This provides sample automation and higher throughput but also has an added level of complexity. Samples are usually injected onto a reverse phase HPLC column such as C18 allowing the concentration and separation of peptides prior to analysis by the mass spectrometer. This analysis produces MS/MS data from the fragmentation of a parent ion (peptide) in the process termed collision-induced dissociation (CID). These CID spectra are high –quality data for pattern matching and interpretation of peptides.

Data analysis:

Peptide mass fingerprints: The ability to rapidly screen many hundreds of gel-separated samples at relatively low costs still makes PMF-based analyses a practical route. Since no sequence data are obtained (unless using PSD), data matching is heavily reliant upon mass accuracy and sequence availability. Most modern TOF mass spectrometers provide a high mass accuracy (±10-30 ppm) which is a crucial aspect to the analysis of PMF data. Thus the real limitation to using PMF in proteomic studies is sequence availability.

Peptide fragmentation data (MS/MS): The use of MS/MS data produced by a mass spectrometer capable of targeted peptide fragmentation provides an unsurpassed method of data matching and analysis in species with both a well characterized genome and those lacking genomic data. The data format provides the mass of the analysed peptide as well as some fragmentation data which can either be used directly for interrogation with a database using available software (e.g. MASCOT of SEQUEST) or can be deconvoluted to provide some sequence information (*de novo sequencing*), which can then be used to match to the sequence databases using the BLAST. The use of either method provides a far greater confidence in the resulting match, but just as with PMF matching, the final interpretation still requires some level of assessment.

Application of plant proteomics

Proteomics is a vital component of functional genomics and systems biology. Proteins being the actual functional entities of the biological systems their in-depth analysis using proteomics approach will be helpful in all the research areas aiming to have deeper molecular insights into

the complex metabolic processes influencing plant growth, development, biomass potential and their interactions with environment.

Metabolomics

Metabolites represent a diverse group of low-molecular-weight structures including lipids, amino acids, peptides, nucleic acids, organic acids, vitamins, thiols and carbohydrates, which make global analysis a difficult challenge. It is estimated that more than 100,000 secondary metabolites are produced by plants, while the total number is estimated to exceed over 500,000. To assess this diversity of structurally complex chemical compounds, various approaches have been initiated largely due to the tremendous advances in the instrumentation and data handling capabilities. The comprehensive assessment of endogenous metabolites and attempts to systematically identify and quantify metabolites from a biological sample is known as metabolomics.

Methodologies for metabolomics

Sample preparation: With the exception of nondestructive nuclear magnetic resonance (NMR)-based, nevertheless still have certain limitations to application with plants, essentially all metabolomics approaches give us what is basically a 'metabolic snapshot' which is specific to the time of sampling. This so-called 'point-in-time-chemistry', provides us with a hugely valuable metabolic insight but it is also important to bear in mind that failing to take full and proper care of the cultivation and sampling of the plants will result in potentially misleading and incorrect conclusions. Full consideration must be given to these temporal and spatial dynamics of plant metabolism when wishing to perform comparative analyses on contrasting samples. Producing and comparing metabolic profiles of any biological materials is always possible but determining the significance and biological relevance of the differences observed is a challenging task.

Extraction, separation and detection: Chemical complexity, metabolic heterogeneity, dynamic range and ease of extraction remain the main challenges in developing an effective metabolomics technology platform. Multiparallel technologies are required to gain the desired broad metabolic picture. Careful selection of suitable combinations of extraction, separation and detection protocols can however, lead to a rapid build-up of complementary biochemical data on the composition of biological samples (LC-MS, GC-MS, high-pressure liquid chromatography (HPLC)-PDA); (GC-MS, LC-MS); (HPLC-PDA, FT-ion cyclotron resonance (ICR)- MS); (CE-MS, CE-PDA)). Consequently, both polar/semipolar (e.g. methanol (MeOH)/water) and lipophilic (e.g. chloroform) extractions are usually analysed and, especially for plants, an additional analysis of the volatile components via solvent extraction (e.g. pentane) or through headspace extraction (e.g. solid phase (micro)extraction, SP(M)E) is often desirable. The main detection technologies are described as follows:

Mass spectrometry (MS): Mass spectrometry is the primary detection method of choice for plant metabolomics due to its sensitivity, speed and broad application. Depending upon the type of extract made, GC (gas chromatography) or LC (Liquid Chromatography) are most routinely used for metabolite separation before the samples pass into the mass spectrometer. Capillary electrophoresis separation is, however, gaining interest.

*Gas chromatography–mass spectrometry (GC-MS) i*s currently proving to be the most popular global analysis method. Popularity stems primarily from the robustness of both the separation and the electron impact spectrometry technique and the availability of some excellent deconvolution and metabolite identification software. Together, these can be used for complete metabolite identification and quantification. Running standards and comparing with commercially available compound databases can be used to confirm metabolite identity. Gas chromatography-MS is the principal technique for separation and detection of metabolites that are naturally volatile at higher temperatures. However, the technology is more broadly applicable to groups of nonvolatile, polar (mainly primary) metabolites, such as amino acids, sugars and organic acids, by converting these into volatile and thermostable compounds through chemical derivatization. These derivatized samples can then be analysed by GC-MS.

Liquid chromatography-MS is a particularly important additional, versatile technology for plants as it also provides a means to analyse many large groups of 'secondary' metabolites often present in plant tissues. Advances in chromatographic technologies (e.g. the ultraperformance liquid chromatography (UPLC) system from Waters Corporation, Milford, MA, USA) together with advances in column chemistry (e.g. hydrophilic interaction chromatography (HILIC) and long monolithic columns) are yielding significantly improved separation potentials. The technology is inherently restricted to molecules which can be ionized, either as positively or negatively charged ions, before moving through the MS. A range of possible techniques is available in order to ensure the ionization of a broad range of (LC-separated) metabolites (e.g. electrospray ionization (ESI); atmospheric pressure chemical ionization (APCI); photoionization (PI)). The high analytical precision of modern LC techniques combined with the high sensitivity and mass accuracy and resolution of MS systems, in particular time of- flight (TOF) and Fourier transform ion cyclotron resonance (FT-ICR) instruments, is proving very useful in the analysis of complex metabolite mixtures typified by plant extracts. Working in positive and negative ion modes can provide broader coverage of molecules which more readily either gain or lose a proton. Rather than performing separate analyses, some machines now have the capacity to switch continuously between positive and negative modes during each run.

Capillary electrophoresis is an alternative separation technology growing in popularity when combined with MS (CE-MS) for extra selectivity and sensitivity. The value of application with the less complex microbial extracts has already been clearly demonstrated and wider use with plants can be expected. High-resolution chromatographic separation and sensitive detection of water soluble extracts make a strong combination suitable for the analysis of a diverse range of primary and secondary metabolites.

Fourier transform-ICR-MS (abbreviated as FTMS) is a much discussed but, as yet, rarely used technology for plant metabolomic analysis. More recent applications are beginning to emerge. This technique has the advantage of providing more accurate molecular mass estimations through a high resolving power. Recently introduced orbitrap FTMS instruments offers not only faster and more sensitive analyses but also are significantly less expensive than the cyclotron FTMS machines are also worthy of attention. The technology is predicted to have a great future in the field of metabolomics, although for many the inability to separate structural isomers which have identical mono-isomeric masses is still seen as a significant limitation to its application. Linking up to some sort of prior isomeric separation strategy will be required to overcome this.

Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) seems currently to be the method of choice for medical metabolomics (metabolomics). For plants, its application has so far been limited because of its currently much poorer sensitivity compared to MS-based methods. Nuclear magnetic resonance analysis is a favored choice for the major metabolites but many other important compounds will typically be missed as they occur in plant extracts at levels below the usual NMR detection threshold. It does, however, have the advantage that it is a more uniform detection system and can directly be used to identify and quantify metabolites, even *in vivo*.

Application of metabolomics in plants:

Plant metabolomics has a potentially broad field of application in plant science in terms of monitoring crop quality characteristics or identifying potential biochemical markers to detect product contamination and adulteration. Metabolomics is expanding the general knowledge of plant materials and is illustrating how organisms function as integrated biological systems. Studies in plant biology involving the four different types of metabolite analysis (target analysis, metabolite profiling, metabolomics, metabolite fingerprinting) may be classified into following five broad research areas viz: (i) Bioengineering of metabolism, (ii) metabolic pathway analysis, (iii) deciphering the complex physiological processes, (iv) development of plant metabolomics methods, and (v) food science.

In bioengineering, metabolite analysis is typically restricted to only a few target compounds or select pathways are profiled to conclude the effectiveness of a certain treatment. If

metabolite profiling or target analysis is used, hundreds of lines involving thousands of analyses can easily be scrutinized to guide the bioengineering efforts because both sample purification and instrumental methods can easily be optimized for this task. The result of metabolomic analyses is a series of measurements of metabolite levels, that is, snapshots of metabolism. However, this is just one way to study the control of metabolism. Metabolic snapshot data are usually not sufficient to directly derive enzyme activities and hierarchical structures of pathways and, even more importantly, the dynamics of carbon partitioning between the different organs or the different metabolic cycles. A range of possible scenarios may explain a finding of altered metabolite levels: anabolic reactions might be faster, the catabolic fate might be different or transport activities may have been changed. For vascular plants, a highly suitable way to analyze fluxes is to use NMR-based techniques. Physiological adaptation to environmental stress has been the focus of several studies. Investigating metabolic relationships has been the focus of a physiological study on sink–source transitions in plant. Novel insights into the control of metabolism go hand in hand with improved methods. For this reason, various research groups validated the usefulness of novel methods or improved protocols by using model plant experiments. Metabolomics has also found application in control of metabolism is food science. Food quality is easily and rapidly deteriorated by a number of different pests, and therefore it must be tightly monitored to prevent major losses. Metabolomics based methods enable a rapid survey during food storage by observing metabolic effects for disease diagnostics rather than trying to understand the biochemical or physiological control mechanisms.

Systems biology approach seems to be very valuable in answering the complex issues related to plant growth, development and their interaction with environment. A natural shift is taking place in the approaches being adopted by plant scientists in response to the accessibility of systems-based technology platforms. Metabolomics is one such field, which involves a comprehensive non-biased analysis of metabolites in a given cell at a specific time. Metabolomics now plays a significant role in fundamental plant biology. Plants collectively produce a huge array of chemicals, far more than are produced by most other organisms; hence, metabolomics is of great importance in plant biology. Metabolomics has gained importance in biotechnology applications, as exemplified by quantitative loci analysis, prediction of food quality, and evaluation of genetically modified crops. Systems biology driven by metabolome data will aid in deciphering the secrets of plant cell systems and their application to biotechnology.

Fundamentals of Vector-Virus Relationships and their Implications for Management

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As obligate parasites, plant viruses need to move from infected to healthy plants in order to survive. This is achieved either by mechanical means or, in the case of most plant viruses, by exploiting biological vectors such as arthropods, nematodes, and fungi. Of the 700 or more plant viruses, about 70% are known, or suspected, to be transmitted by arthropod, nematode, or fungal vectors (Nault, 1997). The aphids (Aphididae) are by far the most important family among these vectors, transmitting many more viruses than whiteflies (Aleyrodidae), leafhoppers (Cicadellidae), or planthoppers (Delphacidae). The majority of reported aphid virus vectors belong to the genera *Myzus*, *Aphis*, *Acyrthosiphon*, and *Macrosiphum* in the subfamily Aphidinae. Aphid-transmitted viruses belong to 19 of the 70 recognized virus genera and comprise approximately 275 virus species (i.e. about 50% of insect-borne plant viruses) (Nault, 1997).

The cotton- or sweet potato whitefly, *Bemisia tabaci* (Gennadius), is one of the most devastating pests in modern agriculture today. The whitefly feeds on plants by inserting the stylets into the leaf and withdrawing sap from the phloem. Feeding by whiteflies causes extensive direct damage to crops through excessive sap removal, excretion of honeydew that promotes growth of sooty mold fungi, and by inducing systemic disorders such as leaf silvering in squash and irregular ripening in tomato. However, the most devastating damage induced by whiteflies is due to virus transmission. Despite the large number of whitefly species, only three, *B. tabaci, Trialeurodes vaporariorum* and *T. abutiloneus* are known as vectors of plant viruses. *B. tabaci* is the most important of these three and has been demonstrated to vector over 150 different viruses in the tropics and subtropics, most of which belong to the *Begomovirus* genus.

Virus–Vector Interactions

Virus transmission by an aphid (or any other vector) involves the transfer of virions from infected to healthy plants. The transmission cycle comprises up to four phases:

1. *Acquisition* – the process by which the vector takes up virions from an infected plant;

2. *Retention* – the carriage of virions at specific sites in or on the vector;

3. *Latency* – an inability to inoculate immediately following acquisition (the vector is able to transmit the virus after the 'latent period' (LP) has passed); and

4. *Inoculation* – the release of retained virions into the tissues of a susceptible plant in such a way that they are able to establish a new infection.

Based on the period of retention by the vector, virus transmission by aphids and whiteflies has been divided into three major categories:

1. *Non-persistent transmission* – where acquisition and inoculation require only very brief stylet penetration (< 1 min). There is no LP and the entire transmission cycle may therefore be completed within a few minutes. Viruses transmitted in this way have also been referred to as 'stylet-borne' and aphids rapidly lose the ability to inoculate them following acquisition (e.g. *Potato virus Y* (PVY)).

2. *Semi-persistent transmission* – where efficient acquisition and inoculation requires longer periods of plant access than for nonpersistent viruses, often at least 15 min. There is no LP and the aphids retain the ability to inoculate for longer periods, and may continue to transmit for up to 2 days following acquisition (e.g. *Citrus tristeza virus* (CTV)).

3. *Persistent transmission* – where virus acquisition and inoculation require relatively long periods of plant access. A LP occurs between acquisition and inoculation, but once it has passed, the vector can remain infective for life (e.g. *Potato Leaf Roll Virus*-PLRV, *Potato Apical Leaf Curl Virus*, PALCV.

These categories are properties of the relationship between viruses and their vectors, but are often be used to describe the viruses. A given virus is always in the same category, regardless of

its vector. A fundamental distinction regarding the mode of transmission is whether virions are circulative or non-circulative within the aphid vector. All viruses transmitted non-persistently and semi-persistently are non-circulative, residing on or in the aphid stylets and foregut, whereas those transmitted persistently are known as circulative, as they pass from the gut into the haemocoel and then to the accessory salivary glands (ASGs) before they can be inoculated. Persistent viruses may be propagative, i.e. able to multiply in the aphid, but are mostly non-propagative.

Aphids as Effective Virus Vectors

Certain characteristics of aphids predispose them to being effective virus vectors. Host selection and feeding behaviour are particularly important factors in virus epidemiology, and the host range and life cycle characteristics of aphid species are also key in determining the rate of spread of viruses. A variety of sensory stimuli may influence aphid host-selection behaviour before plant contact and stylet insertion. However, aphids attempt to make brief (< 1 min) stylet insertions ('probes') as a behavioural reflex that follows tarsal contact with any solid surface (Powell *et al*., 1999), even when repellent or deterrent cues are present. The aphids' propensity for landing on green surfaces and initiating probing behaviour helps explain their extraordinary capacity for transmitting plant viruses. The aphid stylet bundle comprises two pairs of long stylets that taper to a sharp point at the tip. The two inner, maxillary stylets are tightly interlocked by ridges and grooves, forming the food canal (FC) and salivary canal (SC). Although the FC and SC are distinct canals for almost the entire length of the aphid stylet bundle, they merge to form a common duct (CD) at the maxillary stylet tips (terminal 2–4 μm region). The outer pair of 'mandibular' stylets plays an important role in physical penetration of plant cell walls, but it is the maxillary stylets that enter plant. The maxillary FC, SC, and CD are therefore the channels through which aphids acquire and inoculate virions. Probing behaviour is a particularly important feature of host-plant selection by aphids, because it provides a means of assessing internal plant chemistry (Powell *et al*., 2006). The stylets apparently lack chemoreceptors, and aphids need to ingest plant sap through the maxillary FC to the pharyngeal area of the foregut to allow chemosensory assessment using a gustatory organ. Aphids usually initiate stylet penetrations in the anticlinal grooves between epidermal cells and much of the stylet pathway remains intercellular (apoplastic) (Tjallingii and Hogen Esch, 1993). However, DC-EPG reveals that aphids regularly puncture the symplast during their host selection, phloem location, and feeding behaviours. Even initial epidermal probes, lasting less than 30 s, usually include a brief (5–10 s) maxillary puncture of one cell. Such brief cell punctures always include ingestion of a cytosolic sample presumably allowing chemosensory evaluation *via* the gustatory organ in the foregut. Virions of non-persistent viruses are acquired and inoculated during these brief epidermal cell punctures. Aphids typically make several brief probes following contact with a new plant and, regardless of whether the plant is a host or non-host species, this behaviour is often followed by plant rejection (departure). Alate aphids will then often make a short flight before landing on and probing another plant. Flight and landing/ probing continue to alternate as antagonistic reflexes, leading to brief probes on a series of plants. If one of the plants in the series is already infected with a non-persistent virus, the result may be very rapid spread to one or more plants visited later. In newly inoculated plants, virions delivered into cells multiply and spread to neighbouring cells through plasmodesmata and eventually become systemic *via* the phloem, infecting the whole plant (Carrington *et al*., 1996) and providing a new source of virus inoculum and further spread. The virus acquisition and inoculation processes occur as a direct consequence of various stylet activities that enable aphids to select appropriate host plants, suppress plant defensive processes, and extract nutrients. Depending on the mode of transmission, virions may adhere to the cuticular lining of the stylets and the foregut, or be ingested and pass through the gut into the circulatory system and salivary glands and then be inoculated *via* salivation.

Whitefly, *Bemisia tabaci* as Effective Virus Vector

All whiteflies studied are obligate phloem-feeders *Bemisia* feeds primarily on phloem in minor veins, which they usually access from the abaxial leaf surface. Whiteflies ingest sap specifically from sieve elements which comprise the sieve tubes. Adult whiteflies also ingest sap from the plant's other transport system: xylem. In aphids, this behaviour is believed to occur primarily when the insects are dehydrated and has been referred to as "drinking" (Spiller et al. 1990). Adult whiteflies are not known to engage in significant ingestion of any plant cells other than phloem sieve elements and xylem tracheary elements. To initiate feeding, an adult whitefly first lowers its labium and usually rubs the apex of the labium over the leaf surface. It then presses the apex against the leaf surface, secretes a small amount of sheath saliva (the salivary flange), and begins to insert its stylets. Stylet penetration usually is initiated in the anticlinal grooves between adjacent epidermal cells (Freeman et al. 2001). The stylet pathway from the leaf surface to the phloem and xylem is primarily intercellular. The stylets are remarkably flexible and weave their way around and between cells from the plant surface to their target: a phloem sieve element, or a xylem tracheary element, if the insect is dehydrated. In aphids, there are three variations of intercellular penetration: through the middle lamella between adjacent cell walls, between adjacent parallel cellulose lamellae within cell walls, and between the cell wall and the plasmalemma (cell membrane). The latter is referred to as *intramural* penetration because it occurs on the inner side of the cell wall but external to the cell (Tjallingii and Hogen Esch 1993). Scanning electron microscopy (SEM) of fractured leaf sections indicates that in mesophyll, whitefly stylet penetration between adjacent cell walls and through intercellular air spaces is very common. Although the stylet pathway to the phloem is primarily intercellular, plant cells are periodically punctured along the way. This phenomenon has been best studied in aphids, which like whiteflies, also penetrate the plant in mostly an intercellular route on their way to the phloem. Aphid stylets frequently make deviations from their intercellular route to make brief (usually <10 s) intracellular punctures with their maxillary stylet tips. Upon withdrawal of the stylet tips, the cell plasmalemma spontaneously seals over the puncture site and the punctured cell seems little affected (Tjallingii and Hogen Esch 1993). During these brief intracellular punctures, aphids secrete a small amount of watery saliva into the cell and then suck up a small volume of cell sap, presumably for sensory evaluation by the precibarial chemosensillae. It is during brief intracellular punctures, especially those near the beginning of a stylet penetration, that non-persistent viruses are transmitted by aphid vectors. In contrast to aphids, intracellular punctures by whiteflies are much less frequent, and generally occur only after the stylets have penetrated deep into the leaf tissue (Johnson and Walker 1999). Furthermore, salivation and ingestion behavior during intracellular punctures has not been detected in whiteflies. The difference in the frequency and behaviours occurring during these intracellular punctures may explain why the great majority of vectors of non-persistently transmitted plant viruses are aphids and not whiteflies (Nault 1997). Although there is conflicting evidence regarding the mode of penetration through the epidermis, all studies, SEM and EPG, are in agreement that once past the epidermis, the pathway is primarily intercellular until the sieve element is penetrated.

Penetrating the leaf and locating a sieve element is a time-consuming and often unsuccessful process. After landing on a leaf, a whitefly usually makes many stylet penetrations before successfully locating a sieve element (Jiang et al. 2001). Salivary sheaths often have many branches indicating probes in multiple directions before the insect successfully locates a sieve element or gives up and withdraws its stylets. The average time interval from the beginning of a successful probe to the initiation of phloem phase (sieve element salivation and ingestion) varies from 16 to 42 min in various whitefly/plant species combination. Considering that many unsuccessful probes usually precede the first successful probe, it may take an hour or more after landing on a leaf before the whitefly adult begins ingesting phloem sap. After penetration of a sieve element, watery saliva is secreted into the sieve element for a period of up to several minutes before the onset of ingestion (Jiang et al. 2001). The function of this salivation has been hypothesized to "condition the sieve element" for ingestion; specifically to prevent, or reverse,

the sealing response by the penetrated sieve element. Phloem-feeding insects such as whiteflies and aphids have to avoid triggering these plant responses or reverse them if they occur, and salivation into the sieve element seems the most likely way that this occurs. Secretion of watery saliva into the sieve element occurs not only prior to the onset of ingestion, but periodically ingestion is interrupted by bouts of salivation. Nymphs of greenhouse whitefly can apparently remain feeding from the same sieve element, alternating between ingestion and presumed salivation, for an entire instar without ever withdrawing their stylets. While adult whiteflies always precede phloem sap ingestion with salivation , nymphs often begin ingestion almost as soon as the sieve element in penetrated.

Transmission Modes of Plant Viruses by Insect Vectors

A) Non-persistent and Semi-Persistent Transmission

i) Non-persistent transmission

Approximately 75% of viruses with aphid vectors are transmitted in a non-persistent manner. Non-persistently transmitted viruses cause a number of important diseases in cultivated plants and belong to six genera. These viruses are usually distributed in many tissues of their host plants, including the epidermis, and they cause stunting, leaf distortion, vein clearing, vein necrosis, leaf mosaic and mottling, and in some cases, flower breaking. All non-persistent viruses are readily transmitted mechanically under laboratory conditions, and some are seed-borne (e.g. *Lettuce mosaic virus* (LMV) and *Cucumber mosaic virus* (CMV).

Non-persistent transmission is virtually exclusive to aphids and acquisition and inoculation occur optimally during very brief stylet penetrations (< 1 min). During these probes, aphid stylets penetrate the epidermal layer, where virus concentration can be high. As the stylet insertion period increases beyond approximately 30 s–1 min (representing penetration beyond the epidermal cell layer), the subsequent transmission rate decreases rapidly. Pre-acquisition fasting of aphids for 15 min to 4 h can result in increased transmission efficiency (Hull, 2002). Aphids lose their infectivity after moulting since the stylets and cuticular lining of the foregut are shed with any retained virions. The aphids remain viruliferous for only a few minutes to hours, and lose the ability to inoculate more rapidly when given access to a plant (irrespective of whether it is a host for either the aphid or virus) than when. Although the rate of loss tends to be exponential in fasted aphids, it is positively correlated with temperature. In a few cases, aphids can retain their vectoring ability for a longer period if they are not feeding on plants, for at least 18 h in the case of *Maize dwarf mosaic virus* (MDMV).

The retention of virions in the aphid foregut, particularly the maxillary FC indicates that non-persistent viruses are acquired *via* ingestion during maxillary puncture of an epidermal cell. Localization studies have focused particularly on potyviruses, where a virally encoded helper component (HC; or 'helper factor' or 'aphid transmission factor' – reviewed by Pirone and Blanc, 1996) accessory protein is required for transmission. Present evidence suggests that the CD and FC of the maxillary stylets and the cibarium are the sites where virions of potyviruses are attached to the cuticular surfaces of the stylets. However, virions are particularly associated with the distal third of the maxillary stylets. HC acts as a bridge to facilitate binding between a putative aphid cuticular receptor (ACR) and the viral capsid-protein (CP) subunit (Raccah *et al*., 2001). With cucumoviruses, such as CMV, there is no viral HC and only an ACR– CP bridge exists (Perry, 2001). Attached virions provide the inoculums for viral infection of healthy plants. In common with the acquisition process, inoculation of virions requires maxillary puncture of the plasma membrane. Virions are therefore released very rapidly (within 2 s) after puncture of the plasma membrane. However, the inoculation mechanism has been the subject of controversy and two hypotheses have been advocated, presenting alternative scenarios. The 'ingestion–egestion' hypothesis suggests that virions carried in the maxillary FC and more proximal areas of the foregut are expelled into the inoculated cell during egestion (regurgitation). Martin *et al*. (1997) have put forward an alternative ('ingestion–salivation') hypothesis whereby virions are inoculated *via* salivation. They pointed out that the anatomy of the aphid stylets, with the FC and

SC merging near the maxillary tips (forming the CD), provides a point of convergence where retained virions are exposed directly to the flow of saliva.

ii) Semi-persistent transmission

Viruses transmitted by aphids in a semipersistent manner occur in five genera (Nault, 1997). Similar to the non-persistent viruses, some semi-persistent viruses may be acquired within minutes, but the efficiency to inoculate virions subsequently to a new host increases with the length of acquisition access. Efficient virus acquisition appears to be a function of feeding rather than probing. In common with non-persistent viruses, semi-persistent viruses do not require a LP in the vector, are not present in vector haemolymph, and cannot be transmitted after injection into the vector haemocoel. Infectivity may be retained for a few days but it is lost after moulting. However, unlike non-persistent viruses, pre-acquisition fasting does not affect transmission efficiency. Using the DC-EPG technique, Palacios *et al*. (2002) showed that the principal vectors of CaMV (*Myzus persicae* – peach–potato aphid and *Brevicoryne brassicae* – cabbage aphid) are able to acquire virions during brief punctures of epidermal cells. However, the efficiency of acquisition increased significantly if aphids reached a phloem sieve element and ingested phloem sap for at least 15 min on CaMV-infected plants. Other semi-persistent viruses may be limited to the phloem (particularly closteroviruses; Hull, 2002), and their acquisition therefore may have a more rigid requirement for phloem sap ingestion. The transmission characteristics of semi-persistent viruses suggest the functional retention of a much larger number of virions than for non-persistent viruses. Harris and Harris (2001) pointed out that the surface area in the proximal part of the maxillary FC and the pre- and post-cibarial area is extensive, providing for the binding of a large number of virions. Transmission electron microscopy has indicated that semi-persistent viruses are retained in the aphid foregut, whereas Lopez-Abella *et al*. (1988) suggested that they may use two binding sites, one in the aphid stylets and another in the foregut. The exact location of cuticular receptors for semi-persistent viruses in aphids remains unclear and, as with non-persistent viruses, retention of virions at particular sites does not prove that they are functional in the transmission process. It is widely accepted that semi-persistent virus transmission occurs *via* an ingestion–egestion mechanism (Harris and Harris, 2001). However, further research is required to determine when aphids egest during plant penetration.

As all non- and semi-persistent viruses have a simple structure with their nucleic acid (RNA or DNA) encapsidated in simple virus particles (Hull, 2002), it is the CP that is available for interaction with cuticular surfaces of the aphid stylets and foregut. Two forms of interaction have been identified between the CP and aphid retention sites: direct interaction (called the capsid protein strategy), and indirect interaction (the helper component(s) strategy) in which one (e.g. potyviruses) or two (e.g. caulimoviruses) non-structural virus-coded protein(s) are involved. Aphid transmission of potyviruses involves the so-called helper strategy. In addition to virions, the acquisition by aphids of a virus-coded helper protein is required for successful potyvirus transmission. For this reason, aphids cannot transmit purified potyviruses unless they have previous or simultaneous access to HC. The viral HC-Pro (to denote the dual functional role of HC in both aphid transmission and proteolysis) has been identified in many potyvirus–host-plant systems and seems to be a general phenomenon. Potyviral HC appears to act as a link between virions and aphid mouthparts, commonly referred to as the 'bridge' hypothesis (Pirone and Blanc, 1996).

iv) Vector specificity in non- and semi-persistent viruses

'Vector specificity' refers to the degree of interdependence between a specific virus and a particular aphid vector species. As would be expected, the vector specificity of nonpersistent viruses, because of their relatively transient relationship with the vector, is lower than that of the other aphid-borne viruses. Aphids acquire and inoculate non-persistent viruses during brief probes, and many different aphid species (including some that are unable to feed on virus host plants) may be vectors. A given virus may be vectored with varying efficiency according to the aphid species, clone, or biotype. Virus strains may differ in the efficiency with which they are transmitted by a particular aphid species. For non-circulative viruses with a helper strategy, it is

likely that helper proteins play a very important role in vector specificity. It was shown that in the case of potyvirus transmission, specificity was conferred by HC and transmissibility was highly correlated with retention of virus in the stylets. In the P2 helper component of CaMV, changing a single amino acid residue can alter the spectrum of aphid-vector species.

B) Persistent transmission

Persistent viruses are usually, but not exclusively, confined to the phloem tissue (sieve elements and companion cells) of their host plants and cause symptoms such as stunting, leaf discoloration, leaf curl and leaf rolling. Such viruses either replicate (propagative viruses) or not (non-propagative viruses) within the vector.

i) Non-propagative circulative transmission of viruses by aphids

Non-propagative, aphid transmitted viruses belong mainly to the family Luteoviridae, which comprises four genera: *Luteovirus*, *Polerovirus (e.g. PLRV)*, *Enamovirus*, and *Umbravirus*. As luteoviruses are phloem limited, aphids need to ingest phloem sap in order to acquire them. The minimum recorded virus acquisition access period (AAP) therefore reflects the time taken for aphid stylets to reach the phloem, and has been determined as 15 min for *Barley yellow dwarf virus* (BYDV) and 1 h for *Potato leaf roll virus* (PLRV) (Taliansky *et al*., 2003). However, transmission efficiency of both viruses increases with a rise in the AAP to 2 or more days, as in the case of PLRV. The LP is at least 24 h and may extend to as much as 4 days. LP length depends on several factors such as the aphid species, virus concentration in the host plant tissue, and environmental factors such as temperature. An inoculation access period (IAP) of about 10–30 min is required for most virus species, but efficiency is higher when IAP is increased. Aphids retain the capability to transmit luteoviruses for several days, and in some cases throughout their life span. The interactions between luteoviruses and their aphid vectors have been reviewed by Gray and Gildow (2003). For successful transmission, virions have to pass at least two barriers: the gut wall and the salivary gland membranes. Virions first enter the aphid's body *via* the ingestion of phloem sap, then are transported across the gut wall to the haemocoel, and finally accumulate in the ASGs. The particles are delivered into the SC, from where they are injected into a plant during subsequent stylet penetration. Endocytosis and exocytosis at the two epithelial barriers (alimentary tract and ASG) involve specific interactions between virus CPs and receptors present in these two locations within the aphid.

Viruses transmitted by aphids in a persistent manner are usually vectored by one, or only a few, aphid species that colonize the virus host plants. The molecular interactions determining transmission and vector specificity have been researched in some detail, particularly with the *Luteovirus* species BYDV and CYDV. As aphids can acquire and transmit purified luteoviruses, it is likely that no additional proteins, other than the CP, are involved. Studies with different luteoviruses have suggested that both the CP and the minor CP (CPm) structural proteins play key roles in the aphid transmission process as point mutations in or near conserved domains of *Beet western yellows virus* (BWYV) CPm affect aphid transmissibility (Brault *et al*., 2000). There is good evidence that symbionin, a 60 kDa protein produced by the endosymbiotic bacterium *Buchnera* sp. and found in *M. persicae* haemolymph, interacts with PLRV and other *Polerovirus* species to assist virus retention in the haemolymph (van den Heuvel, 1999). Symbionin possibly mediates the longevity of virus particles by binding and protecting them from proteolysis. However, the binding of symbionin to virus particles or to P5 (the coat protein read through structural proteins) has only been demonstrated *in vitro*, and the exact role of symbionin in virus transmission has yet to be elucidated.

ii) Circulative Virus Transmission by Cotton whitefly, Bemisia tabaci

In potato, a variant of Tomato Leaf New Delhi Virus (ToLCNDV-[potato]) causes potato apical leaf curl disease and now it is one of the most important diseases of potato in India. The incidence of this disease was initially observed in western part of Uttar Pradesh state and later in Hisar district of Haryana. The virus was transmitted to potato from sponge gourd and tomato due to overlapping planting period and early planting of potato where the potato is exposed to high temperature and whitefly population. Now, this disease is widely spread almost in all potato

growing states (Jeevalatha et al., 2013). Most of the work in relation to the transmission of Begomoviruses has been done on the Tomato Yellow Curl Virus (TYLCV) and Tomato Leaf Curl Virus (ToLCV), related species of ToLCNDV-potato.

Following landing on a tomato plant, the stylets of *B. tabaci* find their way between the epidermal and parenchymal cells of infected plants before penetrating the vascular tissues and reaching the phloem the insect feeds on during virus acquisition and transmission. Single insects are able to acquire TYLCV and transmit it to tomato plants. The minimum acquisition access period (AAP) and inoculation access period (IAP) of Middle Eastern TYLCV isolates varies from 15 to 60 min and from 15 to 30 min, respectively; efficiency of transmission reaches 100% when 5–15 insects are used. Gender and age affect transmission ability (Czosnek et al. 2001). Once ingested by whiteflies, begomoviruses are not immediately available for infection. They need to translocate in the insect digestive tract, penetrate the gut membranes into the haemolymph, crossing the epithelial cells of the whitefly digestive tract which bridge between the gut lumen and the haemolymph. From there, begomoviral particles reach the salivary systems and finally enter the salivary duct from where they are egested with the saliva. Translocation of begomoviruses from the digestive tract to the haemolymph and from the haemolymph to the salivary gland is thought to be mediated by still unidentified receptors. It is likely that, during the transit of begomoviruses in their whitefly vector, the capsid is the structure that is exposed to the whitefly tissues and interacts with insect receptors and chaperons.

Vector specificity of geminiviruses is determined by the coat protein (Höfer et al. 1997) and there is no evidence for the involvement of other virus-encoded proteins in transmission. Loss of transmission by *B. tabaci* can be caused by a surprisingly small number of amino acid replacements in the CP of begomoviruses. Virions that cross the gut wall into the haemolymph on their way to the salivary gland face a particularly hostile environment. A GroEL homologue produced by the primary endosymbionts of aphids has been shown to play a crucial role in the transmission of luteoviruses. Similarly, endosymbiotic bacteria housed in the whitefly bacteriocytes seem to have a cardinal role in safeguarding begomoviruses in the haemolymph. As demonstrated for TYLCV, the GroEL homologue seems to bind to and protect begomoviruses from degradation in the haemolymph; disturbing the GroEL-TYLCV association leads to the degradation of the virus and to a markedly decrease in transmission efficiency of the virus (Morin et al. 2000). During begomovirus circulative transmission, it is likely that particles interact with whitefly proteins in order to move from the digestive tract into the haemolymph and from the haemolymph into the salivary system. A 16 kDa small heat-shock protein (named BtHSP16) belonging to the HSP20/a-crystallin family was shown to bind to the TYLCSV CP. The gene coding for BtHSP16 has an intron and occurs at a single locus in the *B. tabaci* genome. Full-length BtHSP16 is required for the interaction with the CP of TYLCSV.

Begomovirus replication in its vector remains a controversial issue. The persistence of TYLCV/TYLCSV in *B. tabaci* as infective entities for longer than the latent period, sometimes for the entire life of the insect, raises the question of replication of the virus in the insect. Accumulation of viral DNA in *B. tabaci* reared on a TYLCV-non host plant, after first feeding on plants infected with a TYLCV isolate from Egypt, has been interpreted as multiplication of TYLCV in its vector (Mehta et al. 1994).

Using PCR, Southern blot hybridization and transmission tests, it has been found that TYLCV is transmitted to the progeny of viruliferous insects with various efficiency. Moreover the progeny of viruliferous insects was able to infect tomato test plants. Dissection and analysis of the reproductive system of viruliferous whiteflies showed that both the ovaries and the maturing eggs contained TYLCV DNA (Ghanim et al. 1998). TYLCV can be transmitted between whiteflies of the B biotype in a sex-dependent manner, in the absence of any other source of the virus (Ghanim and Czosnek 2000). TYLCV was transmitted from viruliferous males to non-viruliferous females and from viruliferous females to non-viruliferous males, but not between insects of the same sex. Caging together *B. tabaci* and *Trialeurodes vaporariorum*, two whitefly species that do not mate, confirmed that mating is obligatory for TYLCV

transmission. It has to be noted that while TYLCV is found in the haemolymph of *B. tabaci* after feeding on infected tomato plants, the virus is ingested by *T. vaporariorum*, but it is unable to cross the gut/haemolymph barrier (Czosnek et al. 2002) probably because the later insect does not possess the begomoviral receptors that allow virus to cross the gut wall.

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Farm Mechanization for Potato Production

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Potato cultivation has a series of time bound operations, where, mechanization plays an important role to perform various operations timely, precisely and cost effectively. Planting operation with planters helps in uniform crop rising with desired plant population of 80-85 thousand per hectare. Fertilizer drills, inter-row-cultivators, ridgers and plant protection machines are useful in saving time, cost in different field operations and protect crop from dangerous diseases like late blight of potato. Mechanical harvesting with the help of diggers reduces potato damage significantly. Uniform grading and seed treatment with boric acid can also be done using graders and seed treatment machine. This way almost all the operations in potato production can be completed with different equipment or machines and crop can be managed in short period without much loss in terms of cut and bruising of tubers and delay in marketing and storage.

Potato is one of the most important food crops in developed as well as developing countries. India is the second largest country in potato production after China. Potato cultivation is highly mechanized in developed countries due to labour scarcity and large farm size. In India, till late seventies most of the operations in potato cultivation were performed manually because of non availability of machines. It requires about 1600-2000 man-hours per hectare for complete manual cultivation. During eighties and nineties lot of research work was done for mechanization of potato crop. As a result of which, a number of good machines/equipment have been developed for potato cultivation to perform operations like land leveling, seed bed preparation, planting, weeding and interculture, plant protection, digging, grading and seed treatment. Timely field operations and handling of potato crop are important factors for improving quality and quantity in potato production.

Seedbed preparation

After burying green manure crop (usually *dhaincha*) with mould board plow or disc plow followed by one planking makes field conditions favorable for decomposition of green manure. After 5-7 days of pre-sowing irrigation, 2-3 cross harrowing or 3-4 tiller operations followed by two planking provides favorable soil condition for proper growth and tuberization of potato. All these equipment are easily available in the market and are used for other crops also.

Fertilizer application

Precise application of fertilizer at right place is an important factors for good production of any crop. In potato, fertilizer should be placed 5-7 cms below the seed tubers and should not come in direct contact with the seed. Fertilizer drill-cum-marker has been developed for basal application and band placement of fertilizer in split dosing. Band placement helps to improve efficient utilization of fertilizers which results in increased productivity. In case fertilizer unit is attached with the planter than the use of fertilizer drill can be avoided.

Planting

For good yield of potato crop, about 80-85 thousand plants per hectare are required. It can be achieved by planting 40-60 gm weight 35-40 quintal of seed tubers per hectare. Uniform plant emergence can be achieved by planting with tractor operated potato planters (Fig.1). These machines maintain uniformity of planting in terms of row to row (60 cms) and plant to plant (20 cms) spacing and depth (6-8 cms) of planting. Following type of planters are available in India:

a) Revolving magazine type,
b) Belt and cup type and
c) Automatic planter.

First two are semiautomatic type planters in which one person per row has to put seed tubers in to the seed metering cells of the machines. Seed tubers are dropped in lines and a ridge of soil mass is formed over the tubers. Automatic planters are becoming popular nowadays due to their

distinct advantages over the other types. All other activities like lifting individual tubers, dropping them into rows and than covering with soil mass are carried out by the machine itself.

Weeding, top dressing and earthing

Mechanical and chemical weed control methods are used in most of the crops nowadays. Potato being an underground crop is highly responsive to inter-row-cultivation. Operation of inter-row-cultivator-cum-fertilizer applicator is very popular for inter-culture operations (Fig.1). Function of the machine is to remove the inter-row weeds, apply second dose of fertilizer and makes favorable soil condition for better crop growth. Three row tractor operated inter-row-cultivator is genrally used about 22-25 day after planting or when plant height is 8-10 cms. After operating this weeding machine three or five row ridger is used for reforming the ridges. Ridger makes uniform ridges and accumulates sufficient soil mass around the plants. For light soils and low weed intensity areas another multipurpose machine is in use nowadays. With this machine operation of opening furrows, fertilizer application and re-earthing are completed simultaneously.

Plant protection

Potato crop needs to be protected against different disease carriers, insects and pests. Application of pesticides in potato crop is utmost important to manage sap sucking pest, foliage feeders and other insects. Many times delay in chemical spray can cause huge crop losses. Particularly, in case of late blight disease of potato, delay of 1-2 days can result in total loss of crop. Therefore, high capacity tractor operated sprayers are useful for timely and uniform application of plant protection chemicals. Spray gun is especially effective in case of late blight disease (Fig. 1). Multinozzle sprayers of 12-16 nozzles are also popular for spray uniformity and width of coverage in potato crop. For small holdings, manually operated knapsack sprayer can be used which has field capacity of 0.5 ha/hr.

Potato digging

Before starting harvesting operation haulms have to be removed manually or with chemical methods. Potato digging is a cumbersome process and involves a lot of manpower. About 600-700 man-hours are required for manual digging of 300-400 quintals of potato from huge soil mass of around 10,000 quintals. In manual harvesting spade or *khurpa* is used which results up to 10% tuber cut or bruise which is a huge loss to the farmers and nation. In manual harvesting some portion is always left behind in the field. Tractor operated diggers are the fast, economical and cause least damage to the produce. Digger elevator is an efficient machine which exposes 90-95% tubers in optimum field conditions (Fig.1). Multipurpose potato digger is less expensive equipment. It exposes about 80-85% potatoes and is easy to maintain. It can be used in early crop (60 days without cutting haulms) and also can successfully work in dry field conditions. It is also very useful for research field, where mixing of varities has to be avoided. Potato-sugarcane intercropping is a highly remunerating cropping system. But potato digging from this intercrop is a difficult task. Recently, a new digger has been developed at CPRI for potato-sugarcane intercropping. This equipment is helpful in digging potatoes without damaging sugarcane crop. Simultaneously, interculture of sugarcane crop is also done.

Fig. 1. (a) Field preparation using rotavator, (b) Planting operation with semi-automatic planter, (c) Interculture operation (weeder cum fertilizer applicator), (d) Ridging, (e) Spraying of chemical with gun sprayer and (f) Potato digger cum elevator.

Grading

Graded, sorted and properly packed potatoes always fetch good price in the market. Manual grading is not uniform and it requires a lot of time, manpower and energy which bring down the overall returns. For fast and uniform grading various manual as well as power operated graders have been developed and are bieng used by potato growers.

Seed treatment

To grow a healthy potato crop disease free seed is the most important requirement. For controlling tuber and soil born diseases viz. common scab, black scurf and powdery scab, 3% boric acid treatment can be given with the help of a motor operated seed treatment machine. In this machine boric acid is sprayed on the potatoes moving and rolling over a conveyor. All the

surfaces are coated with the chemical and the used solution is re-circulated. This machine can treat about 300-350 quintals of potatoes per day.

Different equipment and machines for mechanized potato production

S. No.	Machine/equipment	Capacity (ha/day)	Power Requirement(hp)	Approximate Cost (Rs)
1.	Fertilizer drill	4.0-5.0	25-35	7000
2.	Cup and belt type Planter	1.0-1.2	25-30	12000
3.	Revolving magazine type planter			
	(i) Two row	1.0-1.2	20-25	12000
	(ii) Four row	2.0-2.5	30-35	20000
4.	Automatic planter			
	(i) Two rows	2.0-2.5	20-25	20000
	(ii) Four rows	3.0-3.5	30-35	30000
5.	Intercultivator	3.0-3.5	25-30	6000
6.	Fertilizer applicator-cum-interculture	3.5-4.0	30-35	12000
7.	Triple purpose machines(intercultivator-cum-fertidrill-cum-ridger)	2.5-3.0	30-35	16000
8.	Power sprayer (tractor operated)	6.0-8.0	25-30	16000
9.	Digger			
	(i) Multipurpose	3.0-3.5	30-35	4500
	(ii) Digger elevator	1.2-2.0	30-35	25000
10.	Potato seed treatment machine	300-400q/day	2 hp motor	35000

Decision Support System for Potato Production

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Information Technology (IT) is the mantra of modern world. Today IT has pervaded each and every field of human endeavour including agriculture. Unlike medicine, engineering and commerce, its application in agriculture is much slower primarily due to paucity of IT trained manpower in this sector. The institute has so far developed six decision support systems, viz. Indo-Blightcast, The Potato Pest Manager (PPM), Potato growing period and yield calculator, Computer Aided Advisory System for Potato Crop Scheduling (CAASPS), Potato Weed Manager and VarTRAC. One EBook and a potato photographic database are also developed at Central Potato Research Institute Shimla.

Decision support systems:

1. INDO-BLIGHTCAST- A web based Pan India Model for forecasting potato late blight

Late blight is the most dreaded disease of potato causing annual crop loss of about 12 billion € globally. Its appearance and spread is highly dependent on environmental factors. Under favourable conditions its spread is so fast that it can wipe out the crop within a weeks' time. In India it is very serious in the hills where it occurs regularly but in the plains it may or may not appear and even if it appears its time of occurrence would vary. The time of its occurrence and severity determines the yield loss which may exceed 40% country wide in some years. Prevention through prophylactic sprays of recommended chemicals is the best option since once it appears it is very difficult to control. This, however, requires information on the likely time of appearance of the disease and hence the importance of disease forecasting.

INDO-BLIGHTCAST- is a web based forecasting model (http://cpri.ernet.in) developed to predict the first appearance of late blight disease using daily weather data of meteorological stations. This is an improvement over the JHULSACAST model, which requires hourly data of temperature, relative humidity and daily rainfall. The intensive data requirement as well as location specific calibration of JHULSACAST was a serious impediment to its wide spread use. The INDO-BLIGHTCAST, however, is applicable pan India, since, it is web based, it requires only daily weather and does not need local calibration for different regions. Hence it is more robust and its predictions are broader based.

INDO-BLIGHTCAST has two modules one for data entry and the other for the general users to see the status of late blight forecast.

Data entry: The data entry module is user and password protected. The registered users can "Load data"(for viewing already entered data), "Add data" (to save entered data), "Edit data" (to change entered data values) and "Delete data" (to remove data) if required, in addition to running the model. Check late blight appearance status: Through this button, any user (requires no registration) can select any location which would lead to another window with a calendar and map showing the location with default state map. The user can select a date in the calendar and click on the "Run model" button which would then display the status of late blight in a circle through colour. Green colour indicates that late blight is not likely to appear soon; yellow colour indicates that late blight would appear very soon; and red colour indicates that the weather conditions have become suitable for late blight and it can appear any time within fifteen days. Thus depending upon the time required for taking control measures, the user may start preventive measures at yellow or red colour indication. The model has been developed and tested using the data on late blight appearance monitored at CPRI regional stations and AICRP centres over the past several years. Presently the data is available for nine locations in Punjab, Uttarakhand, Uttar Pradesh, Bihar, West Bengal, Assam and Karnataka.

2. Potato Pest Manager

KNOWLEDGE BASE STRUCTURE

For the management of diseases and pests two aspects are involved. The first is to establish the identity of the disease/pest and second is to recommend appropriate preventive and management practices to control them. These objectives are achieved in a sequence of steps as discussed below

Step 1: The photographs showing the symptoms of the diseases/pests are arranged in a photo gallery and displayed in sequence. The user is asked to match the symptoms in the photographs with those he has seen in the field and select the most closely matching one.

Step 2: The appropriateness of the selected photograph needs to be confirmed, because the user may not be fully conversant with the symptoms of different diseases or damage by pests. Information about the biotic/a biotic factors prevailing, together with the symptoms, are necessary for a correct diagnosis. This is done through a set of confirmatory questions.

These are questions about the symptoms of the disease/damage by the pest, or conditions which need to be satisfied for the disease/pest occurrence. This information is arranged in a linear fashion. This arrangement allows insertion/deletion of questions/an option to a question at any level without disturbing the overall structure. Furthermore, this information is presented in a format of questions/statements to the user, while answers are given as options to these questions/statements.

Step 3: Once all the confirmatory questions are answered, the name of the disease/pest corresponding to the photograph selected is displayed along with confidence percentage. The confidence percentage is calculated based on answers given to the confirmatory questions relevant to disease symptoms/pest damage. Each confirmatory question/statement is assigned a certain value such that for all the questions if the option corroborating the disease/pest whose photograph is selected is chosen as the answer, the value adds up to 100.

However, the value allotted to each question may vary depending upon its significance.

Step 4: Many potato diseases/pests can only be controlled through preventive measures taken over a period of time before planting the crop and control is not possible once the disease/pest appears. This is especially the case with diseases/pests where symptoms are seen at/after harvest. Therefore, the preventive measure applicable to the disease/pest identified is displayed in this step. The preventive measures are the set of practices, which would have prevented/mitigated the disease/pest occurrence.

Step 5: In this step information required for suggesting control measures on the standing crop is obtained through a further series of questions. For example, information regarding severity of disease/pest damage, age of the crop, *etc*. is invariably required for deciding the chemicals to be used, their dosage, number of sprays *etc*. This information is again obtained from the user by presenting the questions or statements with various options. The questions or statements are arranged in tree structure and depending upon the answers given to each question a path is followed leading to a recommendation which is attached at the end node.

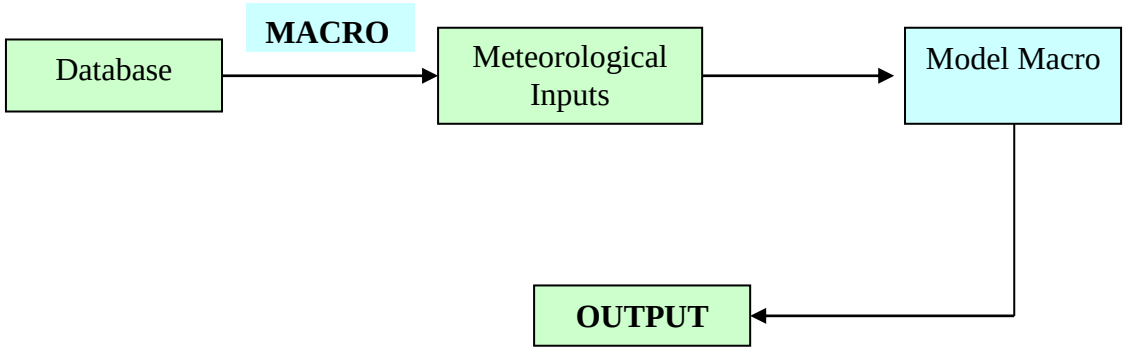
Step 6: This step displays the recommendation based on the options chosen.

This tool/DSS is web based and is developed in ASP.NET and the database is developed in Microsoft SQL. It can be accessed directly by the URL http://14.139.61.86/ppm_test/default.aspx or can be accessed from CPRI web site http://cpri.ernet.in.

3. POTATO GROWING PERIOD AND YIELD CALCULATOR

Potato is a short duration crop grown under diverse growing conditions. It is highly flexible in terms of time of planting as it can be planted early, at optimum time or late in the season. Similarly, as regards harvest time it can be harvested at any time after about 3 quarters of the growing season by which time economic yields are realized. Such flexibility makes it an ideal choice to fit in multiple cropping systems. To exploit this flexibility at any given location

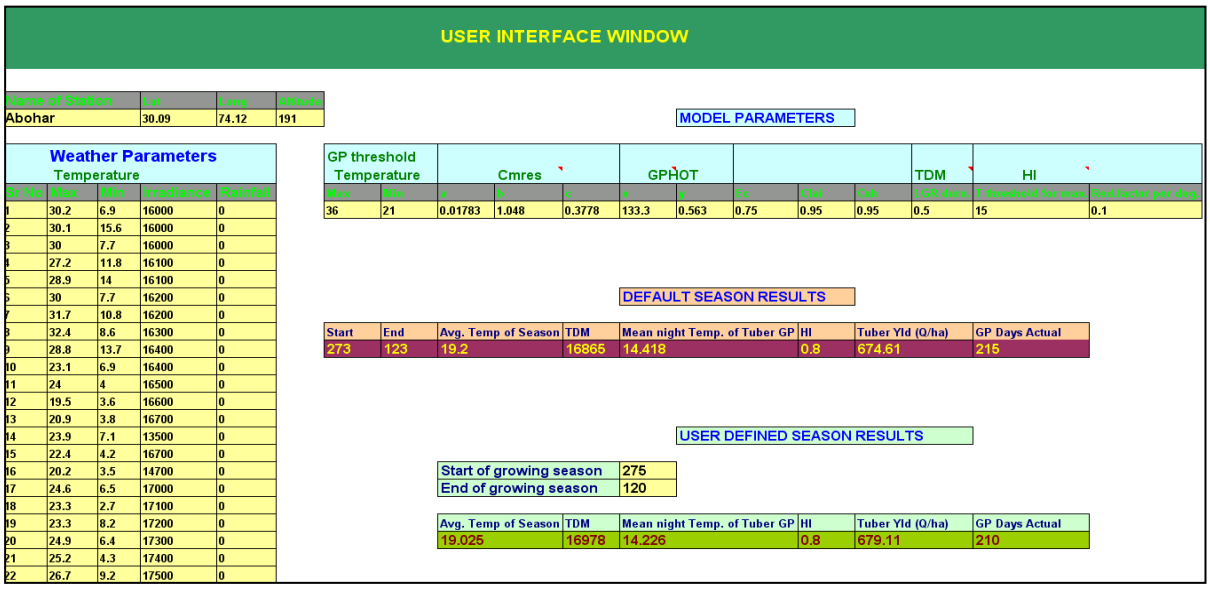
there is need to identify the growing season/s and the length of each growing season/s. Temperature is the primary determinant for potato under sub-tropical conditions of India. The temperature suitability criteria are that the maximum temperature should be less than 35 ºC, minimum temperature should be less than 21 ºC at least 3 weeks after the maximum temperature suitability criteria is met and it should also be more than 2 ºC to ensure frost free season. Identifying the suitable growing period based on these criteria is difficult and there is also a need to know the expected yield in the different season. A PC based tool was developed for delineating the potato growing seasons and the expected yield of any given season for any location as given in the flow chart below.



Methodology: A spreadsheet with macros was created to process the raw data and extract the information required by the model. Four fields each with 365 records were created in spreadsheet and macros for screening the day as suitable or unsuitable for potato based on threshold limits for maximum and minimum temperature was written. The starting day number, ending day number and the total number of suitable days of the longest period, where more than two growing seasons were obtained, were derived as outputs. Further the meteorological variables required by the model *viz*. mean temperature and mean irradiance of the growing season were derived from the data of the thermally suitable days for potato.

Macros for estimation of gross photosynthesis (GPHOT), maintenance respiration, linear growth rate *etc*. (Versteeg & vanKeulen, 1986) utilizing the outputs of the database screening macros were written to derive the expected potential yield.

A Graphical User Interface (GUI) was designed in MS Access/Visual Basic. Fields were created for display of location and its spatial features and also for display of the various outputs as shown below.



4. COMPUTER AIDED ADVISORY SYSTEM FOR POTATO CROP SCHEDULING (CAASPS)

The optimum time of planting, the most suitable variety and the expected yield at different dates of harvest are vital information required by farmers for scheduling their planting and harvesting times as well as for choosing the variety to be grown. Obtaining such information through field experimentation in the diverse agro-climatic conditions in which potato is grown in India is an uphill task, but this information can be derived from crop models which can simulate crop growth, development and yield with reasonable accuracy under diverse situations.

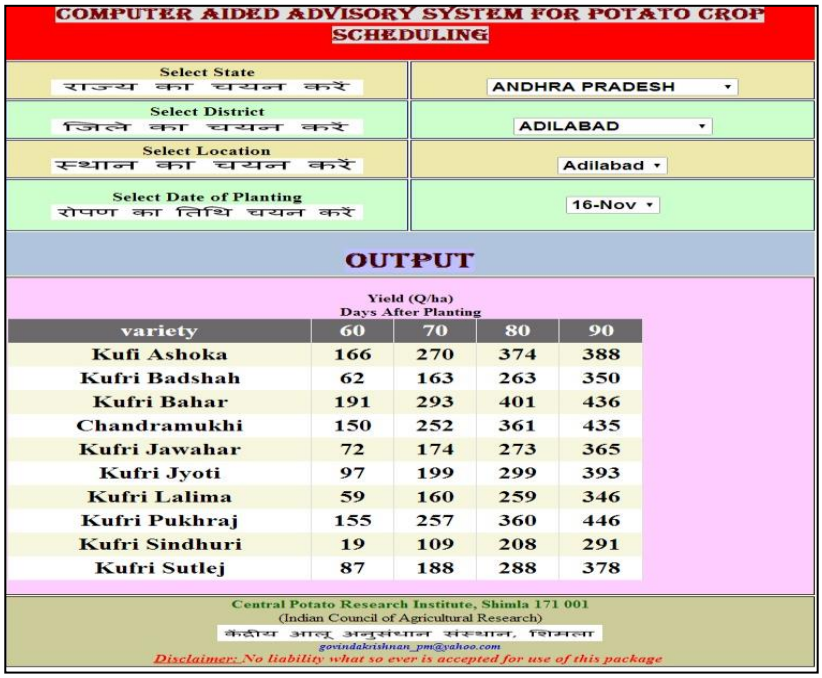
However, use of crop models requires extensive data inputs as well as technical expertise to handle the model. Therefore, world over, models are handled by researchers and off take of models by field level workers is not very satisfactory. Decision Support Systems (DSS) on the other hand provide a method for delivery of information in a user friendly and simple way. Therefore, this DSS "Computer Aided Advisory System for Potato Crop Scheduling (CAASPS)" has been developed with the following purposes:

a) To provide information on the expected yields of different varieties planted at different times to enable farmers to decide on the most suitable one for their respective locations.
b) To help decide the time of harvest based on yield accrued at 60, 70, 80 and 90 days after planting.
c) To indicate the varietal performance under different dates of planting and crop durations and thus help choose the appropriate variety.

This DSS consists of a database and a user interface. The database consists of state, district and location names along with Infocrop-potato model derived yield outputs.

The model outputs were derived as follows:

1) Weather database were created for important locations in India using MARKSIM weather generator.
2) Suitable thermal window were delineated for each location by defining screening rules for maximum temperature (< 35^0C) and minimum temperature (< 21^0C).
3) Infocrop-potato model was run for 5 planting situations starting from ten days earlier to the beginning of the suitable thermal window identified by the screening rules and staggered at 10 days interval.
4) For each date of planting, the model was run for 10 varieties under potential situations and 80% of the potential yield was taken as attainable yield.
5) Yield output of each variety at 60,70,80 and 90 days after planting were linked to orrespondinspatial attributes *viz.* state, district and location names in MS Access.



COMPUTER AIDED ADVISORY SYSTEM FOR POTATO CROP SCHEDULING

Select State राज्य का चयन करें	ANDHRA PRADESH
Select District जिले का चयन करें	ADILABAD
Select Location स्थान का चयन करें	Adilabad
Select Date of Planting रोपण का तिथि चयन करें	16-Nov

OUTPUT

Yield (Q/ha)
Days After Planting

variety	60	70	80	90
Kufi Ashoka	166	270	374	388
Kufri Badshah	62	163	263	350
Kufri Bahar	191	293	401	436
Chandramukhi	150	252	361	435
Kufri Jawahar	72	174	273	365
Kufri Jyoti	97	199	299	393
Kufri Lalima	59	160	259	346
Kufri Pukhraj	155	257	360	446
Kufri Sindhuri	19	109	208	291
Kufri Sutlej	87	188	288	378

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Disclaimer: No liability what so ever is accepted for use of this package

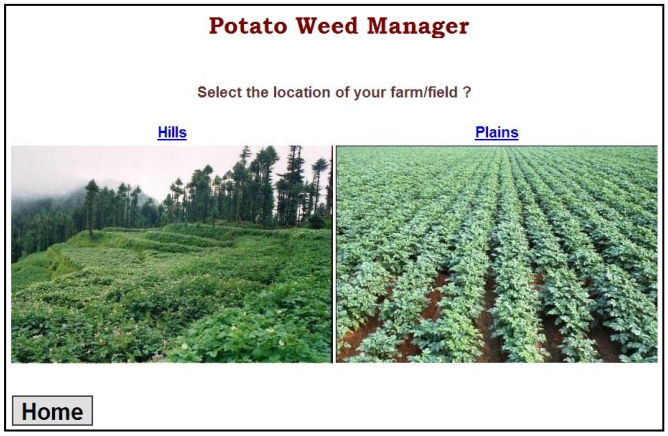
User interface: A simple query system was designed for querying the database.

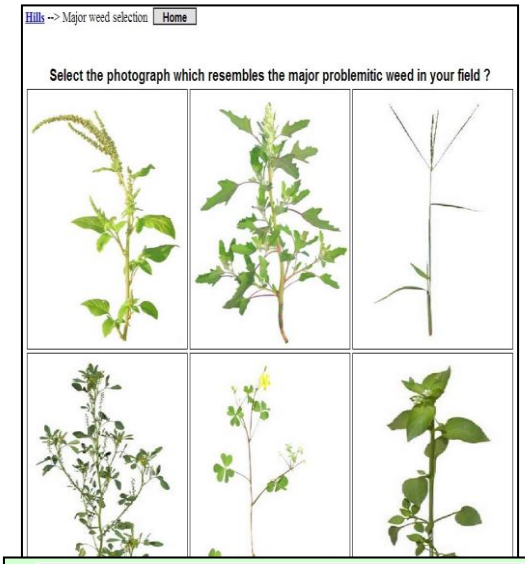
The user first selects the State, and then the Districts of the state. The locations within the district for which information is available are then displayed for selection of one of them.

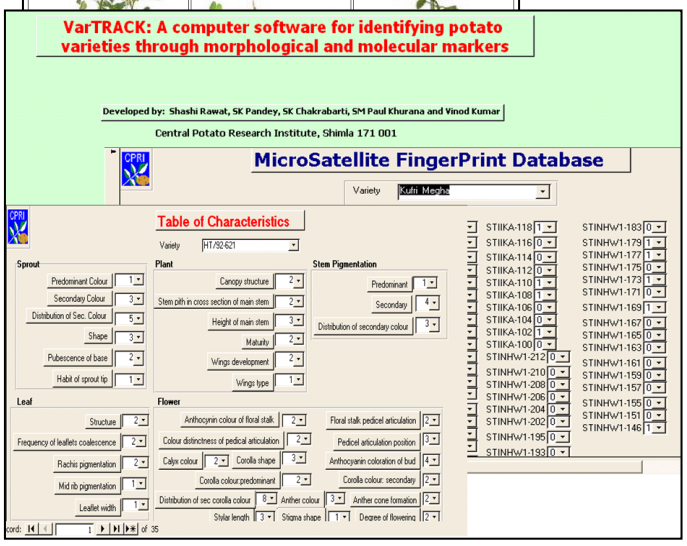
Once the location is selected, the five dates of planting for which model has been run for the selected location is displayed and the user is required to select one of them. When any of the dates is selected, the attainable yield data for all the ten varieties at five durations, corresponding to 60, 70, 80 and 90 days after start is displayed in tabular format.

This tool/DSS is web based and is developed in ASP.NET and the database is developed in Microsoft SQL. It can be accessed directly by the URL http://14.139.61.86/caasps/login.aspx or can be accessed from CPRI web site http://cpri.ernet.in.

5. **Potato weed manager:** Weeds cause enormous loss in potato production. Weeds in potato not only compete for moisture, nutrients, space and light but also harbour several pests and diseases as alternate hosts. Up to 80% reduction in the productivity of potatoes due to weeds is reported. A number of cultural, mechanical as well as chemicals methods are available for controlling weeds in potato crop. Herbicides are available for control of different types of weeds at different stages. The selection of proper herbicide depends upon the type of weed flora and the stage of crop growth. However, weeds prevalent in potato crop vary from region to region and season to season and in the absence of knowledge about weed flora, it is difficult to give precise recommendation for their control. The knowledge of farmer about the weeds is limited only up to its local name and the extent of damage it may cause. Moreover, availability of proper guidance about weed control in the absence of technical advice may lead to improper control method leading to inefficient weed control. To alleviate this problem, a decision support tool "Potato Weed Manager" has been developed. The software is developed in DOT NET technology and the database used is SQL. This software is hosted on CPRI application server and is connected to CPRI website. This software incorporates the photographs of the weeds for identification by the user. This software gives the recommendation for weed control on the basis of situation of potato field, type of weed flora (major and







A combined view of different windows of the "VarTRAC"

secondary/associated weeds) and the stage of the potato crop. Thus the software provide proper guidance to the farmer about the weed control method to be adopted and dispense with the need of technical knowledge.

6. VarTRAC: BIOINFORMATICS-TOOL FOR IDENTIFYING POTATO VARIETIES

Authentic identification of potato cultivars is important for plant breeders, the variety registration and certification agencies, seed producers, merchants, farmers, growers, processors, and other end-users. Currently morphological descriptors are being used internationally for variety identification. However, there is a possibility of utilizing DNA fingerprint data to supplement morphological characters in near future. Central Potato Research Institute, Shimla is, therefore, developing both morphological and DNA fingerprint databases for potato cultivars' identification.

Data on 50 different morphological attributes and DNA fingerprints based on 127 alleles from 4 micro-satellite markers are currently being used at CPRI for varietal identification. Manual analysis of such huge data is not easy. Therefore, a computer software named "VarTRAC" was developed at CPRI for speedy identification of a variety based on the morphological and DNA fingerprint data.

The database was created in MS Access with each morphological character taken as a field. All the characters necessary for the identification of a potato variety have been included. Scores are given for each character in a drop-down menu format and the users have only to select the appropriate score for each character. Further the help has also been provided for proper scoring. As regards DNA fingerprints, the data on 127 alleles have been recorded by giving a score of one for those alleles, which are present while zero for the absent ones.

The software can make generalized abstraction even from the minimum available information. For example, if only 5 morphological attributes of any unknown variety are known, the software can identify the group of varieties having similarity in respect of those 5 attributes.

7. E-BOOK ON POTATO

E-book on the Potato is meant to give a bird's eye view of practical knowledge about the potato production, utilization, *etc*. in India. It is aimed at providing appropriate information for all those interested in knowing about the ways potato is cultivated in different regions in India, the reasons for the adoption of the various agro techniques and the major abiotic and biotic stresses. This is expected to provide insights about the scientific cultivation and utilization of potato. This e-book is also meant to be a supplement to many excellent publications on potato, which could not be fully illustrated with photographs due to limitation of cost of printing. This lacuna is overcome in this e-book since cost factors are minimum in this case. Thus, this e-book apart from being used as a book *per se* would also be a pictorial supplement to other publications available in print. Through this e-book, it is hoped to further strengthen the cause of potato R&D in India using the electronic media, the use of which is becoming rampant.

8. DIGITIZED PHOTOGRAPHIC DATABASE OF POTATO

The creation of photographic database is a very important activity because information can be presented very easily and concisely through photograph rather than text. Therefore, a digitized photographic database was developed. It can be used by professionals in their presentations, extension lectures to the farmers/industry entrepreneurs, in publication of scientific books, technical bulletin *etc*. The database contains more than 600 photographs pertaining to all aspects of potato research and development. The use of this database does not require any specialized skill.

Fungal diseases of potato and their management

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Potato, an important food has the potential to meet food demand of the fast growing human population. This is going to be the future food crop for the millions especially in the third world countries. Potato production and consumption is accelerating in most of the developing countries including India primarily because of increasing industrialization. Potatoes in India are grown under varied climatic conditions as a result the spectrum of insect-pests and diseases is very large. Therefore, management of diseases and pests is important to realize full potential of the crop. Scope of this chapter is limited to the important fungal diseases of potato, which causes considerable losses to the growers. A brief description of these diseases and their management is given in this chapter.

FOLIAR DISEASES

Late blight (*Phytophthora infestans* (Mont.) de Bary)

It is one of the most devastating diseases of potato and losses up to 85% have been reported if crop (susceptible cultivar) remains unprotected. In India, losses are more in hilly regions where the crop is grown under rain-fed conditions as compared to the plains. Disease appears every year in epiphytotic form in hilly regions whereas in the plains, although it usually appears every year but its intensity is low (traces to 25%). It is only in few years that it assumes epiphytotic form. Recently, late blight has become a serious problem in *kharif* grown potatoes and tomatoes in Karnataka state. The annual average losses to the tune of 15% have been estimated in the country.

Symptoms: Late blight affects all plant parts *i.e.* leaves, stem and tubers. It appears on the leaves as pale green, irregular spots which enlarge into large water soaked lesions. In moist weather the spots enlarge rapidly with central tissue turning necrotic and dark brown or black. Often, the spots have a purplish tinge. On the lower side, white mildew (cottony growth) ring forms around the dead areas. In dry weather the water soaked areas dry up and turn brown. On stems and petioles light brown elongate lesions develop often encircling the stem/petiole. Under favourable conditions, the whole vine may be killed and blackened and the disease spread rapidly killing the entire crop in a few days. Tubers are readily infected while in soil by rain borne spores from blighted foliage. Initially the tubers show a shallow, reddish brown dry rot that spreads irregularly from the surface through the flesh. At low storage temperatures, the lesions usually remain firm and frequently show a metallic tinge especially at the border of healthy tissues.

Epidemiology: Tubers carrying the pathogen are the real carriers and serve as the source of the disease in the subsequent season. In the plains, the pathogen over summers through infected seed tubers in cold stores. Infected seed tubers grow into healthy plants but under conditions favourable for the disease (temperature 10-20^{o}C and RH>80%) the resting pathogen develops within the infected seed and affects the stem base/lower leaves. Such infected stems and leaves serve as the primary source of inoculum. The pathogen sporulates on the primary lesions and the sporangia so formed are carried over by wind currents/rain splashes to other plants/fields thereby setting a chain reaction. Fungal sporangia are also washed down to soil with rain water or dew and infect the new tubers.

Appearance and buildup of late blight depend solely on weather conditions. There are specific requirements of temperature and humidity for initiation and further buildup of disease. Temperature requirements are different for fungus growth (16-20^{o}C), spore production (18-22^{o}C), spore germination (10-20^{o}C) and for infection and disease development (7.2-26.6^{o}C with optimum 18+1^{o}C). Spore germination and infection requires 100 per cent humidity and spores

get killed under low humidity (<75%). Fungal spores are produced during the night and are sensitive to light. Cloudiness favours disease development.

Late Blight Forecasting

Development of late blight mainly depends on moisture, temperature and cloudiness. In India, the rains are heavy and the weather is cool and cloudy/foggy during summer in the hills but in plains the weather is generally clear with scanty rains (during autumn or spring) and therefore, the disease epidemic is not a regular feature. The monsoon moves from East to West in the Himalayas. Therefore, the blight occurs early in the eastern Himalayas. Taking weather parameters in account, Bhattacharyya *et al*. (1982) developed forecasting models for Shimla, Shillong and Ootacamund i.e. i) if the 7-day moving precipitation (30 mm for Shimla, 28.9 mm for Ootacamund and 38.5 mm for Shillong observed to be critical rainfall lines) associated with mean temperature of 23.9ºC or less continues for 7 consecutive days, late blight would appear within 3 weeks. Once these conditions are met, then more accurate prediction based on RH and temperature was developed. It states that if hourly temperature remains in between 10-20^0 C associated with the RH ≥80 % for continuous 18 hr for at least 2 consecutive days, late blight would appear within a week. This model has been put to successful use for predicting late blight in Shimla hills since 1983 and it is still working very well.

Late blight forecasting in the sub-tropical plains is different to that of temperate highlands. In the hills, environmental conditions (temperature, RH, rainfall) favourable for late blight appearance are assured. There are plenty of rains during the crop season which led to high RH (>80%) for most of the crop season. Temperature remains moderate and congenial throughout. It is therefore, possible to rely on weather parameters like, rainfall, RH and temperature for making disease forecasts. Such situations, however, do not exist in the sub-tropical plains, where there are scanty rains during the crop season. In such a situation, role of micro-climate, fog, dew and sunshine becomes critical for the appearance of the disease. A computerized forecasting model 'JHULSACAST' has been developed for western Uttar Pradesh using temperature, RH and rainfall data. It consists of two models, one each for rainy and non-rainy years. For rainy years, if i) measurable rains (0.1-0.5 mm) for a minimum of two consecutive days, ii) 5-day moving >85% RH period 50 hrs or more, iii) 5-day moving congenial temperature (7.2-26.6ºC) for 105 hrs or more, blight would appear within 10 days time. For non-rainy years, if 7-day moving >85% RH period 60 hrs or more and 7-day moving congenial temperature (7.2-26.6ºC) for 120 hrs, blight would appear within 10 days time. Besides, decision rules for predicting first appearance of late blight in Punjab under non-rainy conditions have also been developed recently using JHULSACAST model as template. The model specifies that 7-day moving sum of RH ≥ 85% for at least 90 hr coupled with a 7-day moving sum of temperature between 7.2-26.6ºC for at least 115 hr would predict appearance of late blight within 10 days of satisfying the conditions. JHULSACAST has also been calibrated for Tarai Region of Uttarakhand and the plains of West Bengal. Based on JHULSACAST, Decision Support System (DSS) has also been developed which has three components i.e.(i) prediction of first appearance of disease, (ii) decision rules for need based fungicide application, and (iii) yield loss assessment model. Recently, INDO-BLIGHTCAST, a web based model has been developed to predict first appearance of late blight using daily weather data of meteorological stations. This is an improvement over JHULSACAST model as it is applicable pan India and requires only daily weather data and does not need local calibration for different regions.

Disease Management: The disease can be contained if farmers follow the integrated management schedule as follow:

Use of healthy seed: Only disease free seed should be used. Avoid seed from the field which has been infected by late blight in the previous year. The infected tubers should be thoroughly checked for late blight infection. The infected tubers should be removed and buried in the soil. This practice of sorting out late blight infected tubers can also be done at the time of planting. The late blight symptoms are easy to be identified in cut-pieces where bronzing of the flesh can be seen easily.

Use of resistant cultivars: Select varieties which have moderate to high degree of resistance to late blight.

Cultural methods: Important cultural methods include:

i) Selection of well drained soils for potato cultivation.
ii) High ridging to prevent exposure of infected seed tubers which serve as primary source of the disease. Besides, it helps in the reduction of new tuber infection.
iii) Scouting of the field for identifying primary infection foci and their destruction by removal of the infected plants after drenching them with recommended fungicides. Nearby plants should also be sprayed.
iv) As soon as the weather conditions become congenial for late blight, irrigation should be stopped wherever applicable. Only light irrigation may be given later, if required.
v) Destroy and remove the haulms from the field when the disease severity reaches >80% to reduce tuber infection.
vi) Harvest the crop 12-15 days after haulms cutting, sort out the late blight infected tubers and store the seed after treating it with boric acid (3%).

Chemical control: A spray schedule of minimum of four fungicide sprays is recommended. However, the number of sprays may be increased or decreased depending on disease pressure.

I spray: As a prophylactic measure, spray the crop with contact fungicides like mancozeb 75%WP (0.2%), propineb 70% WP (0.2%) or chlorothalonil (0.2%) as soon as the weather conditions become congenial for late blight, or about a week in advance of canopy closure whichever is earlier. Do not wait or allow late blight to appear and establish in the field. Always use a sticker @0.1% for proper sticking and uniform spread of fungicide on leaf surface.

II spray: As soon as the disease is noticed in the field, apply any of the systemic fungicides viz., cymoxanil-based (0.3%) or dimethomorph-based (0.3%) or fenamidone-based (0.3%) fungicides.

III spray: Apply contact fungicides viz. Mancozeb (0.2%), propineb (0.2%) or chlorothalonil (0.2%) after 8-10 days of 2nd application of fungicides. However, if weather is highly congenial, repeat application of systemic fungicides may be resorted to.

IV spray: Apply systemic fungicides or contact fungicides as mentioned above depending on disease severity and weather conditions. Ensure thorough coverage of plants top to bottom with fungicides. Special attention should be given to lower leaves which need to be covered with fungicides.

Early blight (*Alternaria solani* (Ell. & Mart.) Jones & Grout)

Early blight occurs in all the potato growing areas but is common in central India and plateau of Bihar, Jharkhand, Chhatisgarh and Maharashtra. The disease has been reported to cause significant losses (up to 20%) in *Kharif* crops in Ranchi and adjoining plateau region. In north-western and north-easten hills and plains, the disease appears regularly but in lesser significant form since late blight takes over.

Symptoms: Initially the symptoms occur on the lower and old leaves in the form of small (1-2 mm), circular to oval, brown spots. These lesions have the tendency to become large and angular at later stage. Characteristic 'target board' concentric rings of raised and depressed necrotic tissue can be observed, often with a chlorotic halo surrounding the lesion. The tuber symptoms comprise brown, circular to irregular and depressed lesions with underneath flesh turning dry, brown and corky. Lesions tend to enlarge during storage and affected tubers later become shriveled.

Epidemiology: The fungus can survive in soil and plant debris particularly in temperate climate. The infected tubers form the primary source of inoculum. The disease is favoured by moderate temperature (17-25^{o}C) and high humidity. Intermittent dry and wet weather is more conducive for early blight.

Phoma leaf spots (*Phoma exigua* Desm., *P. sorghina* Doerema, Doren & Kest.)

Leaf spots caused by *Phoma* spp. also occur widely both in hills and plains. Depending upon the severity, these leaf spots may cause significant yield losses.

Symptoms: Leaf spots due to *P. exigua* are larger (1-2.5 cm) with broad alternate light and dark concentric zones. Affected tubers have grey to greenish black depressed lesions (up to 3cm) on the surface. Leaf spots due to *P. sorghina* are characterized by pin head size spots, which may be oval, circular or irregular (not exceeding 4mm). Infected tubers show grey large lesions (up to 1.7cm).

Epidemiology: These fungi can survive in soil and plant debris and on infected tubers during storage. The infected tubers form the primary source of inoculum. Infection usually appears on the lower leaves near ground level and results in the infection of young immature tubers if not covered by the soil. The disease is favoured by moderate temperature (17-25ºC) and high humidity.

Management: The integrated management of early blight and leaf spots is as below:

i) Use disease free tubers for raising the crop.
ii) Removal and burning of haulms of the affected potato crop help in reducing the inoculum in the field.
iii) Cultivation of solanaceous crops, being collateral hosts, nearby potato field must be avoided.
iv) Spray the crop with mancozeb (0.2%), chlorothalonil (0.2%), copper oxychloride (0.3%) and Bordeaux mixture (1.0%).

SOIL AND TUBER BORNE DISEASES

Soil and tuber borne diseases are multifaceted in nature. Most of the pathogens have a very long soil phase and also carried through potato tubers. These diseases may cause disfiguring of tubers thereby impairing the quality, tuber rots in storage & transit, and wilts and stem rots in field.

Black scurf (*Rhizoctonia solani* Kuhn)

Symptoms: Almost all plant parts are affected. The fungus attacks young sprouts through epidermis and produces dark brown lesions thereby killing the sprout before emergence, which result in gappy germination. Elongated reddish brown lesions develop on the stem at or below soil surface that may girdle the stem. When the girdling is complete the foliage curl and turn pinkish to purplish. Often aerial tubers are formed as a result of interference in starch translocation. Towards the end of the season, the fungus produces numerous hard, small, dark brown to black sclerotia on the surface of mature tubers. These sclerotia when get deposited continuously, form a black encrustations on the tuber surface. The fungus also causes foliage blight of potato.

Epidemiology: Seed tubers serve as the main source of the disease. In the hills, the fungus survives in the soil throughout the year and is a potential source of the disease. However, high summer temperatures are not conducive for the production of sclerotia and their survival. Therefore, *R. solani* has to over summer either as saprophytic mycelium or by infecting the crops grown during summer period. The soil temperature governs production of sclerotia on the tuber surface. The optimum temperature for growth of the fungus is 25-30ºC and for the germination of sclerotia is 23ºC.

Charcoal rot (*Macrophomina phaseolina* (Tassi) Goid

Symptoms: The pathogen produces three types of symptoms i.e. stem blight, charcoal tuber rot and dry tuber rot. The charcoal tuber rot phase is important under Indian conditions. The first visible symptoms are black spots (2 to 8 mm) surrounding the lenticels and eyes. As the disease advances, the tissue underneath the skin becomes uniformly black up to a depth of 2 to 5 mm. No sclerotia are formed.

Epidemiology: Both tubers and soil may serve as primary source of inoculum. However, soil is the main inoculum source. Soil temperature at or preceding harvest is the most crucial factor for

disease development. Temperature below 28ºC almost completely checks the disease. Therefore, in sub-tropics, tuber rottage is less in crop lifted before middle of February. Disease buildup is faster in sandy-to-sandy loam soil as compared to clay soil.

Black dot (*Colletotrichum coccodes* (Wallr.) Hughes (Syn.: *C. atramentarium* {Bek. & Br.} Traub.)

Black dot is commonly found in most potato growing regions. It is generally considered to be a surface blemishing disease of tubers, which downgrades potatoes, destined for table purposes and may affect seed tuber sales due to disease tolerance restrictions. Recent studies indicate that the fungus may be associated with the potato relatively early in the growing season, and with many plants over a wide geographic area. Therefore, yield effects may be more significant than formerly assumed.

Symptoms: Symptoms on leaves are less common than stem or tuber symptoms in the field. Infection of vascular tissue and girdling stem lesions can induce yellowing and wilt like symptoms, which generally progress from plant apices to lower portions of the plant. Wilt symptoms may be confused with those caused by *Fusarium* or *Verticillium*. Small, black, dot-like sclerotia (microsclerotia) are formed abundantly in stem lesions, particularly late in the growing season, and are visible to the naked eye. Sclerotia may form in internal tissues as well. On roots and stolons silvery brown lesions are formed on which characteristic microsclerotia are readily formed-aiding to diagnosis. Infected remnants of stolons oftn adhere to tubers at harvest. Tubers infected with *Colletotrichum* develop dark, grayish lesions which appear similar to silver scurf. However, black dot lesions are more irregular, with undefined margins. They also usually contain microsclerotia which are smaller than those on stolons. Extensive tuber blemishes may increase tuber respiration, resulting in shriveling and tuber shrinkage.

Epidemiology: The pathogen overwinters as microsclerotia occurring free or on colonized plant debris in the soil. The fungus can persist in the soil for at least 8 years. The fungus may also overwinter as sclerotia on infected seed tubers and, therefore, infection of plants may be due to tuber –borne and/or soil borne inoculum. Conidia probably serve as the primary inoculum for infection. Conidia do not germinate at 7^0C, the optimum temperature for germination and infection is between 22 & 28^0C. Roots are the organs most susceptible to infection; stems generally become diseased only after the fungus is well established on the underground stem of the plant. Black dot is commonly associated with high temperature, poor soil drainage and sandy soils, and low nitrogen levels. Other solanaceous plant species and several weed species also act as hosts for *C. coccodes*. In storage, infection and symptom development are favoured by warm, humid conditions.

Management

i) Long rotations (at least five years between potato crops without solanaceous crops) and good irrigation management.
ii) Use disease free tubers for planting.
iii) Deep ploughing will bury infected debris and encourage decomposition.
iv) Soil application of azoxystrobin have shown efficacy against soil borne inoculum.
v) Incorporation of *Trichoderma* through fortified FYM.

Silver Scurf (*Helminthosporium solani* Dur. & Mont.)

It is a common storage disease and occurs wherever potatoes are grown. Now, it has become an economically important disease through reduction in cosmetic quality of washed fresh-packed potatoes. Silver scurf does not usually cause yield loss, but severe seed infection can affect vigour. The disease is also becoming important in potato processing, because crisps made from potatoes with severe silver scurf infection may result in blackened edges, making the product unmarketable. Fresh weight reduction of tubers may also occur due to excessive moisture loss from the tubers through lesions.

Symptoms: There are no above ground symptoms and on roots. However, lesions can be observed on stolons soon after tuber initiation. The most conspicuous symptoms are produced on tuber periderm. The lesions are roughly circular in size, expanding upto several centimeters. The edge of the lesion is regular. The disease gets its name because the lesions are mostly silvery in colour. In soil, established lesions expand rapidly within a few weeks of planting infected seed tubers. Lesions on progeny tubers spread slowly on the surface when in soil. The lesions are usually small at the harvest but enlarge during storage.

Epidemiology: Perpetuation of the disease takes place through soil as well as tuber borne inoculum. Therefore, transmission of silver scurf can occur through infected seed introduced into soil or through conidia present in soil. Conidia produced in storage conditions are released and carried to other tubers via circulating air. Under favourable conditions – moderate to warm temperatures (10-32^{o}C) and very high humidity or free water-conidia germinate on plant tissue by polar germ tubes and cause infection of tubers.

Management

i) Ensure planting of silver scurf free seed.
ii) Avoid delay in harvest and exposure of tubers to the pathogen in the soil.
iii) Follow rapid drying of tubers after harvest.
iv) Tubers treated with fungicides (benomyl, thiophanate-methyl, thiabendazole) at planting, at harvest, or at both times can reduce infection but their effects do not usually extend into storage.

Fusarium wilt and dry rot (*Fusarium* spp.)

Symptoms: Variety of symptoms is produced on potato including wilt, stem rot and damping off of seedlings. On tuber they produce spots, necrosis, dry rot and seed piece decay. In wilting, lower leaves turn yellow and the affected plant dries off rapidly. Both stems and tubers at stolon end show vascular browning. In some cases wilting may be accompanied by rotting of stem base. It may cause damping off of seedlings if planted early in the season when temperature is high.

Dry rot is a storage disease and does not become evident until 2-3 months of storage. Rot may occur in any part of the tuber but wounded site and stolon end are the most vulnerable. Initially the infected tissue develops slight depression, which increases, and the skin develops wrinkles in the form of irregular concentric circles. Underlying tissue assumes mealy and brown fungal mycelium.

Epidemiology: Infected tubers and soil are the primary source of inoculum. Dry rot development is affected by tuber age, tuber size, storage conditions, tuber damage and degree of curing. Dry rot infection gets aggravated 5-6 months after harvest. Store temperature ranging 20-28^{o}C is congenial for dry rot development. Wilt is mainly affected by soil temperature and relative humidity. High wilt incidence in early planted crop is mainly associated with high soil temperature.

Powdery scab (*Spongospora subterranea* (Wallr.) Lagerh

Symptoms: The fungus attacks all underground parts of the plant without showing any adverse effect on plant growth. The damage to the tubers is however, more serious. The disease does not affect the potato yields but disfigures tubers, reducing its commercial value and renders them unsuitable for seed purpose. Pimple like spots appears on the surface of young tubers. These spots are circular, smooth and light brown which gradually increase in size and ultimately rupture, exposing a cavity containing a brown powdery mass of spore balls. Deep pustules of powdery scab resemble deep pitted common scab lesions. However, powdery scab pustules are filled with mass of fungal spore ball whereas common scab lesions are empty.

Epidemiology: The fungus over winters through spores in soil and on infected seed tubers. The spores germinate during crop season and produce zoospores in soil, which infect the tubers through lenticels or directly through epidermis. Soil temperature and moisture are the main factors affecting the disease. Low soil temperature (0-15^{o}C) coupled with high soil moisture is

ideal for disease development. This disease is a high altitude disease and is seldom noticed below 2500 m amsl and its incidence increases with the increase in altitude.

Wart (*Synchytrium endobioticum* (Schilb) Perc.)

Symptoms: It is a disease of potato tubers and is usually not recognized in the field until the tubers are dug out. The disease is characterized by prominent warty protuberances resembling cauliflower or bunches of 'cocks comb' like proliferated outgrowth on tuber. Sometimes small greenish warty growths on the stalks may be observed near the ground level.

Epidemiology: Wart disease is both soil and tuber borne. Once the soil is contaminated with the resting sporangia, it becomes an important source for the spread of the disease, as winter sporangia are known to remain viable for many years. The chief means by which the disease spreads is through the transportation of material containing resting spores. The disease is worst in wet season. Both winter and summer sporangia can germinate over a wide range of temperature (12-18^{o}C) if the moisture is favourable.

Wilts

Verticillium wilt (*Verticillium alboatrum* Reinke & Berth.)

Symptoms: The infection starts from the roots and the fungus grows into the stem and colonizes the xylem vessels thereby disrupting the water and mineral supply to the aerial parts as a result plants remain stunted, lack vigour, lower leaves tend to droop and there is loss of turgidity. Vascular bundles of stem and tuber become brown. In tuber initial infection is seen as yellowish discolouration at the stolon end. In tuber, initial infection is seen as yellowish discolouration at the stolon end.

Sclerotium wilt (*Sclerotium rolfsii* Sacc.)

Symptoms: Infection starts at the stem base in the form of 1-2 cm dark brown lesions, which gradually enlarge and encircle the stem base resulting in the collapse of plant. The pathogen produces white fungal mat and mustard sized sclerotia on the underground parts within the hyphal mat.

Sclerotinia wilt (*Sclerotinia sclerotiorum* (Lib.) de Bary)

Symptoms: The disease occurs on the stem either at the soil line or at the junction with leaf petioles. Early symptoms on stems are the appearance of water soaked areas on which white fluffy mycelial growth subsequently develops, which gradually enlarge and encircle the stem base resulting in the collapse of plant. Rotting of the stem may extend up to 5 cm above the ground. In the later stages of symptom development, large, dark, compact resting sclerotia are formed in stem pith.

Epidemiology: All the wilt causing fungi survive in the soil and plant debris although infected seed tubers my also act as the primary source of inoculum. *Sclerotium* survives in the soil in mycelial as well as in sclerotial form. The fungus may also survive on collateral hosts. The fungus gets aggravated at high soil temperature (25-30^{o}C) and requires alternate periods of wet and dry soil. Flooding of soil kills *S. rolfsii* thereby reduces the wilt.

Infected tubers and contaminated soil serve as the source of primary inoculum for *Verticillium* wilt. For the perpetuation of the disease the seed surface contamination has been reported to be more important than the internal seed borne inoculum. The pathogen requires comparatively low temperature and therefore, restricted to cooler parts of the country. *S. sclerotiorum* overwinters as mycelium in dead or living plants, but primarily in the form of sclerotia. In soil it can remain viable up to 5 years. It also requires comparatively low temperature (10-27^{o}C) and therefore, restricted to cooler regions of the country.

Management of soil and tuber borne diseases

Soil and tuber borne diseases primarily perpetuate through infected seed tubers and soil. Their management therefore, requires elimination or lowering down of the inoculum load on the tubers as well as in soil. Management strategies therefore, have to be many fold for combating these diseases.

Cultural practices

Crop rotations and green manuring: When potato crop is planted year after year in the same field, the survivability of the pathogens and their buildup gradually increases over the years. Although, most of the diseases, which infect the potato crop, have wide host range, it is still possible to keep the pathogens population within manageable limits by practicing suitable crop rotations. It has been found that long-term rotation of maize or sun hemp with potato significantly reduced black scurf and charcoal rot incidence. Sesbania, sunhemp and pearl millet are also effective against black scurf. *Verticillium* wilt can be effectively managed if potato crop is grown after two years of Kulth cultivation. Intercropping of potato with maize, rotated with bean or radish was quite effective in the management of potato wart.

Amendment of oil cakes: Oil cakes have mostly been tried for the management of black scurf and *Fusarium* wilt. Buildup of the *Fusarium* population was least in groundnut cake amended soil followed by mustard cake and cotton seed cake.

Adjustment in planting and harvesting time: Some of the soil and tuber borne diseases are temperature sensitive and can be effectively managed by altering the planting and harvesting dates. By advancing the harvest from February 16 to January 30, the incidence of black scurf was brought down by more than 50 %. Similarly, harvesting of potato tubers before the soil temperature crosses 28^{o}C reduces charcoal rot incidence in endemic areas. By delaying the planting from October 1 to October 30 resulted in 36% reduction in *Fusarium* wilt.

Sanitation: Use of disease free seed, weed control and removal of diseased plants/debris from field are some of the cultural practices that reduce soil and tuber borne inoculum.

Soil solarization: Soil solarization by the use of transparent polyethylene sheet is an effective, simple and ecofriendly way of managing soil borne diseases. This method could be useful in tropical and sub-tropical plains where summer temperatures are very high and is practicised during the hottest period of the year. Solarization was superior to deep summer ploughing as it reduced black scurf incidence by 55.6% and russet scab by 58.4%.

Biological Control: Use of *Bacillus subtilis* (B-5) has been found effective against black scurf, common scab, Fusarium wilt and bacterial wilt. A combination of soil solarization and seed treatment with boric acid or *Trichoderma viride* improved black scurf.

Host Resistance: Disease resistant or immune varieties are the best methods to check soil and tuber borne diseases, however, such varieties are available only against few diseases. Varieties immune/resistant to wart disease are Kufri, Sherpa, K. Kanchn, K. Jyoti, Kufri Muthu, Kufri Bahar, Kufri Chmatkar, Kufri Khasigro, Kufri Kumar, Kufri Giriraj, Kufri Chipsona-2, Kufri Anand, Kufri Pukhraj, Kufri Jawahar and Kufri Sutlej. The early maturing varieties like Kufri Chndarmukhi and Up-to-Date are less prone to charcoal rot and may be cultivated in spring. Most of the varieties in India are susceptible to black scurf. However, Kufri Dewa, Kufri Bahar and Kufri Sherpa are comparatively less susceptible.

Chemical Control: Dipping of infected tubers in boric acid (3 %) for 30 minutes or spraying on tubers has been recommended for the management of tuber borne diseases (black scurf & common scab).

Suggested Reading:

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Bacterial Diseases of potato and their management

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Potato (*Solanum tuberosum* L.) is the world's fourth most important food crop after wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.). It provides a balanced source of starch, vitamins and minerals to many communities in the global village and holds promise for food in the scenario of ever growing population. Potatoes were first introduced outside the Andes region four centuries ago, and since then have become an integral part of much of the world's cuisine. Potato can produce more food per unit area per unit time than the conventional cereal crops. However, the full potential of this crop can only be realized if diseases and pests are kept under control.

Potato is a vegetatively propagated crop and as a consequence seed tubers suffer from many diseases and pests. Therefore, the availability of good quality planting material is a major constraint in successful production of potatoes. Moreover, successful production of quality potato can be undertaken only in those areas and fields which are free from serious soil borne pathogens like wart, cyst nematode, bacterial wilt, black scurf, and common scab. Suitable strategy to manage bacterial wilt and other diseases may enable quality seed production in such areas. Adoption of management strategy along with innovative seed production technology like micro-propagation may bring breakthrough in quality seed production. Potato production is seriously compromised due to prevalence of a number of diseases. For example, potato wart restricted seed production and movement from Darjeeling hills and its adjoining area in West Bengal; cyst nematode infestation restricted seed movement from Nilgiri hills. Bacterial wilt is a primary factor restricting seed production in states of West Bengal, Karnataka, Maharashtra, Orissa, NEH Region, and Bihar that constitutes about 50-55% potato area.

Potato is affected by relatively a few bacterial diseases namely, bacterial wilt or brown rot, soft rot of stem and tubers, ring rot, common scab, pink eye and leaf spot. In India ring rot (*Clavibacter michiganensis* sub sp. *sepedonicus* (Spieck & Koth) Devis *et al.*) and pink eye (*Pseudomonas* species) do not occur. The leaf spot (*Xanthomonas vesicatoria* (ex Doidge) Vauterin *et al.*) is a disease of minor importance. However, bacterial wilt/ brown rot is the most destructive bacterial disease followed by common scab and soft rot.

1. Bacterial Wilt

Bacterial wilt or brown rot is caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* 1995. It is one of the most damaging pathogens on potato and has been estimated to affect potato crop in 3.75 million acres in approximately 80 countries with global damage estimates exceeding $950 million per year. Strains of this pathogen affect more than 450 plant species in over 54 botanical families throughout the world, including a wide range of crop plants, ornamentals and weeds. In India, Losses up to 75 per cent have been recorded under extreme conditions. With increase in global temperature, the disease is likely to spread to new areas and affect potato cultivation. The disease causes damage at two stages; (i) killing the standing plants by causing wilt and (ii) causing rot of infected tubers in field, storage and transit. Another indirect loss is spread of the disease through planting of healthy looking tubers harvested from infested fields. Bacterial wilt poses a serious restriction to seed and processing potato production. Potato breeder seed production cannot by undertaken in those fields having even slightest bacterial wilt incidence. There is nil tolerance to this disease in most international seed certification systems.

Symptoms

The earliest symptom of the disease is the slight wilting in leaves of top branches during hot sunny clear days. The leaves show drooping due to loss of turgidity followed by total unrecoverable wilt **(Fig. 1a).** In well-established infections, cross-sections of stems may reveal brown discoloration of infected tissues **(Fig. 1b).** In advanced stages of wilt, cut end of base of the

stem may show dull white ooze on squeezing. Bacterial wilt in field can be distinguished from any fungal wilt by placing the stem cut sections in clear water as shown in **Fig. 1c**. Within a few minutes, a whitish thread like streaming can be observed coming out from cut end in to water. This streaming represents the bacterial ooze exuding from the cut ends of colonized vascular bundles. The same test can also be carried out to see infection in tuber.

Fig. 1: Symptoms of bacterial wilt (a); brown discoloration of stem tissues (b); bacterial streaming in clear water from stem cut section of potato (c) infected with *R. solanacearum*. In tubers, two types of symptoms are produced; they are vascular rot and pitted lesion on surface. In vascular rot, the vascular tissues of transversely cut tuber show water soaked yellowish brown to brown circles and in about 2-3 minutes, dirty white sticky drops appear in the circle **(Fig. 2a)**. In advanced stages of wilt, bacterial mass may ooze out from eyes (**Fig. 2b**). Such eyes may carry soil glued with the bacterial ooze. Second kind of symptom is the lesions on tuber. The lesions are produced due to infection through lenticels (skin pore). Initially, water soaked spot develop which enlarges in the form of pitted lesion **(Fig. 2c)**. The tubers may not rot in storage and also may not show vascular browning. These symptoms on tuber surface are more common in north eastern region of India**.**

Causal Organism

Ralstonia solanacearum (Smith 1896) Yabuuchi *et al.* 1995, is a Gram-negative, rod-shaped, strictly aerobic bacterium that is 0.5-0.7 x 1.5-2.0 Cm in size. For most strains, the optimal growth temperature is between 28 and 32°C; however some strains have a lower optimal growth temperature of 27°C. Strains of *R. solanacearum* have conventionally been classified as races and biovars. Brown rot of potato is caused by either race 1 or race 3 of *R. solanacearum*. Recently, a new classification scheme has been described for strains of *R. solanacearum*, based on variation of DNA sequences. Four phylotypes were identified within the species that broadly reflect the ancestral relationships and geographical origin of the strains. These phylotypes can be further subdivided into sequevars. In India, the bacterial wilt of potato is known to be caused by strains of phylotype I, IIB and IV of *R. solanacearum*.

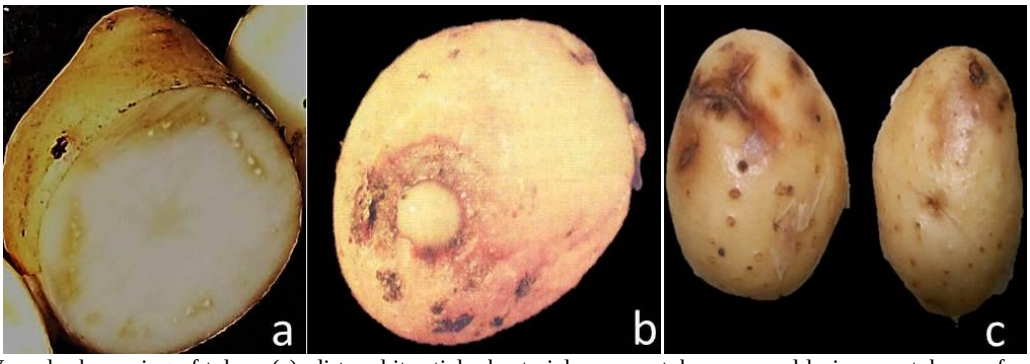

Fig 2. Vascular browning of tubers (a); dirty white sticky bacterial ooze on tuber eye; and lesions on tuber surface (c) due to *R. solanacearum* infection

Disease Occurrence and Distribution

Bacterial wilt or brown rot has a worldwide distribution. It is a destructive disease of potato especially in tropical and subtropical parts of Asia, Africa, South and Central America and in

some soils and waterways in Europe and Australia. In India, the disease is endemic in Karnataka, Western Maharashtra, Madhya Pradesh, eastern plains of Assam, Orissa and West Bengal, Chhota Nagpur plateau, north-western Kumaon hills, eastern hills of West Bengal, Meghalaya, Manipur, Tripura, Mizoram, Arunachal Pradesh and in Nilgiris, Annamalai and Palani hills of Tamil Nadu. Bacterial wilt is a serious problem in Malwa region and adjoining areas in Madhya Pradesh where potato is grown for processing industry. However, it has not been noticed in the north-western high hills (excluding Kumaon hills) and in the North-western and North-central plains which are major seed producing zones of the country and need to be protected from the introduction of the disease.

Disease Cycle

Infected tubers and plant debris in infested soil are two major sources of inoculum. The pathogen infects roots of healthy plants through wounds. Nematodes such as *Meloidogyne incognita* which affect potato roots and tubers increase wilt incidence. Inoculum potential of about 10^7 cfu/g soil favours infection which however is dependent on other predisposing factors. Mean soil temperature below 15ºC and above 35ºC do not favour disease development. Several other factors that may affect pathogen survival in soil and water may also favour disease development, including soil type and structure, soil moisture content, organic matter in soil, water pH and salt content, and the presence of antagonistic microorganisms.

Survival

The pathogen survives through infected seed tubers and in plant debris in soil. Symptom-less plants may harbour the bacterium and transmit it to progeny tubers as latent infection. This could lead to severe disease outbreaks when the tubers are grown at disease free sites. High soil moisture, temperature, oxygen stress and soil type affect the survival of the pathogen. The pathogen population decline gradually in soil devoid of host plants and their debris.

Spread

Transmission of *R. solanacearum* from one area to another occurs through infected seed, irrigation water and farm implements. Under favorable conditions, potato plants infected with *R. solanacearum* may not show any disease symptoms. In this case, latently infected tubers used for potato seed production may play a major role in spread of the bacterium from infected potato seed production sites to healthy potato-growing sites.

Management

The control of bacterial wilt has proved to be very difficult because of seed and soil borne nature of the pathogen, and especially for race 1 with its broad host range. Chemical control is nearly impossible to apply. Soil fumigants have shown either slight or no effects. Antibiotics such as streptomycin, ampicillin, tetracycline and penicillin also showed hardly any effect; in fact, streptomycin application increased the incidence of bacterial wilt in Egypt. Biological control has been investigated, but is still in its infancy. Potato cultivars developed in Colombia with a *Solanum phureja* and *S. demissum* background showed resistance to *R. solanacearum*, but the race and strain diversity of the pathogen made it difficult to utilize these in different countries. The absolute control of bacterial wilt, at present, is difficult to achieve, however, economic losses certainly can be brought down considerably using the following eco-friendly means:

Healthy seed: Use of healthy planting material can take care of almost 80% of bacterial wilt problem. We fortunately have bacterial wilt free areas in western and central Indo-Gangetic plains. Obtain seed from these areas. Do not cut tubers; if the tuber is infected, the cutting knife spread the disease and also cut tubers contact disease from soil easily.

Field sanitation: Where the field is already infested, the best way to minimize the disease is to adopt the following agronomic practices:

Crop rotation: Follow 2-3 years' crop rotation using crops like maize, cereals, garlic, onion, cabbage and sanai. Do not rotate vegetables like brinjal, ginger, chillies and other solanaceous crops. Paddy and sugarcane although are not host, still they carry pathogen and contribute to the disease perpetuation.

Avoid tillage operations: Pathogen enters in plant through root or stolen injuries. Such injuries cannot be avoided during intercultural operations. Therefore, restrict tillage to the minimum and it is advisable to follow full earthing-up at planting.

Off-season management of field: The pathogen perpetuates in the root system of many weeds and crops. Clean the field from weeds and root/foliage remnants and burn them. The pathogen in remnants can be exposed to high temperature above 40 ^{0}C in summer in plains and plateau and low temperature below 5 ^{0}C in hills by giving deep ploughing. This may cause extinction of pathogen from the field.

Chemical control: Application of stable bleaching powder @ 12 kg/ha at the time of potato planting in furrow along with fertiliser reduces pathogen population from field and gives effective control. Based on intensive ecological and epidemiological studies at Central Potato Research Institute, Shimla, the following practices are recommended for checking the bacterial wilt in different agro-climatic zones of the country.

Zone I: It comprises of non-endemic areas like Gujarat, Maharashtra, north-western and north-central plains. This zone is characterised by hot and dry summer with scanty vegetation (April-June); temperature may go up to 40-43^{0}C. The bacterial wilt is no more a major problem. Therefore, deep ploughing in summer and use of disease free seed is adequate for the disease control.

Zone II**:** It includes north-western mid hills (up to 2200 masl), north-eastern hills and the Nilgiris. This zone comprises complex, diverse and risks prone areas. The zone is characterised by mild summer, profuse vegetation with a maximum temperature range of 26-30^{0}C. Winter temperature may go as low as 3-6^{0}C. Many weed hosts can provide perpetual niche for colonisation and survival of the bacteria. The use of disease free seed plus application of stable bleaching powder @ 12kg/ha mixed with fertiliser at planting, ploughing the field in September- October and exposing the soil to winter temperature are adequate for disease control. The application of bleaching powder can be substituted by 2 year crop rotation with crops like wheat, barley, finger millet, cabbage, cauliflower, knol-khol, carrot, onion, garlic etc. Early planting and early harvesting are also recommended.

Zone III**:** It includes eastern plains and Deccan plateau. Here, potato is cultivated as short day crop during winter months (October-March). Day temperature sometimes reaches 38°C. Heavy precipitation occurs due to western disturbances. The area is relatively rich in vegetation. Eastern plains and Deccan plateau have many symptomless carriers of the pathogen. Therefore, management of the disease is most difficult. However, the disease can be kept under check with practices like use of disease free seed, application of bleaching powder, blind earthing-up and ploughing in March and leaving the soil exposed to summer temperatures during April- May and crop rotations along with clean cultivation.

Zone IV**:** It includes north western high hills (above 2200 masl excluding Kumaon hills). This zone has a temperate climate with severe winters; daily temperature ranges from –10 to 5°C during December–January. Snow is common during these months. Bacterial wilt is not endemic and the use of disease free seed alone is adequate.

2. Soft Rot or Black Leg

Bacterial soft rot can cause significant loss of potato tubers at harvest, transit and storage. Losses under bad handling of the produce, poorly ventilated storage or transit may go up to 100 per cent. Soft rot bacteria usually infect potato tubers which have been damaged by mechanical injury or in the presence of other tuber borne pathogens. Bacterial soft rot develops much faster under warm and humid conditions. The disease also results in blackleg of foliage during the crop growing season.

Symptoms

Initially a small area of tuber tissue around lenticels or stolon attachment point becomes water soaked and soft **(Fig. 3a)**. Under low humidity, the initial soft rot lesions may become dry and sunken. Under high humidity, the lesions may enlarge and spread to larger area. Tubers in

advanced stages of decay are usually invaded by other organisms and the decaying tissue becomes slimy with foul smell and brown liquid ooze. The tuber skin remains intact and sometimes the rotten tubers are swollen due to gas formation. At harvest, many small rotten tubers with intact skin can be seen. The infected seed tubers rot before emergence resulting in poor stand of the crop. In another kind of symptoms called black leg phase, which develops from soft rot infected seed tubers in cooler regions, the affected haulms become black at collar region just above the ground **(Fig. 3b)**. Infected plants develop yellowing, start wilting and die early without producing any tubers. Stem and petiole has been observed in some locations. Water soaked lesions develop on succulent stems, petioles, and leaves. On stem and petioles, the lesions first enlarge into stripes, turn black and then invade the affected parts causing soft rot and toppling of the stem and leaves.

Fig. 3: Soft rot symptoms on tubers (a); black leg symptoms on stem near soil line (b)

Causal organism

Pectobacterium atrosepticum (syn. *Erwinia carotovora* sub sp. *atroseptica*), *Pectobacterium carotovorum* sub sp. *carotovorum* (Jones) (syn. *Erwinia carotovora* subsp. *carotovora*), *Dickeya* spp. (including *D. dianthicola, D. dadantii, D. zeae*) (syn. *Erwinia chrysanthemi*), *Bacillus polymyxa, B. subtilis, B. mesentericus, B. megaterium* de Bary, *Pseudomonas marginalis* (Brown) Stevens, *P. viridiflava* (Burkholder) Dowson, *Clostridium* spp., *Micrococcus* spp., and *Flavobacterium* have been found to cause soft rot. *Pectobacterium atrosepticum* the primary enterobacteria causing soft rots are gram negative bacteria, rod shaped with peritrichus flagella which can grow both under aerobic and anaerobic conditions, produce pectolytic enzymes and degrade pectin in middle lamella of host cells, breakdown tissues and cause soft rot and the decay. The decaying tissue become slimy and foul smelling and brown liquid oozes out from the soft rot affected tubers. About 1500 strains of pectinolytic *Erwinia* have been isolated from infected plants and tubers. The pathogen produce certain volatile compounds such as ammonia, trimethylamine and several volatile sulfides and early detection of such volatile compounds in storage could be used as a method to detect the disease at initial stage.

Disease Occurrence and Distribution

Bacterial soft rot of potato is found wherever potatoes are grown. The disease affect the crop at all stages of growth but it is more serious on potato tubers under poor storage conditions especially in warm and wet climate. Black-leg (*Pectobacterium atrosepticum*) phase of the disease is not common in India. It occurs only rarely in the Shimla hills in HP, the Kumaon hills in Uttarakhand, Ootacamund in Nilgiris and also in Bihar plains. Stem and petiole rot due to *Pectobacterium carotovora* sub sp. *carotovora* has been observed in Shimla, Jalandhar, Ambala, Panipat, Meerut, Agra, Kanpur, Allahabad, and Burdwan.

Disease cycle

Soft rot bacteria may be carried latently in lenticels, wounds and on surface of tubers without any visible symptoms and spread to healthy tubers in stores, during seed cutting, handling and planting. Water film on surface of tuber which cause proliferation of lenticels and creates anaerobic conditions and injury on surface of tuber predispose potatoes to soft rot. From soft rot infected seed tubers bacteria may enter vascular tissues of developing stems and can develop

black leg under favourable conditions. From black leg infected plants the pathogen can reach daughter tubers through stolons and initiate tuber decay at the site of tuber attachment. Decaying tubers in soil could serve as source of contamination for healthy tubers. The threshold level for disease development is about 103 cells of *E. carotovora* sub sp. *atroseptica* per tuber. Tubers harvested in wet soil, poor ventilation in transit and storage promotes the rot.

Survival

Soft rot bacteria may survive in soil, on tuber surface, lenticels, periderm, cortex, ground tissue and vascular tissue. Rotting and decay of infected tubers in fields or stores may cause extensive contamination of adjacent healthy tubers, which serves as the most important source of primary inoculums. Contaminated irrigation water, aerosols of rains, farm implements, soil micro-fauna, nematodes, earthworms, larvae and adults of some insects etc. also help in secondary spread of the disease. Excessive moisture creating anaerobic condition, high temperature, excess nitrogen, tuber injuries and poor ventilation during storage are the important factors helping in disease development.

Spread

In warm climates, where one potato crop follows another or where only short rotation cycles are applied, the bacteria can pass easily from one crop to the next, especially in poorly drained soil. The bacteria can be disseminated in the potato fields by irrigation water, insects, rain or bacterial aerosols. The pathogen may also spread through water during washing of the produce with contaminated water. Soft rot causing bacteria can also spread easily from diseased to healthy tubers during storage, handling and grading. Insects especially maggots of *Hylemyia* species may also transmit the bacteria from one tuber to another.

Management

Soft rot bacteria are carried deep inside the tuber, in lenticels and surface wounds making it difficult to eradicate. These quiescent bacteria proliferate in high moisture condition and require water film that cause anaerobic conditions leading to disease development. Surface injury predisposes the tubers to soft rot infection. Based on ecology and epidemiology of the disease following management practices have been worked out:

- In field, avoid excess irrigation, provide proper drainage and restrict nitrogen dose to 150 kg/ha.
- Adjust planting time to avoid hot weather during plant emergence. Harvest the crop before soil temperature rises above 28°C.
- Harvest the crop only when the tuber skin is fully cured.
- Avoid injury to tubers and sort out bruised/injured tubers.
- Treat tubers (for seed purpose) before storage with 3% boric acid for 30 min. and dry under shade.
- Store the produce either in well-ventilated cool stores or cold stores.

3. Common Scab of Potato

Common scab of potato caused by *Streptomyces* species cause superficial lesions on surface of potato tubers and affect quality of the produce. The affected tubers fetch low price in market due to bad look and also because deeper peeling is required before consumption. Seed lots exceeding 5 per cent incidence is rejected by seed certification agencies (in India) causing huge loss to seed industry (Shekhawat *et al*., 1999; Shrivastava and Sahai, 1997). This disease was first recorded in Patna during 1958. Since then, it has become endemic in various potato growing states. Its real impact is felt in state like Punjab and Lahaul valley of Himachal Pradesh where potato production is for seed industry.

Symptoms

Scab begins as small reddish or brownish spot on the surface of the potato tubers and its initial infection takes place during juvenile period of tuber. Infection takes place mainly through lenticels and surrounding periderm turns brown and rough. Lesion becomes corky due to

elongation and division of invaded cells. Under Indian conditions multiple kinds of symptoms have been recorded and they are grouped as (1) a mere brownish roughening or abrasion of tuber skin (2) proliferated lenticels with hard corky deposition, might lead to star shaped lesion (3) raised rough and corky pustules (4) 3-4 mm deep pits surrounded by hard corky tissue (5) concentric series of wrinkled layers of cork around central black core **(Fig. 4).**

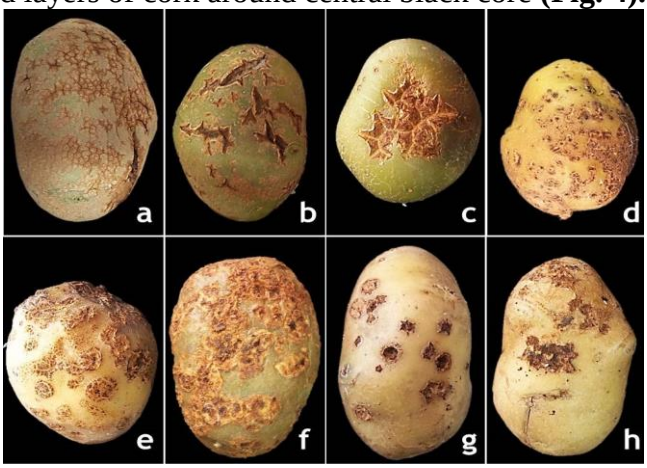

Fig. 4: Various types of scab symptoms caused by *Streptomyces* species on potato tubers

Causal organism

At least 13 different *Streptomyces* spp. have been found to cause common scab on potato worldwide. The prominent among them are *Streptomyces scabis* (Thaxter) Lambert and Loria, *S. acidiscabies* Bambert and Loria, *S. turgidscabis* Takeunchi, *S. collinus* Lindenbein; *S. griseus* (Krainsky) Waksman & Henria, *S. longisporoflavus*, *S. cinereus* , *S. violanceoruber*, *S. alborgriseolus*, *S. griseoflavus*, *S. catenulae* and others. *Streptomyces* are bacteria which resemble fungi due to formation of vegetative substrate mycelium that develop aerial filaments. However, the filaments are of smaller dimensions than the true fungi. These filaments produce spores through fragmentation. *Streptomyces* spp. may be pathogenic or non-pathogenic. The pathogenic species produce thaxtomins which are phytotoxins and cause hypertrophy and cell death. Considerable variation exists within the pathogen with respect to their pigment production in media, colour and shape of sporulating filaments and use of specific sugars. *S. scabies* form grey, spiral spore chains on several media and produce brown pigment whereas *S. acidiscabis* produce peach coloured wavy chains of spores and brown pigment in medium. The identification and taxonomy of *Streptomyces* spp. has been based on morphological and physiological characteristics combined with thaxtomin production and pathogenicity tests *in vitro* and *in vivo*. Ability to produce thaxtomin toxin is strongly correlated with the pathogen's pathogenicity.

Disease Occurrence and Distribution

Common scab occurs in most potato producing areas in Africa, Asia, Europe, North and South America. In India, the disease is prevalent in almost all potato growing regions. It is spreading fast in some areas in Indo Gangetic plains due to cultivation of potato year after year in the same land.

Disease cycle

Potato is physiologically most susceptible to *Streptomyces* spp. in the period following tuber initiation. *Streptomyces*species infects the newly formed tubers through stomata and immature lenticels. Once the periderm has differentiated, tubers are no longer susceptible to the pathogen. The pathogen is both seed and soil borne. It can survive in soil for several years in plant debris and infested soil. Soil conditions greatly influence the pathogen. Favourable conditions include pH between 5.2 to 8.0 or more, temperature in the range of 20 to 30^{o}C) and low soil moisture. The pathogen is aerobic in nature and maintaining high soil moisture for 10 to 20 days after tuber initiation can help in reducing the common scab.

Survival

The organism is tuber-borne and is well-adapted saprophyte that persists in soil on decaying organic matter and manure for several years.Infected tubers serve as inoculum foci in the field, giving rise to infected progeny tubers.

Spread

The pathogenic *Streptomyces* species are both soil and tuber-borne. Tuber-borne inoculum is likely to be involved in the distribution of new strains or species.

Disease management

The pathogen is difficult to eradicate because of long survival both on seed tubers and in soils. Therefore, following practices to minimize the inoculum and creating adverse condition for pathogen spread/disease development are recommended.

- Use only disease free seed tubers.
- Give tuber treatment with boric acid (3% for 30 min.) before or after cold storage; dry under shade before storage or planting.
- Irrigate the crop repeatedly to keep the moisture near to field capacity right from tuber initiation until the tubers measure 1 cm in dia.
- Maintain high moisture in ridges at least for a few weeks during the initial tuberization phase.
- Follow crop rotation with wheat, pea, oats, barley, lupin, soybean, sorghum, bajra, and adopt green manuring to keep the disease in check.
- Plough the potato fields in April and leave the soil exposed to high temperatures during May to June in the North Indian plains.

Suggested Reading

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Nutritional value of potatoes

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Potato (*Solanum tuberosum*) is an economical food as they provide a cheap source of available energy irrespective of every age's human diet. Potato does better than rice and wheat as edible energy source. Consuming potatoes just once or twice each day lowers high blood pressure almost the same as oats without resulting in increase in weight. Besides potatoes have more in store than just those unwanted calories, ensuring staying healthy. It is a staple component of the diet in many human cultures and source of many essential nutrients and is available all year round moreover may be cooked with various means (e.g. boiling, baking, and frying) prior to consumption.

Carbohydrates

Carbohydrate is almost universally the major dietary source of metabolic energy. The main energy-providing nutrient in potatoes is carbohydrate, in the form of starch. Potatoes contain both carbohydrate types' i.e. simple and complex carbohydrates that provide primary source of energy for the body and supply at least half of calories for the day. Sucrose, fructose are the main sugars present in potatoes. The advantage of getting carbohydrates from potatoes is that one can get a considerable amount of few micronutrients as well. Although, potato starches are less completely digested when fed raw but their digestibility is similar to that of the cereal starches after cooking.

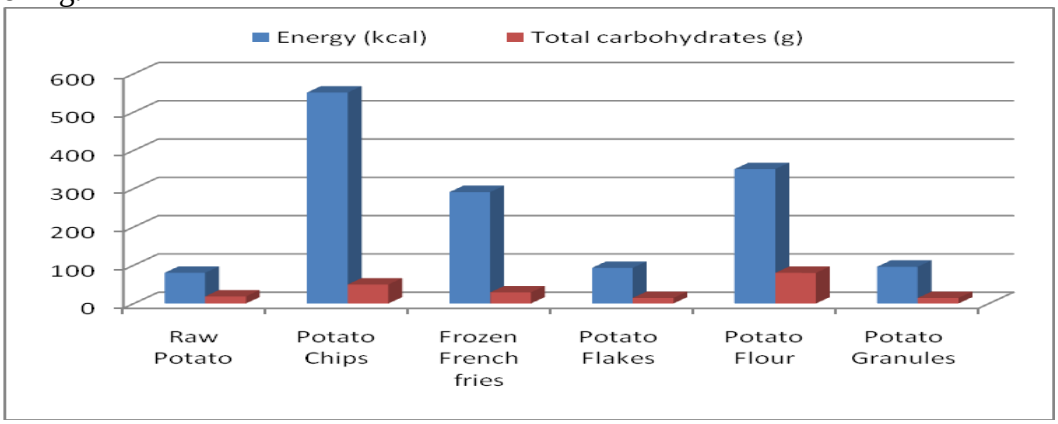

Fig.1. Energy and total carbohydrates provided by raw and processed potato products

Fat

When boiled or baked, potatoes are a virtually fat-free food and average fat content of potato is 0.1%. The little fat present in tuber gives palatability. About 60-80% of the fatty acid content is composed of unsaturated fatty acids (linoleic acid).

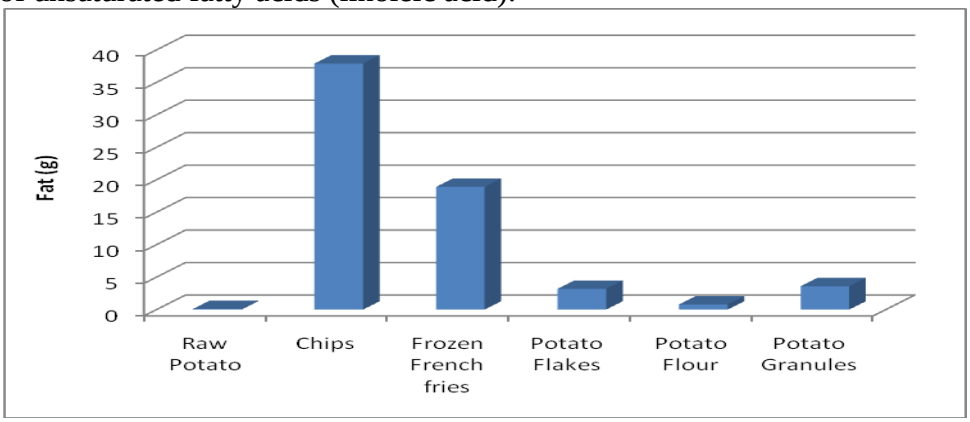

Fig. 2. Fat content in raw and processed potato products

Protein

Proteins are important constituents of cellular membranes as well as various cytoplasmic structures. Also the enzymes present in potatoes are made up of proteins. The essential nitrogen fraction in a potato tuber is protein nitrogen. Potatoes are commonly perceived as a carbohydrate source only, but they do contain high quality protein. On a fresh weight basis they contain only about 2% protein; the value increases to about 6-8% on a dry weight basis that makes potatoes comparable to cereals, such as rice or wheat. In countries, where potato consumption is high potatoes can make a significant contribution to health as a protein source. Potato proteins comprise 18-20 amino acids present in varying quantities. More than two-thirds of the non-protein nitrogen present in potatoes is present as free amino acids. A number of different amino acids are present in highest quantities in potatoes viz. lysine, aspartic acid, glutamic acid and valine.

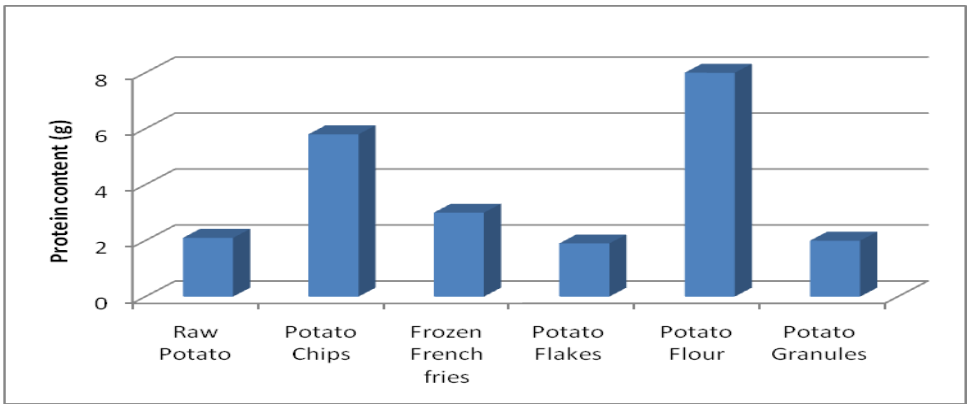

Fig.3. Protein content in raw and processed potato products

Vitamins

Of all the vitamins, potatoes contain vitamin C in the highest amount. Vitamin C is a water-soluble and its main component is ascorbic acid. However, dehydroascorbic acid has properties similar to vitamin C if it is reduced to ascorbic acid in the organism. It acts as an antioxidant stabilizing free radicals, thus helping prevent cellular damage. It aids in collagen production; assists with iron absorption and help support the body's immune system. Potatoes are a good source of vitamin B6, a water soluble vitamin that plays important roles in carbohydrate and protein metabolism. It helps the body by making amino acids that is later used to manufacture various proteins. There are several different B group vitamins, and potatoes are a source of some of these. A medium serving of boiled potatoes (180 g) contains more than one sixth of the adult daily requirements for vitamins B_1, B_6 and folate. These B group vitamins have many functions in the body including being essential components in the metabolism of carbohydrates to provide energy, and maintaining a healthy skin and nervous system. Folate is needed for cell growth and development.

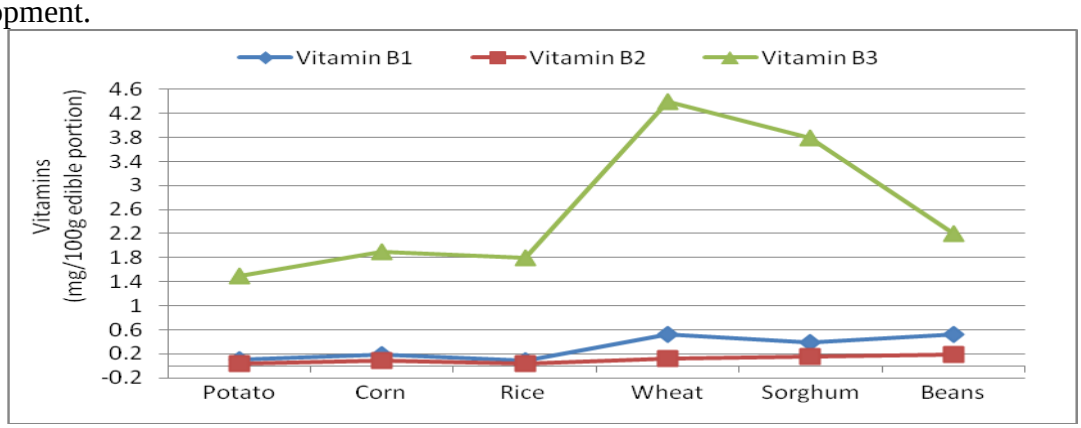

Fig. 4. B-Complex vitamins in raw potato and other plant foods

Minerals

Various important minerals and trace elements are present in potato. Potatoes are among the top sources of potassium. In fact, potatoes have more potassium per serving than any other vegetable or fruit, including bananas, oranges, or mushrooms. Diets rich in potassium and low in sodium (which together with chloride forms salt) reduces the risk of hypertension and stroke and help lowering blood pressure. Potatoes are a rich source of iron and this, coupled with the high vitamin C content, helps in its absorption. It is also a good source of potassium, phosphorus and magnesium. Zinc is an important trace element found in potato. Because of low phytic acid content of potato zinc availability is very high.

Phytochemicals

Phenolic acids and flavonoids are the most prominent phytochemical groups present in the potato. Bioactive compounds or phytochemicals are secondary plant metabolites found in the potato have been the subject of interest for researchers due to their promising role as health-modulators. Potatoes due to its high consumption is considered as the third largest source of phenolic compounds in the human diet after oranges and apples, thus potatoes can act as 'delivery mechanisms' for bioactive compounds. Colored-flesh potatoes are gaining popularity due to the potential health benefits of anthocyanins. Numerous health benefits such as antioxidant activity, anti-cancer and anti-inflammatory properties, have been attributed to consumption of anthocyanin-rich foods.

Dietary fibre

Potatoes are a good source of fibre, which contributes to the feeling of fullness, and supports healthy digestive functions. A 180 g portion of boiled potatoes provides about 3 grams of fibre, which equates to more than 10% of the daily recommended intake of fibre. Dietary fiber has been shown to have numerous health benefits, including improving lowering the risk of heart disease, diabetes, and obesity, and increasing feelings of fullness, which may help with weight management. Some people enjoy the stronger taste of eating cooked potatoes with skins on, and in this form they contain even more fibre.

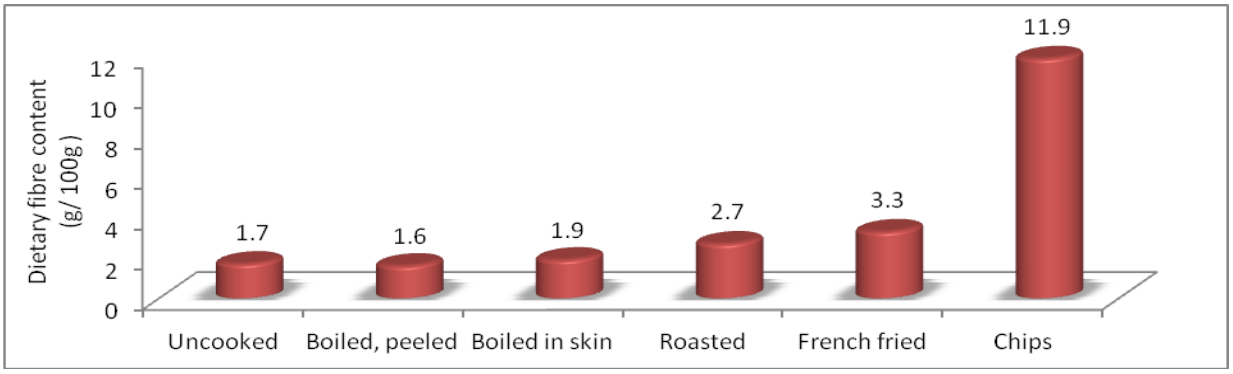

Fig.5. Dietary fiber content of potatoes when cooked by different methods

(Source: Ezekiel et al (1999) CPRI Technical Bulletin No. 49)

The main components of dietary fiber are non-starch polysaccharides (NSP), lignin, resistant starch and non-digestible oligosaccharides. Potatoes are a good source of resistant starch. A small amount of the starch in potatoes resists digestion that is called as 'resistant starch'. Amount of resistant starch depends upon the time and temperature during processing or cooking and amount of added water. Resistant starch acts in the body in a similar way to fibre, and too aids in the control of blood glucose and blood lipid levels.

Potatoes! awesome for good health

Potatoes are a very popular food source for human health. Food ranking system qualified potatoes as a good source of vitamin B6 (involved in more than 100 reactions), vitamin C, copper, potassium, manganese, and dietary fiber. Besides that, potatoes also contain a variety of phytonutrients that have antioxidant activity. Among these important health-promoting compounds are carotenoids, flavonoids, and caffeic acid, as well as unique tuber storage proteins, such as patatin, which exhibit activity against free radicals. UK scientists at the Institute

for Food Research have identified blood pressure-lowering compounds called kukoamines in potatoes. Potatoes are gluten-free and therefore can be consumed freely by people who need to avoid gluten (so cannot eat many common foods including bread, most breakfast cereals). It is a protein which is found in wheat and rye. Therefore, potatoes can be served as very important food for them.

Fig.6. Resistant starch content of different food materials

Misconceptions

There are numerous misconceptions about the nutritional value of the potato. It is often believed that the potato is a high-energy food that provides little else in the way of nutrients. Most people eat potatoes in the form of French fries or potato chips. Preparation and serving potatoes with high-fat ingredients raises the caloric value of the dish. Such treatment can make even baked potatoes a potential contributor towards fattening. Potatoes are not contributing towards fattening because potato itself contains very low quantity of fat (0.1%) and it is absorbing oil only when deep fried.

Nutritional value of potato processed products

Demand for processed potato products is increasing and potato products are liked well worldwide. Since potatoes need to be baked, boiled, fried, or otherwise cooked before consumption, it is of interest to determine to what extent exposure to heat affects nutritive value of the potatoes. Losses during chipping and canning are considerable but appear to be minor during boiling and frying. Processing up to some extent, have deleterious effect on nutrients because during cooking and processing diffusion of nutrients from the tuber make them low in concentrations. But it does not mean that processed potato products become low source of nutrients. Frying also avoids loss of water-soluble vitamins through leaching into the cooking water. For example, vitamin C concentrations of French fried potatoes can sometimes be as high as in raw potatoes, and thiamine is well retained in fried potato products as well as in fried pork meat.

Conclusion

Potato, a starchy tuberous crop and is an essential food item in the recipes of many countries around the world. While no vegetable dish is complete without the presence of potatoes and there are several countries where only potato is the principal food and is an excellent source of energy-rich diet. Potato is very easy to digest as well as contains mild roughage. The vitamin C content in potatoes is far better than most of the vegetables and fruits and is helpful for preventing the dreaded deficiency disease scurvy and frequent viral infections. Well, the fact is that potatoes are filling and non-fattening, contradictory to the popular belief. Potato is such a wholesome food that one can live by eating potato alone.

True Potato Seed technology

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Sexual or botanical seed of potato commonly called "True Potato Seed" (TPS) is a radical alternative to seed tubers for raising a commercial potato crop. Low multiplication rate, high storage and transportation costs, carry-over of pathogens, and physiological degeneration are some of the constraints associated with the use of seed tubers. Inevitably, performance declines as viruses accumulate due to which an elaborate and complex system of disease-free seed production is necessary. Costs of healthy seed tubers may account for 50 percent of the total production costs. Compared to seed tubers, TPS offers several advantages, such as low cost of planting material, reduced transmission of pathogens/pests, and convenience as well as inexpensiveness of storage and transport etc. With rare but important exceptions (e.g. potato spindle tuber viroid), TPS is indeed free of virtually all diseases including systemically transmitted viruses. By using TPS, a considerable amount of valuable potato stocks that is used as seed can be saved for consumption as food.

The possibility of raising commercial crop from TPS was first explored in India in late 1940s. The attempts, however, were not successful because efforts were mainly made to use self-seeds of cultivar Phulwa that flowers naturally under short days when potato crop is grown in plains. The produce of this population was highly heterogeneous for most of the economic characters and also had low yields due to inbreeding depression (Ramanujam, 1954). Renewed interest and formal research efforts to exploit the TPS as low cost alternative for potato production in developing countries were initiated by International Potato Center (CIP) in 1977 (Mendoza, 1984). At present, there are few potato producing countries in the developing world where TPS has not been tried. This paper reviews the developments in TPS technology vis-à-vis its problems and prospectus.

Production of hybrid TPS

The advantage of TPS technology has extended the use of botanical seed from mere breeding to raising of commercial crop. For this, production of TPS in large quantities becomes imperative. Generally, potato flowers under long photoperiod conditions. Such conditions are available in hills of our country where the crop is grown during summer. The TPS production in hills is however hampered owing to the regular occurrence of late blight and premature foliage killing of susceptible parents. Therefore, efforts were done to induce flowering under short day conditions. The main steps involved in hybrid TPS production:

i) Planting of hybridization block: Cut seed pieces (23-30g) or whole tubers of desirable parents are planted at 50 x 25 cm. Male and female lines should be planted in separate blocks under artificially extended photoperiod with the help of high density Sodium Vapour Lamps of 100/250 W per 100 sq.m. The extra light is given from germination till berry setting for 5 to 6 hours immediately after sunset. Male lines should be planted one week earlier and two to three batches to ensure continuous supply of pollen.

ii) Hybridization: Trim the female flower bunches to retain 6-8 large size buds per bunch. Collect right stage flowers from the male parents in the morning and remove anthers for storing overnight. Extract the pollen next morning by shaking anthers in a nylon tea sieve to separate the pollen and store in a refrigerator at 6-10^{o}C if not required immediately. However, the use of fresh pollen every day is more effective. Pollinate each receptive stigma twice by dipped in pollen at 8 hourly intervals.

iii) Harvesting of berries and seed extraction: Harvest berries 6-7 weeks after pollination and allow them to ripen at room temperature till they become soft. Macerate the berries by hand or using reverse screw juice extractor into pulp. Treat the pulp with 10% HCl and stir for 20 minutes to separate the seeds from debris. Wash the seeds with water 3 to 4 times to remove the

acid. Treat the seeds with 0.05% sodium hypo-chlorite for 10 minutes for surface disinfecting. Dry the clean seeds in shade followed by sun drying to reduce moisture contents to 5-6%. Pack the seeds in double polythene bags and store over calcium chloride as desiccant under temperature below 20 ^{o}C in a refrigerator.

Use of TPS

TPS can be sown directly in the field or in nursery beds, and seedling grown from TPS can be left in place or transplanted. Seedling tubers (i.e. tubers harvested from TPS seedlings) can either be sold as ware potatoes or used as seed tubers. Since plants grown from TPS generally produce more and smaller tubers per stem than plants from seed tubers, seedling tuber production is especially suitable to form the basis of a seed tuber system.

***Direct seeding*:** Potato true seeds are small and seedlings rather delicate, and as such make severe demands on horticultural skills. Thus, raising the crop from direct seeding (Fig.1) prone to risks involving failure due to poor germination and mortality of seedlings. This method is thus rarely recommended for raising a commercial potato crop. However, under experimental conditions direct sowing of 200 g TPS per ha and an emergence of 75% in well-controlled field conditions was reported to produce a yield of 28-30 tons of seedling tubers with an average weight of 25-30 g (Renia, 1995).

Fig **1. Direct seeding TPS crop**

***Seedling transplants*:** Raising seedlings in nursery beds and transplanting them to field is the possible alternative of TPS use for potato production (Fig.2). Nursery beds allow proper water and soil management, and ensure rapid germination and seedling growth. Sowing in nursery beds also permits off-season production as nursery beds can be protected against extreme temperatures and watered optimally. This shortens the growing period of the crop in the field. Transplanting requires good field conditions, assured irrigation and carries considerable labor costs. Direct seeding or transplanting for ware tuber production seems to have potential only in areas where consumers accept small tubers.

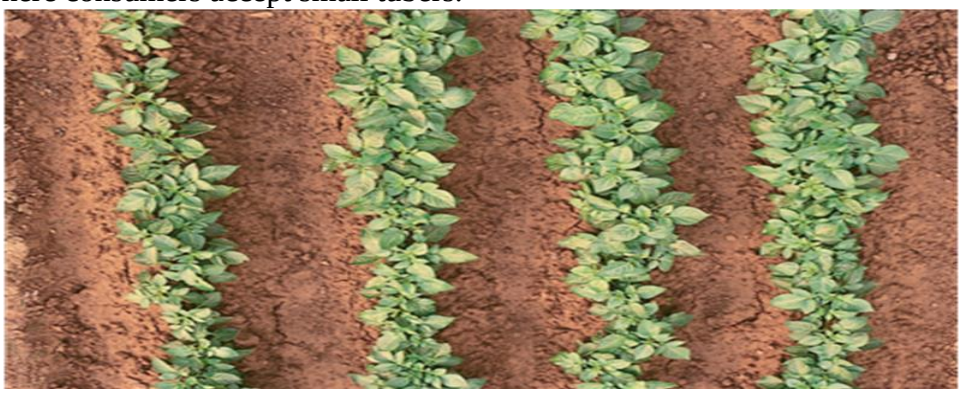

Fig 2. TPS seedlings transplant crop

***Seedling tubers*:** Seedlings raised in the nursery beds can be allowed to grow to maturity in the nursery beds itself to produce seedling tubers (Fig.3). Seedling tuber production in nursery beds or otherwise well-controlled environments is the most feasible way of using TPS at present. Maximum yields of seedling tubers in nursery beds are obtained at plant densities of 80-100 seedlings per m^2 resulting in seedling tubers of 1 to over 40 g with an average size of 10-15 g. Only 750 kg of seed tubers of an average tuber size of 15 g suffice to plant one hectare of potatoes, compared with 2-3 tons for 40-60 g seed tubers. This means that 6.0-7.5 g of TPS (0.60-0.75 mg per seed, 80% emergence) and 80 m^2 of nursery bed can produce seedling tubers for 1 ha potato crop.

Fig 3. TPS seedlings in nursery bed and seedling tubers

The use of seedling tubers or later-generation tubers from TPS- varieties is culturally similar to the use of tubers from conventional cultivars (seed rate, initial crop development, number of tubers per stem etc.). Also the yield potential of seedling tubers and later generations of selected TPS varieties compares well with that of clonal cultivars. The variation in performance of the tubers derived from selected TPS varieties is not significantly larger than the tubers from a clonal cultivar.

TPS Quality

The quality of TPS is determined by genetic constitution of the parents, stage of seed development, nutritional status of mother plant and biochemical components of the seed. Seed vigour generally defined as the superior performance of a genotype after planting compared to the same genotype or other genotypes under defined experimental conditions is a good indicator of quality of seed. Upadhya *et al* (1981) described an additional quality component of TPS concerning the type of embryo shape of the seed. Seeds with a cercinnate and inverted "U" shaped embryos are heavier than seeds with other types of embryos (rod shaped, aborted etc.) and give a higher percentage of germination and more vigorous seedlings with higher yield. However, we found that more than 90 percent seeds of both open and hybrid families have cercinnate and inverted "U" shaped embryos and these two classes of embryos were at par to each other for various seedling characters (Gopal, 2004). By eliminating the poor vigour seedlings before transplanting the seeds with undesired embryos get eliminated and help in improving the productivity of the seedlings.

Breeding TPS Families

The most dominating difference between potato crops grown from TPS and from normal sized tubers is the slow initial development of plants grown from TPS. As a result, a TPS crop has a slow development of the ground cover. It also gives the crop a longer and more vulnerable establishment in the field. Although there is variation between progenies, typically, a stem grown from TPS produces a larger number of tubers with a lower average tuber weight than a stem from a normal sized tuber. This characteristic is further accentuated when a larger number of stems per unit area are used in a crop grown from TPS to compensate for the slower ground-cover. Identification of TPS progenies having quick seed germination and fast seedling growth

speed up the seedling establishment and development of ground-cover, thereby improving on the most important drawback in the use of TPS.

Genetics and Breeding of TPS: The cultivated potato is a complex tetraploid which exhibits tetrasomic inheritance. As a result the segregating population of potato is heterogeneous for various characters. So selection of TPS families with acceptable level of plant to plant variability particularly for tuber characters is the major objective in developing productive TPS families. While inbreeding can give the highest level of gametic uniformity and homogeneity, resulting in completely uniform progenies, it also causes significant yield reductions and poor performance stability. Deleterious effect of inbreeding is also due to the fact that most of characters including tuber yield in potato are determined by genes with non-additive effects (Gopal, 1998) and increase in homozygosity results in the loss of such interactions including epistasis.

Hybrid vs. Open-pollinated Families: In potato, hybrid families are more productive than the open-pollinated (OP) ones. For the production of hybrids, Tuberosum-Andigena crosses are particularly useful, as heterosis for yield is spectacular in such families (Gopal *et al*, 2000). However, OP families from some genotypes can be as productive as the best hybrid families because OP seed in potatoes is not exclusively the product of selfing, it also results from out crossing, thus offering us the possibility of using them in a TPS breeding program with expected favorable results.

Short Duration Families

As mentioned above, TPS crop needs longer duration and does not fit into the prevailing crop sequences particularly in tropical and sub-tropical countries where this technology is considered to have best potential Two alternatives i.e. early foliage maturity and early tuber bulking exist for developing short duration families. However, the choice would depend upon the comparative TPS production potential of the parent(s) so selected.

Gopal (2004) found that foliage maturity has positive association with duration of flowering as well as intensity of flowering indicating that late maturing genotypes flowered profusely and for longer duration. Very early clones generally did not flower at all. On the other hand, early bulking as judged from average tuber weight had no association with any of the flower production attributes. Thus unlike early maturing genotypes, early bulker may flower profusely, early as well as for longer duration. Hence out of foliage maturity and tuber bulking, later is a better criterion for selection of parental clones for developing short duration (early bulking) TPS families.

Hardy Families

Seedling mortality after transplanting is the major risk to which TPS transplant crop is exposed to after seed germination. Gopal (2004) found that transplant survival was highly correlated with seedling vigour. Hardy seedlings thus can be indirectly selected before transplanting based on their vigour. Other characters namely leaf area, root area, root length and seedling dry weight were also associated with transplant survival to varying extent but seedling vigor was a better indicator of seedling transplant survival than the root area or root length. Vigor as indicator of hardiness also has the advantage that it can be recorded visually and effectively, without any damage to the seedlings, whereas recording of leaf area, root area, root length and dry weight is cumbersome and time consuming besides being destructive.

Seedling vigor and other characters governing hardiness can be improved genetically, as enough variability was present for these in a population of wild and cultivated species (Gopal, 2004). Heritability and genetic advance values were also reasonably high. However, it was found that seedlings of wild species were less hardy than those of the cultivated ones. Wild species, in general, also had poorer performance for all characters associated with transplant survival i.e. seedling vigour, leaf area, root area, root length and dry weight. Majority (70%) of the tuber-bearing wild species are diploids These are self-incompatible and set seeds only when crossed with some other strain possessing compatible S gene combinations. Triploid and pentaploid species are mostly sterile. Self-fertile potato species too prefer to reproduce vegetatively as theoretically infinite number of exact replicas of the initially successful biotypes

can be produced by this method (Hawkes, 1958). Predominance of vegetative reproduction thus might had led to poor seedling hardiness in tuber-bearing wild species. On the other hand, high vigour in cultivated potatoes might be because these had been subject to selection since domestication initially by farmers and later by potato breeders.

Advantages of TPS:

i) TPS requirement for planting one hectare land cost 20 times less than the seed tubers (TPS costs approx. Rs. 3000-3500/ whereas, seed tubers costs RS. 60,000-65,000/ for one hectare land)

ii) Several viruses, bacteria, fungi and nematodes which contaminate seed tubers get filtered during the process of pollination and fertilization. TPS is thus disease free and saves investment on seed health testing

iii) TPS crop comprises of segregating population which gives multiline effect providing better safe guards against disease epidemics

iv) TPS can be stored at room temperature under dry conditions without the loss of viability for many years

v) Unlike seed tubers which are bulky, there is no transportation problem with TPS

vi) By using TPS a considerable amount of valuable potato stocks which are used as seed can be consumed as table potato. Currently the country is using 3.1 million tonnes seed tubers which by 2020 will be 6 million tonnes

vii) Tuber seed production for present area under potato requires 40,000 ha land whereas for the production of TPS for the same area only 2000 ha land is required

viii) Disease free potato tuber seed can be produced only in northern plains and north-western hills, whereas, TPS can be produced in most parts of the country

ix) In tuber seed propagation varieties are likely to undergo pathological and physiological degeneration but in hybrid TPS the hybrid vigour is ensured.

Constraints in adopting TPS technology:

Rising of crop through TPS is labour intensive, needs constant attention and is more prone to damage by climatic vagaries like high temperature, heavy rains and draught etc. than the crop raised from seed tubers. TPS crop also takes about 20-25 days more for maturity than the conventional potato crop raised from seed tubers. Limited choice of hybrid TPS populations is also coming in the way of adoption of this technology. TPS being a segregating progeny, its produce is not so uniform as of the conventional potato crop and hence has lower market value.

Concluding Remarks

If we summarise the experience gained with this technology, we can safely conclude that direct seeding (drilling) as method to raise a TPS crop has failed, transplants were better, but seedling tubers, still better. Technically, a crop raised from seedling tubers cannot be regarded as a TPS crop. In my opinion, the only practical way to raise a truly TPS crop is through seedling transplants. Further, for production of seedling tubers too, raising a successful seedling crop either in nursery beds or as transplants in field is mandatory. In its present state a successful crop of TPS seedlings can be raised at the most in restricted niches where small farmers grow potatoes with irrigation and intensive management in small plots. Those who have experience of raising vegetables' seedlings and enough family labor could better manage potato seedlings crop.

Breeding for hardiness and early bulking using conventional as well as non-conventional approaches is thus absolutely essential if this technology is to be popularized on a larger scale. On the other hand, a little variability within the produce of a TPS crop might not matter particularly for consumers in developing countries. Rather this might even be biologically advantageous for achieving good disease resistance. Identification of parents producing open-pollinated seeds under varying thermo-photoperiodic conditions and having reasonably uniform tuber progenies with high tuber yield is another aspect that needs to be further researched upon. This will help farmers to produce their own seed at a low cost and getting good returns for their efforts and time spent on production of seedling tubers and then raising normal potato crop from

the seedling tubers. All this needs sustained efforts to overcome various intrinsic problems associated with TPS technology. The past long experience with this technology, however, makes one pessimistic about its future.

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Seed Potato Production Scenario in India

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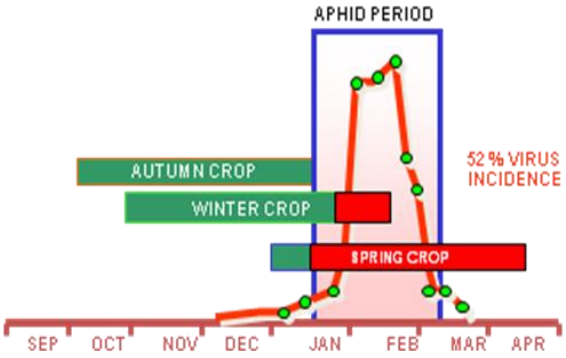
Availability of quality planting material has always been a limitation in vegetatively propagated crops. Potato being a vegetatively propagated crop is subjected to large number of viruses and seed-borne diseases responsible for degeneration of seed stocks over the years. It is therefore imperative to use good quality seed for economic production. Till 1935, the seed potato was being imported from various European countries on yearly basis, but during Second World War, European countries put a blanket ban on the export of potato seed to India. Therefore, Imperial Agriculture Research Institute started potato breeder seed production scheme at Shimla and Kufri during 1935. It was the beginning of seed potato production in the country. The seed potato from the hills used to be dormant for planting in the plains, therefore, either the dormancy was broken artificially or the system of late planting, somewhere in last week of December, was adopted to grow late winter or spring crop of potato. The spring crop was exposed to high population of aphids leading to very high viral infestation resulting in poor productivity in the subsequent generations.

In addition to this, the area under potato was quite marginal in the hills. It was therefore not possible to feed large area in the plains. Therefore, intensive survey was made in the plains to find out vector free period/low vector suitable for potato seed production. This laid the foundation of development of "seed plot technique" in 1959 which helped production of good quality seed in the plains so as to meet the bulk seed requirement for ware potato production in the sub-tropical plains. This technique drastically improved the health standard of seeds produced in plains. During the first three decades (1958-59, 1968-69 and 1978-79) a linear increase in area, production and productivity could be achieved largely because of the seed plot technique and improved varieties. The seed technology research and innovative quality seed production programme by public and private sector has played a vital role in potato production revolution in the country.



Seed Plot Technique

Refinement of Seed Plot Technique

All neighboring countries viz., Pakistan, Bangladesh, Nepal and Sri Lanka are importing seed from Holland, paying very high price. Seed Plot Technique not only benefited our farmers, our country also saved crores of rupees (approx. 3500 annually) on foreign exchange which would have gone for purchase of costly seed from foreign countries. The varieties released before 1963 ran out quickly because small quantities of seed were released to State Departments and progressive farmers, without them linking with the production of disease-free seed stock at the field level. It happened primarily because of low multiplication rate of potato seed, which is as low as 1:5 per generation. Because of high seed rate (25-30 q/ha) and low seed multiplication rate, newly released potato varieties took very long period for reaching to the farmers' field. This

necessitated a technology for quick production of healthy breeder seed of the released varieties in sufficient quantity. Moreover, very strict control was needed to maintain the health status of the vegetatively propagated crop.

Constraints in Seed Production

- Low rate of multiplication and high seed rate.
- Failure of State Department to multiply the Foundation seed.
- Limited land and infrastructure for breeder seed production.
- Limited area for production of quality seed.

Therefore, a well organized scientific strategy of breeder seed production was envisaged in 1962-63 through clonal selection, tuber indexing and stage-wise field multiplication of healthy indexed tubers in subsequent four generations. Indexing of tubers against contagious and insect transmitted viruses is done by ELISA against PVX, PVS, PVM, PVA, PVY and PLRV whilePALCV and PSTVd by PCR. Crop inspection, roguing of diseased plants and immune diagnosis are the regular features of the programme to improve the seed quality. The breeder seed produced by CPRI is supplied to various state Govt. organizations for further multiplication in three more cycles' viz., Foundation-1, Foundation-2 and Certified seed under strict health standards. However, the current status of breeder seed multiplication by the state government is not as per the desired seed multiplication chain. There is a huge shortage of certified seed in the country. The conventional system has the following limitations; i) low rate of multiplication, ii) requires more number of disease free propagules in the initial stage, iii) development of 100% healthy seed stock from infected material is slow and time taking, iv) progressive accumulation of degenerative viral diseases is there in each field exposure, v) require several field Multiplications of initial disease-free material (7 years).

The only way-out to overcome the above said limitations is augmentation of seed production through hi-tech system to improve the quality and to reduce the field exposure. Therefore, Central Potato Research Institute is gradually shifting from conventional system of seed production to hi-tech seed production system. Potato has readily responded to the totipotent nature of plant tissues in micropropagation and it has become easy to export/import disease free planting material in tissue culture form without any risk of importation of deadly diseases. The process of micropropagation has become much more important in the case of potato for the purpose of production of disease-free plants from infected one. There is a tremendous scope to increase healthy seed production vertically by adopting aeroponic technology where increase in multiplication rate from 5:1 to 50:1 can be achieved. We do not need any excess area for aeroponic based healthy seed production. Only one percent of conventional water usage is required which is basically recycled water. It is the ideal technology for cost-effective production of quality seed in the present era. The adoption of high-tech seed production technologies developed by the Institute has led to opening of more than 20 tissue culture labs throughout the country. Several private seed companies had been taking virus-free *in vitro* plantlets since last several years from ICAR-CPRI, Shimla of important released varieties for further multiplication in their seed production programme.

Currently CPRI produces 3186.82 tons of nucleus and breeder seed of 25 potato varieties, out of which 70% is through conventional system whereas, 30% through high-tech system, which is only sufficient to meet the demand of healthy seed potato in the country. However, keeping in view the production of 125 million tones of potatoes from 3.62 million ha by 2050, this supply of breeder seed is likely to fall short of the demand. ICAR-CPRI, targets to produce 4200 tonnes nucleus and breeder seed during 2050. As there is limited scope to increase quantity of breeder seed at ICAR-CPRI farms, due to limitation of additional availability of land for seed production therefore, possibilities are being explored with the help of SAUs/KVKs/Pvt. farmers to identify the new areas of seed production, multiplication of breeder seed into FS-I, FS-II and Certified Seed under MoU and to produce seed through hi-tech system with the help of entrepreneurs/private companies.

Future Challenges in Seed Production

- ❖ Impact of Climate Change on Vector dynamics and shortening of growing window.
- ❖ Emerging New Vectors like white fly, thrips, *A.gossypi* (aphids), hoppers, Psyllids.
- ❖ Emerging new virus diseases like PALCV, CMV, PAMV, PVYn
- ❖ Increasing pressure of soil & tuber borne diseases like common scab, russet scab, black scurf, brown rot, Sclerotium wilt, Sclerotinia stem rot, Verticillium wilt and nematodes.
- ❖ Monoculture of potato as well as Increasing cropping intensity.
- ❖ Production of quality seed in non-traditional areas

Possible Solutions

1. Multiplication of breeder seed in three clonal cycle i.e. Foundation Seed-1, Foundation Seed-2 and Certified Seed by all the state governments as per Seed Act, 1966 .
2. Multiplication of breeder seed under Public Private Partnership.
3. Adoption of tissue culture based high-tech system of seed production mainly Aeroponics.
4. Explore possibility of area expansion in non-traditional seed growing regions.
5. Increased participation of private sector.

Innovative extension strategies for technology transfer in potato

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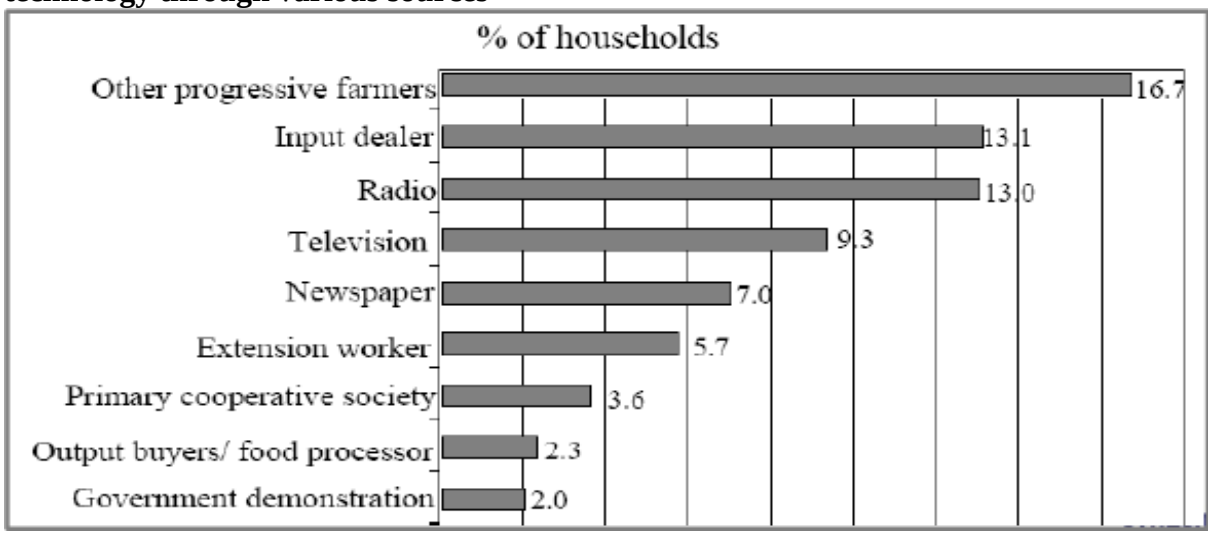
Diffusion of agricultural technologies to end user *i.e* farmers and other stakeholders, is of utmost importance for development of agriculture sector in the country. During the Green Revolution Period, Extension System focused on dissemination of technologies for major cereal crops lke rice, wheat and maize only and extension activities were largely carried out by State Departments of Agriculture (DOA). Other line departments, like Animal Husbandry (DAH), Horticulture (DOH) and Fisheries (DOF), had very limited extension capacity and primarily focused on the provision of subsidized inputs and services to farmers. According to the 2003 survey (NSSO, 2005), the major information sources for farmers on modern agricultural technologies was other progressive farmers (16.7 percent), followed by input dealers (13.1 percent), radio (13 percent), TV (9.3 percent), and newspapers (7 percent). Public-sector extension was used for information by only 5.7 percent of survey respondents. Most of the farmers sought information on seed for cultivation, followed by veterinary care in animal husbandry, and then management and marketing in fisheries.

Figure: Percentage of farmer households accessing information on modern agricultural technology through various sources



Source: Derived from data reported in NSSO (2005)

Agencies involved in diffusion of potato/other crops technologies in India

The last two decades witnessed declining support for public extension and emergence of a wide range of extension service providers in the private sector all over the world. The number and types of organizations providing extension services in India have also shown an increase. Potato is the third most important food crop of India after rice and wheat. Worldwide 325 million tones of potatoes were produced during 2009-10. India has now become the second largest producer of potato in world with a production of 36.57 million tonnes from an area of 1.83 million ha, thus contributing 11.25 per cent of total world production. The average productivity of potato in India is 19.9 tonnes/ha which is a little higher than world average of 18 tons/ha (FAO website). However, it is much lower than many European and American countries such as Netherland (45.6 t/ha), United States of America (44.4 t/ha), France (43.6 t/ha) and Germany (43.8 t/ha). One of the important reason of low productivity is lack of knowledge about scientific methods of potato cultivation and its storage practices among farmers and low level of adoption of improved varieties and technologies. Therefore, to increase the productivity of potato, proper diffusion of

potato technologies and its adoption among potato growers is necessary. Technology transfer in any crop including potato involves following agencies:

1. **Public-Sector Extension Agencies:**
   a. **State Departments of Agriculture:** Extension is implemented at the state level through the state Department of Agriculture (DoA). The extension staff of the DoA operate at the district and block levels, which are administrative subdivisions. The information is transmitted from the district and block extension staff to the village levels through contact farmers or para-extension workers.
   b. **Central Potato Research Institute (CPRI):** CPRI is a central institute of ICAR working on growth and development of potato sector in India. Its main aim is to develop new varieties and other technologies for improved potato cultivation and to increase its productivity. But, social sciences division of the institute conducts various extension activities to transfer modern potato technologies to farmers as well as other stakeholders. The institute organizes various training programmes for extension functionaries of different states as well as progressive potato growers round the year. In addition to training, it organizes kisan melas, exhibitions, seminars and conferences to diffuse the latest knowledge and technologies to farmers and other stakeholders like privates processing companies, input dealers etc.
   c. **Agricultural Technology Management Agency (ATMA):** This new institutional arrangement for agril. extension was designed to help farmers diversify into high-value crops and livestock enterprises as a means of increasing farm incomes and rural employment. The key elements of the ATMA model included organizing small-scale farmers, including women, into farmer interest groups (FIGs);linking these groups to markets; decentralizing extension decision-making down to the district and block levels and taking a more "farming systems" approach.

   d. **State Agricultural Universities (SAUs):** Almost, each state has a state agricultural university (SAU), which provides extension and training activities through the Directorate of Extension. The other extension activities of ICAR are achieved through the 40 Agriculture Technology Information Centres (ATICs) which works as single window delivery system for farmers and provide consultancy services and some inputs such as seeds and planting material and publications to farmers.
   e. **Krishi Vigyan Kendras (KVKs):** KVKs or farm science center, is a multidisciplinary educational institution situated at the district level, with funding and technical supervision from ICAR. There are currently 569 KVKs, almost one for each district in India. Each center is under the administrative control of a state agricultural university, NGO, or central research institute.

2. **Public-Private Partnership: Agriclinics and Agribusiness Centres (ACABC) Scheme:** Agriclinics and agribusiness centers (ACABC) provide agricultural advisory services to farmers through technically trained agricultural graduates at the village level, known as "agripreneurs." The objectives of the program are to supplement the public extension system, increase the availability of inputs and services for farmers, and provide employment to agriculture graduates. The role of an agriclinic is to provide expert services and advice to farmers, while agribusiness centers provide inputs and farm equipment hire.

3. **Private-sector Extension:** A large number of private agencies provide advisory and other support service to farmers engaged in agriculture and allied sectors. These include input agencies, farmer organizations, producer cooperatives, agro-processing companies, agri-marketing firms, NGOs, agri-business companies, individual consultants, consultancy firms, financial institutions, media and internet services. However, the

presence of these private extension providers is generally skewed towards well-endowed regions and high value crops. They are mostly profit oriented and therefore, rarely meets the need of poor farmers.

4. **Civil Society Organizations:** Due to the number of smallholder farmers, farmer-based organizations (FBOs) and self-help groups (SHGs) are key organizations to make extension demand-driven. This approach reduces many generic problems of public-sector extension, namely accountability to farmers and incentives for extension staff. Advantages of FBOs include the possibility of farmers achieving economies of scale and the shortening of the supply chain. Additionally, because the main source of information for farmers is other progressive farmers, belonging to an FBO or SHG may be an effective way of sharing information.

5. **Mass Media and Information and Communication Technology(ICT) Approaches:** To improve the quality and accelerate the transfer and exchange of information to farmers, ICT need to be given a high priority, particularly as a tool for improving the marketing aspects of farm enterprises. ICT has the ability to reach farmers directly, can enable two-way information sharing processes, has greater storage capacity, is faster, and can increase market efficiency by addressing information gaps and blockages. The mass media support scheme launched a Kisan Channel on Doordarshan, which telecasts agriculture-related programs. Kisan Call Centres is another central government scheme introduced to provide information to farmers on demand. Mobile phone based extension services are also found to be effective at some places and need to be promoted by govt.

Thus, it can be seen that India has a pluralistic extension system and many organizations are involved in transfer of technology activities.

Adoption of Agricultural technologies

The learning which is expected to result from the communication activities called extension is, in turn, expected to lead to desirable voluntary change in behaviour. When we look at extension as a function of Agriculture Knowledge and Information System (AKIS), its goal is to ensure adoption of agriculture innovations by the end users (farmers). This goal is achieved through transfer of agriculture information from the technology generating system to the technology utilizing system by using appropriate methods/media. The innovation-decision process is the process through which an individual (or other decision making unit pass from first knowledge of an innovation to forming an attitude towards the innovation, to a decision to adopt or reject, to implementation

of the new idea and to confirmation of this decision. Thus, the innovation –decision process consists of five main steps:

1. **Knowledge:** It occurs when an individual is exposed to the innovation's existence and gains some understanding how it functions.
2. **Persuasion:** It occurs when an individual forms a favourable or unfavourable attitude towards the innovation.
3. **Decision:** It is the process by which an individual engages in activities that lead to a choice to adopt or reject the innovation.
4. **Implementation:** It is the process by which an individual puts an innovation to use.
5. **Confirmation:** it is the process by which an individual seeks reinforcement of the decision already made.

Thus, at the knowledge stage an individual becomes aware of the innovation or technology through selective exposure. Mass media plays important role in creating awareness of the technology. At the persuasion stage, attitudinal change in the individual occurs through selective perception. The individual evaluates the attributes of the innovation or technology and

decides to adopt or reject it. At this stage the role of extension agents is very critical. The extension agents may persuade the individual to adopt the technology by bringing about favourable attitudinal change. Adoption is the process by which an individual make sure the use of an innovation to the best course of action available (Rogers, 1983). However, an individual may also decide to discontinue the adoption of a technology. Discontinuance occurs when the individual is not satisfied with the performance of the technology or when a superior technology replaces the existing one. Dissatisfaction ours when the individual lacks proper knowledge of using the technology or when the technology do no have perceived relative advantage. When discontinuance occurs due to dissatisfaction it, becomes impossible for the extension agent to persuade the individual to again adopt the technology.

Adopter categories

Based on relative earliness in adopting a technology, the ends users or farmers in a social system are classified in to different categories called adopter's categories. There are five major **adopter's categories** in any social system.

Innovators: They are the first to adopt a technology and do not require any persuasion from extension agents. These people are venturesome, cosmopolite and possess substantial financial resources to bear the risk associated with the adoption of a new technology. The constitute about 2.5 per cent of the total population.

Early Adopters: Principles they adopt the technology after the innovators have adopted it. They are localities opinion leaders and serve as role model for others in the society. They constitute about 13.5 per cent of the total population. The extension agent has focus on this category of people who may serve as local missionary for technology transfer.

Early Majority: They adopt the technology just before the average members of the social system. Their innovation-decision period is relatively longer than that of the innovators and early adopters. However, they serve as an important link in the technology transfer process between the very early and the relatively late to adopt the technology. The extension agent has to persuade these people through individual and group contact methods in order to make them to adopt the technology. Since, they provide interconnectedness in the systems network, they cannot be ignored at all. This category of people constitutes 34 per cent of the total population.

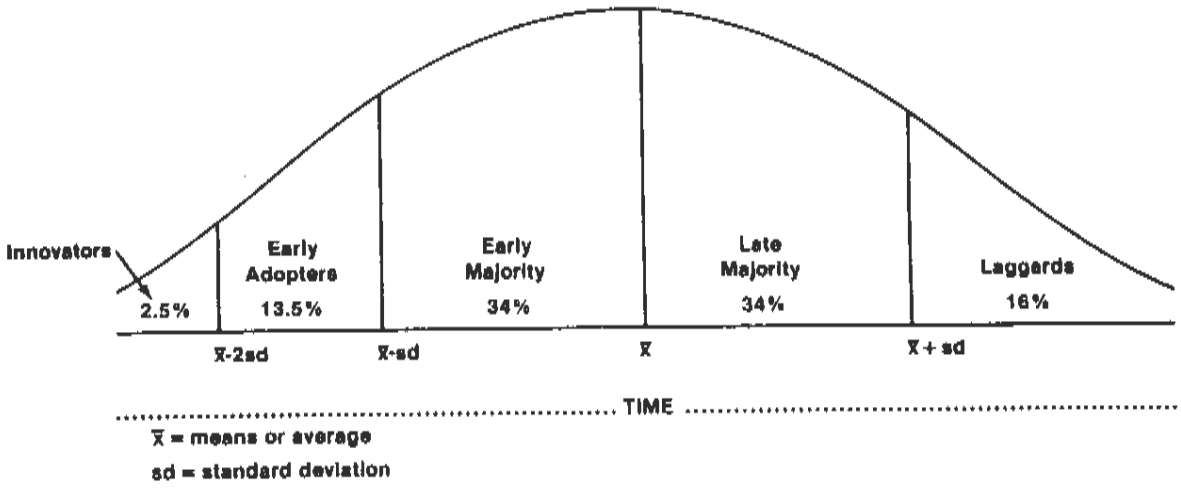


Fig: Adopters category with respect to earliness or lateness in adoption of innovation

Late Majority: They adopt the technology just after the average member of the social system. They are generally skeptical about a new technology and do not adopt it until most others in their social system have done so. They can be persuaded by extension agents to adopt a new technology, but the pressure of peers is necessary to motivate them.

Since, they possess relatively scarce resources to bear the risk, they adopt a new technology only when all their doubts are removed and they feel safe. They can be persuaded to adopt a technology only though individual contact method. This category of people constitutes 34 per cent of total population.

Laggards: They are the last in the social system to adopt a new technology. They possess limited resources and lake capacity to bear any risk. They are highly influenced by societal norms and traditions. They are also suspicious of innovations and change agents. They constitute about 16 per cent of the total population. When laggards finally adopt a technology, it may already have been superseded by another more recent technology. The laggards' precarious economic situation forces them to be extremely cautious in adopting a new technology.

Thus, in any social system 100 per adoption of technology cannot be achieved at time. Specific strategy has to be followed for each category of adopters in order to ensure grater adoption of the technology. The adoption behaviour of farmers very much conforms to the normal curve-limited percentage of farmers. According to Rogers (1983), the adopter of a technology fall on a normal distribution curve in which the percentage of different adopters categories may vary in different social system depending on the education, social economic status and other factors of the members.

Potato Pests and their Management

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Potato crop is attacked by a number of pests that can completely destroy the crop if left unattended Potato could be a home for many pests which includes insects, nematodes, rats, wild animals etc. In the order of importance, a wide variety of insects can damage potato crops either directly, through feeding on tubers, spoiling the produce and reducing the yields or indirectly by feeding on leaves or stems or transmitting the pathogens which also results in poor yield and quality both. In potato seed production, many need based suitable IPM programmes are there to control the insects but they can be different in different geographical areas for e.g. aphids are important in India but Colorado potato beetle is not important; nematode problem is being quarantined in Nilgiri Hills only and potato wart is restricted to Darjeeling area only.

Sap sucking pests

Aphids: Green peach aphid (GPA) *Myzus persicae* (Sulzer): *M. persicae* which is known as green peach aphid or peach-potato aphid is most important aphid worldwide. *M. persicae is* highly polyphagous and colonizes hundreds of plants belonging to more than 40 families. This aphid also attacks many ornamental crops such as carnation, chrysanthemum, flowering white cabbage, poinsettia and rose.

Feeding process of aphids: Aphids feed from the phloem of 3 main parts of the plant, stems, leaves, and roots with the stylets of their proboscis. Their stylets are contained within the proboscis when the aphid is not feeding. Stylets could suffer damage while being pushed into the plant phloem as they are very thin. Therefore aphids protects their stylets by secreting a special protective liquid from the tips of their stylets which starts to harden as soon as it leaves the styletsand forms a hard protective sheath around the stylets as they are slowly pushed into phloem tubes Aphids insert their stylets slowly and it takes quite a bit of time to tap into a phloem tube, it can be anywhere from 25 minutes to 24 hours from starting to insert the stylets to actually getting something to eat.

Transmission of viruses: *M. persicae* is a green or slightly reddish aphid which has peach as its primary host and a wide range of secondary hosts including many brassicas. *Myzus persicae* is cosmopolitan in temperate climates. It is highly polymorphic with varying colours from green to red and has complex type of life cycles. Aphids produce both apterous (wingless) and alate(winged) form. Alates have a shiny black dorsal abdominal patch. Adult females give birth to approximately 50 nymphs. It is very potential vector of more than 100 viruses which affects thequality and quantity of potato crop.Nymph and adults both are equally capable of transmitting viruses.

Flying abilities of aphid GPA: Aphids are weak/passive fliers and in still air they move at about 1.6 to 3.2 km per hour. Their migrations can be quite extensive and they often take advantage of favourable winds to enhance their flight efficiency. Aphids which fly upwards to get above the planetary boundary layer (PBL) early on in the night can end up more than 1000 meters above sea level and are carried by the low level jet streams that occur at these heights. Aphids can travel more than 400 km in 9 hours as a result of travelling on low level jet streams. When coming into land from these great heights the aphids home in on leaves reflecting long wave light, they are particularly attracted to the yellowish light emitted from young actively growing crops or older senescing ones.

Monitoring of aphids: Monitoring is must as aphids can disperse and reproduce rapidly anywhere, anytime as and when they found suitable conditions. There is good correlation between these 3 traps. When aphid count is 20 for by visual count then the corresponding figure for water traps and sticky trap would be 9.4 and 3 respectively.

Management: The most important challenge for potato breeder and seed potato grower is to produce seed tuber of high quality as the level of per cent virus infection level has been set up low for seed propagation programmes. It is a well known fact that aphid management is tough as it develops resistance by repeated chemical sprays so the best way is to keep seed material clean by removing unhealthy stocks even showing slight deformation on plant and the combination of mechanical, chemical, cultural control practices may be adopted for the aphid control. Vegetable/mineral oil could be a better option as it inhibits virus acquisition (i) inhibit virus attachment to mouth parts (ii) reducing probing behaviour. Monitoring of aphids is important which is done by placing the traps of yellow colour so that the arrival of the winged aphids is known and If big size traps are being used the number would be one trap/900m^2 for monitoring the aphid. For aphid control the use of mineral oil application is the most favoured tactics when population levels are low. Among insecticides oxydemeton metyl (1.25lit/ha) and imidacloprid (300ml/ha) gave good control.

Aphis gossypii: *Aphis gossypii* is extremely polyphagous has 700 host plants world-wide. Among cucurbits, it can be a serious pest on watermelons, cucumbers, and cantaloupes, and to a lesser degree squash and pumpkin and hence the common name "melon aphid." In the south, cotton is an important host, which explains the second common name, "cotton aphid." It is yellow to dusky green in colour, antennae and siphunculi are black, oval in shape, 1.5mm long and feeds on the underside of leaves, or on growing tips, sucking nutrients from the plant and forms dense colonies on the underside of the leaf. Their feeding also causes a great deal of distortion and leaf curling, hindering photosynthetic capacity of the plant. In addition, they secrete a great deal of honeydew which provides a substrate for growth of sooty mold which affects photosynthetic capacity of foliage.

Management: If insecticides are used to suppress melon aphid, care should be taken to obtain thorough cover of foliage. Leaf distortions caused by aphid feeding provide excellent shelter for the insects, so systemic insecticides are useful. Early in the season, aphid infestations are often spotty, and if such plants or areas are treated in a timely manner, great damage can be prevented later in the season. Use of insecticides for other, more damaging insects sometimes leads to outbreaks of melon aphid. Inadvertent destruction of beneficial insects is purported to explain this phenomenon. The wide host range of melon aphid makes crop rotation a difficult tactic to implement successfully. Also, crops grown down-wind from infested fields are especially susceptible because aphids are weak fliers and tend to be blown about. Infested crops should be destroyed immediately after harvest to prevent excessive dispersal, and it may be possible to destroy overwintering hosts if they are weeds. Continuous cropping systems may result in retention of aphid populations in that case a crop-free period is needed. Change in time of planting may influence build of aphid population.

It is difficult to disrupt transmission of non-persistent viruses with insecticides, so total dependence on insecticides is not advised. Row covers, whitewash sprays, and reflective mulches or coarse net covers are helpful in delaying or reducing disease transmission, but these are expensive options on large scale. Both aluminum and plastic mulch are reported to be useful for suppression of watermelon mosaic virus. Transmission of non-persistent viruses such as cucumber mosaic virus can sometimes be reduced by coating the foliage with vegetable or mineral oil. Oil is postulated to inhibit virus acquisition and transmission by preventing virus attachment to the aphid's mouthparts, or to reduce probing behavior. Oil seems to be most effective when the amount of disease in an area that is available to be transmitted to a crop is at a low level. When disease inoculum or aphid densities are at high levels, oils may be inadequate protection. This can be controlled by same insecticides which are used for *M. persicae*.

Whitefly, *Bemisia tabaci*: Whitefly was described over 100 years ago and has since become one of the most important pests worldwide in subtropical and tropical agriculture as well as in greenhouse production systems. Being highly polyphagous it feeds on large number of plant families. Over 900 host plants have been recorded for *B. tabaci* and it reportedly transmits 111 virus species.. The population build up of whitefly is heavy in September and October planted

crop in Indo-gangetic plains of India. These plains were identified as seed producing areas of potato because of low aphid periods in winters and that time *Myzus persicae* used to be a major problem in seed production of potato. Both adults and nymphs of whitefly suck the sap from leaves and many tiny adults could be seen on underside of the leaf. Now it has been proven a potential vector of a geminivirus which produces apical leaf curl virus disease in potato. The Geminiviruses/begomoviruses also circulate in the body fluids of whiteflies. It is a known fact that to control/manage whitefly is not easy but a challenge to grow virus free crop. The disease was successfully controlled by controlling the vector population with seed treatment with imidacloprid (0.04%) and first foliar sprays of imidacloprid (0.03%) at 85% germination and second of thiamethoxam (5gm/10lit of water) after 10days of first spray, adult population was also captured on yellow sticky traps.

Leafhoppers: There are several leafhoppers-*Amrasca biguttula biguttula* (Ishida), *Alebroides nigroscutulatus* Distant, *Seriana equata* Singh, *Empoasca solanifolia* Pruthi, *Empoasca kerrimotti* Pruthi *E. fabae* Harris, and *E. punjabensis* Pruthi which damage potato crop. The most important is potato leafhopper *Amrasca b.biguttula* which is polyphagous and phloem/mesophyll feeder. The adults and nymphs are somewhat wedge-shaped with heads that are slightly broader than the rest of their bodies. The newly hatched nymph is yellow in colour and can be located just along the midrib on the lower side of the leaf. Bigger nymphs and adults are pale green in colour, have piercing sucking type of mouth parts and can be easily identified as they move back and forth and hence the name hopper. Eggs are laid in the petiole and life cycle is completed in one month. Adults and nymphs both suck the sap from underside of the leaves hence they do the damage by direct feeding. Late instar nymphs are more harmful and feeding results more than twice yield losses compared with similar number of adults. The feeding results in drying of leaves which is known as hopperburn. Symptoms can be easily identified as wedge-shaped (triangular mark of burn) burning from the tip or may be cupping of leaves.

Management: Because of wide host range and highly dispersive nature of potato leafhopper the crop rotation is of very little use. Yield losses can be tremendous if it is not controlled within 30-40 days of planting. In early crop foliar sprays are recommended as the duration of crop is short it is harvested within 60-70 days. Foliar spray with imidacloprid is applied as soon as adult is seen.

Mite (*Polyphagotarsonemus latus*): This is commonly known as yellow mite or broad mite and polyphagous in nature. It has been reported on more than 100 plant species. Has very small size can not be seen with naked eyes. With very high fecundity it completes its generation in very short time (approximately 5 days).The injury it produces is often confused with diseases and phytotoxicity. It feeds by sucking the plant sap and inject toxic compounds in tender plant tissues. It usually feeds on lower surface of the leaf and causes leaf edges to become rigid and roll under. The symptoms can be identified by carefully observing the lower surface of the leaf which develop bronze colour. The plant, under heavy attack of mite cease to grow and die. Under heavy attack, losses are more and plant may yield nothing. Control with dicofol @0.2% or spiromesifen 0.04%.

Thrips, *Thrips palmi*: They are small insects. Life cycle may be completed in about 20 days at 30^{o}C.When crops mature their suitability for thrips decline. Eggs are deposited egg in leaf tissue, in a slit cut by the female. Larvae resemble the adults in general body form though they lack wings and are smaller. Larvae feed in groups, particularly along the leaf midrib and veins, and usually on older leaves. Adults are pale yellow or whitish in color, the slender fringed wings are pale. Thrips can be successfully controlled by the chemicals used for other sucking pests.

Root and tuber damaging pests

White grubs: They are polyphagous and cosmopolitan in nature. This was most destructive insect threatening potato production in hilly regions, but now it is becoming a problem in potato producing areas in plains too. In pains, white grub has long been associated with sugarcane crop and now causing the damage to potato crop. Twenty species of white grub have been reported on potato from India. The adult emerges with the first shower of rain in May. The damage is done

by second and third instar grub (larval) which feeds on underground part of the plant by making large shallow and circular holes in the tubers The grub stage which can be easily identified creamy white in colour with dark brown head and attains 'C' shape of English letter when disturbed. Tubers infested by grubs have low market value.

Management: 1.Deep plowing after harvest of potato is the best way to expose white grubs to high temperature and natural predatory birds. Similar plowing should be done at the time of planting also. 2. Removal of weeds from bunds around the field will reduce the chances of egg laying as eggs are laid on the grass bunds. 3. Deep placing of seed tuber is recommended. 4. Only well rotten Farm Yard Manure should be applied to the fields as this acts as attractant for grubs to feed on if not fully rotten. 5. Light traps can also be useful to catch the adults as soon as they emerge and kill them in water mixed with kerosene/summer oil. 6. For chemical control, damage can be minimized by the application of phorate 10G @ 15.0kg/ha at the time of planting or at the time of earthing-up which is a regular practice for potato production. Spray of chlorpyriphos 20 EC (0.1%) immediately after first monsoon showers on weeds and bunds around the field will reduce the number of grubs emerging out of eggs. Spray the crop (ridge portion) with chlorpyriphos 20 EC @ 2.5 l/ha after earthing-up to kill the larvae.

Cutworm (*Agrotis segetum* and *A. ipsilon*): Cutworms are polyphagous and most destructive insects. *Agrotis segetum* is commonly found in hills and *A. ipsilon* is common in plains. Peak activity occurs during May-June in Shimla hills, in August in peninsular India and in March April in Bihar and Punjab. In Bihar the tuber damage was 12.7 and in Himachal Pradesh 9.0-16.4% tubers were found to be damaged by cutworm. *Agrotis spinifera* occurs in Punjab, Bihar, Andhra Pradesh and Karnataka. Crop damage is caused by caterpillar (larva) stage only. They cause damage by- (i) young larvae feeding on leaves (ii) mature larvae by cutting the stem of the plant just near the ground and (iii) making irregular holes in the tubers. Smooth, grayish-brown, greasy and plump looking caterpillars are found hiding in the soil near to the stem of the plant during day time. Tuber damage can be from 9.0 to16%.

Management: 1. Exposing the larvae to bird predators is the best way. 2. For effective chemical control one should be able to identify the small larvae and the chemical should be sprayed at the appropriate time. The best time is when caterpillars are small and still feeding on the haulms. Once the caterpillar is big enough and moved to the soil, it is difficult to control as older caterpillars are generally less susceptible to insecticides than young caterpillars. Chemical control would be more effective when soil is dry and weather is warm.. For efficient chemical control thorough coverage of foliage with good amount of water is needed. Chlorpyriphos, cypermethrin and triazophos are used to control cutworms on potato.

Potato tuber moth: This insect has been causing damage in both potato storage houses and in fields in the country. The damage has been reported from Maharastra, Bihar, Madhya Pradesh, Uttar Pradesh, Kangra valley (Himachal Pradesh), Tamil Nadu, North Eastern hill states and plateau region and Karnataka. The range of infestation can be 30-70% in stored potato. The damage is severe under low rainfall high temperature conditions.

The adult moth prefers to lay eggs on green foliage than on tubers. Eggs are laid on underside of the leaf in the field the young larva feed inside the tunnel between two layers of leaf tissue and later on to leaves. The larvae destroy the crop by injuring the leaves and boring into petioles and terminal shoots causing wilting. After tuberization, the eggs are laid in the eyes of tubers through cracked soil or if tubers are exposed. The larvae enter into the tubers and feed on them causing mines. Because of the heat produced by the activity of larvae in the heaps the losses become huge due to tuber rot also. In country store the larvae bore into stored potatoes causing 18-83% tuber damage in NEH hills. Life cycle of PTM is completed in 21-30 days at 27-35^{o}C. Upper and lower threshold temperatures for PTM are 40^{o}Cand 5^{o}C.

Management of Potato Tuber Moth (PTM)

Physical and cultural: Storage of healthy, uninfested potato tuber is the best way to control potato tuber moth. Cultural practices can contribute significantly to reduce PTM infestation at harvest. Use of healthy seed tubers, Deep planting (10 cm) followed by proper earthing-up,

lifting of all tubers from field at harvest, destruction of self-grown potato plants would reduce the initial infestationand subsequent population build-up in storage.

Use of botanicals/biopesticides: Covering of potato heaps with 2.5 cm thick layer of chopped dried leaves of Lantana or Eucalyptus can prevent tuber infestation. Use of Granulosis Virus (GV) is extremely effective in reducing PTM damage. Use of sex-pheromones can be made bymass trapping PTM male adults. Chemicals-dusting of the tubers with 5% Malathion or 1.5% quinolphos (125g dust/100 kg potato)will result in good control of PTM but these potatoes should not be consumed.

Termites: The damage done by termite is more in rain fed crops than to frequently irrigated crops. They can be easily identified with brown head and dirty white soft body. These insects are social insects. It is the worker caste of the termite which damages the crops by damaging the roots as a result the leaves of the plant turn yellow and plant starts wilting and ultimately dries as well as making holes in the tubers. The tubers become hollow and filled with soil and that is the typical symptom of termite. The queen lives 5-10 years and lays large number of eggs 70,000-80,000 per day. Best method to control termite is to locate and destroy the termite nest and kill the queen. Crop residues should not be left in the field as they provide food to the termites.

Red Ant (*Dorylus orientalis*): They also have a habit of attacking the underground parts of the plant but do not avoid light. They can be seen in large numbers in the field. The ants seen in the fields are workers. Workers of red ant feed on tubers by nibbling and making small but deep circulars holes. The damaged plants wilt in sunlight and eventually dry up. The pest is very difficult to eradicate. Spraying of the crop and drenching of the ridges with chlorpyriphos 20 EC @ 2.5 l/ha

Wireworm: Wireworms are the larvae of various click beetles. The major damage occurs from the time of tuber initiation until harvest and reduces the marketable quality of potatoes. Wireworms bore into the tubers making cylindrical holes and bigger larvae do more damage. The economic threshold is low. The treatment may be initiated if wireworms are detected in a pre-planting soil sample. The presence of wireworms can be monitored by using baits (i) pieces of carrot can be buried in the soil about 7.5 cm deep and 2-3 days later they can be checked for the presence of wireworms (ii) 2-3 tablespoon of coarse whole wheat flour is taken in a small tightly tied nylon netting and if more than one wireworm/m^2 is found then field should be treated before planting or potato should not be planted in that field. Phorate used at the time of planting takes care of these worms.

Mole cricket, *Gryllotalpa africana*: This is sporadic in nature and reported from Bengal. Young plants/seedlings are attacked more. The damage can be 5-6% in plants and 10-15% in tubers. Eggs are laid in rainy season deep in the soil, nymphs live underground in branched burrows and feed on roots of cultivated wild plants. They can also damage newly planted seed tubers by tunneling inside. Both nymphs and adults come out in the night and feed on the leaves of the plants.

LEAF EATING CATERPILLARS

They are all highly polyphagous and there are few which cause damage to leaves only but cutting and chewing them but few not only feed on leaves but damage tubers too.

Bihar hairy caterpillar, *Spilosoma obliqua* (Walker): This insect is polyphagous and very common in mild winters and spring season. The larva can be identified easily as it has hair all over its body. The newly hatched larvae feed gregariously, in groups skeletonizing the leaves but in later stages as they grow up they feed in segregation completely devouring the leaf, by moving plant to plant and field to field.

Tobacco cutworm, *Spodoptera litura* (Fab.): It is sporadic pest and highly polyphagous. This is widely distributed world over. The caterpillar has the habit of feeding at night and during day time it hides in cracks and crevices. Freshly hatched tiny larvae feed gregariously by scraping the leaves. As they grow they feed singly. The infestation can be very serious if number is more. It has been observed in case of potato that bigger larvae enter the tuber and feed on it if leaves were not available.

Gram pod borer, *Helicoverpa armigera* (Hubner): behaviour is same as above two.

Management: These can be managed easily in early instars or when the larvae are small. Alternate host plants can be destroyed where they pass their younger stage and then move to potato crop. Eggs can be located on the underside of the leaves and even tiny larvae could be killed mechnically. To the kill the pupae, expose them by plowing fields. In severe attack foliar sprays of monocrotophos, quinalphoschlorpyriphos or malathion is recommended.

Leaf eating beetles, Epilachna beetles: Two species are common in India 12 spotted, *Epilachna ocellata* found in higher hills and 28 spotted *E. vigintioctopunctata* common in lower hills. Plant damage is caused by larvae and adults both. Eggs are laid on the lower surface of the leaf which are yellow in colour and easily identified and destroyed. For good control malathion, chlorpyriphos can be applied. Control is easy when larvae are small.

Flea beetle, *Psyllodes plana* Maulik: Adult feed by making small holes on leaves by chewing can be easily identified. The damage starts as soon as plant comes out of the soil. Adults could be identified as they jump immediately if disturbed. Weeds around the fields serve as their protected homes when crop is not there, adult feeds on leaves and larvae on roots of weed plants. They can be controlled by foliar spray of malathion, and chlorpyriphos (2.5 ml/lit of water).

There is no need to spray the crops separately for all these leaf-eating caterpillars as most of them can be controlled by malathion and chlorpyriphos.

Literature Search and Information Retrieval in Agriculture Sciences

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The ability to perform efficient literature search for retrieving most relevant information has always been a challenge for researchers. This challenge has been further complicated in the present era of exponential growth of scientific information in volume and variety of formats. This paper will cover essential aspects of literature searching, including devising a search strategy and selecting sources to search. Examples will be drawn from the field of agricultural research in general and potato in particular. The implications of the semantic web, Web2.0 and Open Access on literature searching will be discussed, and the role of agricultural libraries in present day agricultural information landscape will be highlighted.

Introduction

"Literature search and information retrieval is a well thought out and organized process to search and retrieve all literature published on a given topic". A well-structured literature search is the most effective and efficient way to locate evidence based information on the subject being researched. Evidence may be found in books, journals, government documents and on the internet.

The literature search in today's agricultural information landscape differs strikingly from that of a generation ago. The abundance of scientific information in volume and formats make researchers simply confused as what to search (which information), where to search (source) and how to search (techniques) to acquire the relevant and authentic information. To avoid this confusion one has to formulate an efficient search strategy. Search strategy here mean to the operations and decisions involved in the search process from the time a user approaches the system with some information need till he construct a search statement that adequately and accurately expresses the information need of the user.

Why Literature Search?

Basically literature searching has two main goals:

(a) To retrieve only those references/papers that are relevant to the search question (this is called "precision/specificity"); and

(b) Not to miss any of those relevant papers (this is called "recall/sensitivity").

These goals are especially important in conducting the rigorous searches and retrieving the relevant information to support the decisions making and implementation of the best practices in agricultural research and farming by facilitating the evidence based authentic knowledge generated from systematic agricultural research that has been conducted using sound methodologies.

However to realize the aforesaid goals of literature search adoption of an efficient search strategy is a prerequisite.

An Efficient Search Strategy

In devising a search strategy that is both sensitive and specific, it is helpful to identify the different components of the question at hand, and formulate as many search terms (including synonyms) for each component as possible. A separate search is then performed for each component, combining all synonyms with the appropriate Boolean operator AND, OR, NOT. These steps, if executed properly, enhance sensitivity and ensures that only those references that match all components of the search question are retrieved, thus enhancing specificity.

In identifying search terms and synonyms for each component, it is useful to consider cross-cultural differences in nomenclature and spelling, trade names, and nomenclature that have changed over time.

Many search engines offer extensive thesauri/indexes to facilitate searching. In employing such thesauri, such as the AGROVAC (Agricultural Vocabulary) used by FAO as subject headings in

its AGRIS database and CAB Thesaurus for CABI database- a search is automatically broadened to include synonyms and word variants of the term that is searched. As citations/references are usually indexed based on the full-text of the article, a search that includes use of the thesaurus could yield citations that might otherwise have been missed. This is especially relevant in cases where no abstract is included in the database itself. However, using thesaurus for formulating search strategy has certain drawbacks also. In most databases a thesaurus based search is by default "exploded" to include all terms lower in the thesaurus hierarchy. This inevitably increases the number of retrieved records and often reduces the specificity of the search. Also, there is a time lag between the date an article is published and the date it is indexed. Consequently, when only thesaurus terms are used in a search, the most recent relevant articles might not be retrieved. Because previously indexed articles are often not retroactively modified to reflect changes made to the thesaurus. Thus, articles published before a certain term was added to the thesaurus will not be retrieved when that thesaurus term is used in a search. As such the use of thesaurus terms might decrease the specificity of a search and should always be accompanied by a search for free text terms or terms occurring in title and abstract only. This can be achieved by adding so called "field tags" to the selected search terms. An example of a field tag in AGRIS and CABI is "[ti]", which limits searches to the title fields. Adding the field tag [ti] disables automatic mapping of the search term to the thesaurus. In addition, it prevents searching in fields like "author name" and "affiliation", thereby increasing specificity.

Where to Search (Source)?

Like any other scientific discipline, the most comprehensive source for literature search in agriculture science is the electronic databases – bibliographic or full text - online or on CDs. Now a days they are considered to be the core source of literature search in agriculture.

The Electronic Databases:

It is important to consider the electronic databases in which the formulated search strategy will be executed. Although AGRIS and CABI, with over 10 million references from 1973 onwards having 3, 50,000 annual addition of records in agriculture and biosciences, is often used as the starting point for literature searching in the agricultural and biosciences [1, 2]. Still to make the search more comprehensive the other databases e.g. AGRICOLA, ASFA, Biological Abstracts, Current Content, Derevent Biotechnology Abstracts, PubMed and many more should also be taken into account for literature search.

All electronic bibliographic databases like CAB ABSTRACTS, AGRIS, or AGRICOLA can be thought of simply as very large collection of electronic files of research articles references/citations and their indexes. Although one can't physically see the records and the indexes en-mass within the database, the two are structured in the same way. They each have a section that contains the records as well as a separate section that contains the indexes that allow the user to "find" or "search" for the records in which the researchers are interested. In a typical printed abstract journal, such as Forestry Abstracts or Potato Abstracts published by CABI, the journal will have a section containing all the records as well as an Author and a Subject index at the back.

An electronic database works exactly in the same way as printed journal index. It has a section that contains all the records, each with its one unique record number, and a section that contains all the indexes used to search for those records. The one major difference is the size of the electronic database and the large number of indexes that can be created without the limitations that a print journal imposes. In the case of the CAB ABSTRACTS database, for example, there is an index of Title words, an Author index, a Source index, an Address field index, a Language index and several Descriptor indexes. In addition to these individual indexes, that each corresponds to an individual field within a CAB ABSTRACTS record, there is also what is called a Free-Text Index which combines many of these individual indexes into one large index and which allows the user to search for words and phrases from the complete record. The Free-Text index is the one that is most commonly used for quick and simple searches and is the index that is searched by default.

When we search for a term (keyword) in one of the search indexes, the system simply looks for the term in the index and, when it finds it, it counts the number of record numbers and returns a result to the search screen that says something like:

#1 7553 Potato

The exact format of the returned results depends upon the search interface being used. This is an example from CABCD where #1 is the number of the search, the 7553 is the number of records that the search has found, and the word Potato is the term that have been searched for. The reference numbers, within the search index, are used to locate and display the records on the screen.

How to search and retrieve (Technique)?

While going for literature search and information retrieval one has to follow the following steps.

Keywords and their Selection:

One of the most important aspects of searching is the selection of all the appropriate search terms or Keywords, as they are often known, and the building of the search profile. The steps to creating a search profile are as follows:

Understand the question:

Write down exactly what information you as an end-user want. If you are performing a search for someone else, make sure that you are absolutely clear about what it is they want.

Select all the important concepts:

From the search question, select all the search terms. A typical search question might be:

"I want 50 recent references about the irrigation of rice in South East Asia." Experienced searchers will often try and search for the phrase "irrigation of rice in South East Asia". If you do this, the system will look only for records that contain exactly that phrase and it is likely to find very few records if, indeed, it finds any at all. Instead, we need to select out the different concepts and keywords expressing those concepts, then search for each separately. In this example, the key concepts are:

Irrigation, Rice and South East Asia

What we now need to do is to instruct the search system to look for records that contain all three of these concepts or "Keywords"

Add synonyms, singulars and plurals:

Once you have selected the keywords from the search question, you should consider if there are any alternative words, scientific names, or spellings variants of those words that you should include in the search. Remember, the system only looks for what you ask it to. Typical examples are singular words or plurals, American versus British spelling, common names like Potato or scientific names like Solanum tuberosum L. The more variations that one can add to the search profile, the more records are likely to be retrieved, particularly when performing a **FREE-TEXT search**. Many databases will use a controlled indexing vocabulary like the CAB Thesaurus and, if this is available, it can be a valuable source of Keywords and alternative terms. For singulars and plurals, most search engines offer a technique known as truncation. You simply enter the singular term followed by a truncation symbol (usually an *, a $ or?) and this symbol tells the search engine to look for all words that start with the term entered.

As an example, searching Nutri*, on CAB Direct, would retrieve all the records containing the words Nutria, Nutrient, Nutrients, Nutrition and Nutritional. On CAB Direct, the Question Mark (?) can also be used for truncation where each? represents up to one character. For example, searching for CAT? Would retrieve records containing the word CAT or the word CATS but not the word CATTLE. The ? may also be used for character "masking" within a word, e.g. Int??net would search for Inernet or Intranet.

Creating the Search Profile:

When searching, each time a term or group of terms is searched, the system creates and stores a set of the records that it finds. In the very simplest of searches, the user may only create one set of records and then print or display them. However, in most cases, the search will need to be a little more complex than a single keyword. Searches for more complex subjects that contain

many keywords will require the creation of a more complex search profile, and will usually result in the creation of many sets of records, where these sets themselves are then combined to obtain the final result. Let's look at our example of Irrigation of Rice in South East Asia. We have already selected our three keywords as:
Irrigation, Rice and South East Asia
In a simple search, we would search for each term separately. This would result in the creation of three separate sets as in the following example:
#1 20972 irrigation
#2 27332 rice
#3 20072 south east Asia
This is the "Search History" and shows the three sets of records, the numbers of records found for each of the terms searched and the terms themselves. In order to complete the search to find the records that contain all three of these search terms, we now need to combine these three sets together.

Combining Sets of Records:

Sets of records are combined together using one or more of the three search operators AND, OR and NOT. These three words are often referred to as "Boolean Operators". So what do these words do?
AND is used to instruct the search system to retrieve only records that contain all the terms chosen. In our example, we want only records that contain all three search terms Irrigation AND Rice AND South East Asia. We don't want records that only contain Rice and Irrigation. The AND operator narrows down a search. It reduces the number of records but increases the relevance of those records.
OR is used to broaden a search; to make the set of records bigger. An example might be a search for all records about sheep or goats. Here the user wants all records that are just about goats plus any records that are just about sheep as well as all the records that might be about both sheep and goats. Here the search would be Sheep OR Goats
NOT is used to exclude certain terms from a search as in a search for records about Rice where the user is not interested in papers about the diseases of rice. Such a search would be performed as Rice NOT Disease*.
These Boolean Operators can be used directly with keywords, as in these examples, as well as with set numbers. If we go back to our example, the final search would be to combine the three sets with the AND operator:
#1 AND #2 AND #3

Note: In the case of a search on CABCD (Ovid's version using the Silver Platter search interface) you must include the # symbol, otherwise the system would simply search for records containing the number.
This final search creates a set of records, #4, which contains all the records that include all three of our search terms. So, to finish, let's take a look at the complete search history:
#1 20972 irrigation
#2 27332 rice
#3 20072 south East Asia
#4 197 #1 and #2 and #3
This shows that we have found 197 records about the irrigation of rice in South East Asia. Once the search has been performed, one can see the records, it is a good idea to check the records for other possible keywords that you may have missed in your original profile. Because you are able to create sets when searching, it is perfectly possible to continue to modify a search by adding in new Keywords, and creating and combining new sets of records until you are satisfied with the obtained results.

Text Mining and Semantic Search

All search techniques mentioned so far are based on finding specific terms within a database,

either directly, through the use of a thesaurus or filter, or by using the results of searches conducted by third-party sources. With the exponential growth of scientific information on the web, there is growing interest in different ways of searching using

Text mining techniques. In text mining, free text is searched for patterns, rather than individual search terms, to retrieve high-quality information. One approach to this effect may be on the proposed architecture of the semantic web [3] that focuses on the relationship between terms, thus taking into account their contextual meaning. Searching for relationships, rather than individual search terms, could enhance search specificity. Recently, an experimental biomedical search engine was launched that makes use of semantic search algorithms [4]. In addition, text mining could be used to improve cross-linking of information in biological and agricultural databases – for instance, those containing gene and protein sequences – with evidence in the literature [5]. There is ongoing debate in this field as to whether text mining should be facilitated a priori – for example, by letting authors prepare structured abstracts in a computer-readable format [6] – or whether it should be done a posteriori using natural language processing[7]. Finally, a triangle of publications, databases and end-users can be envisaged, with users annotating information in a Web2.0 environment like Facebook and Tweeter fashion [8]. Besides web publishing of scientific information, there is growing possibility of open access to scientific contents in agricultural sciences as it is gaining very active momentum world over.

Open Access

Knowing that information exists (for example, finding a citation/reference in AGRIS/CABI) is of little significance if the information itself is not available (i.e. the full-text article cannot be accessed). For example currently, only 53% of citations in CABI contain a link to the free full-text article [9], either through the publisher directly, or through open access journals and institutional digital archive/repository on agriculture. Over the last few years, the Open Access publishing model [10, 11] has been gaining momentum, resulting in a growing number of open access journals and institutional repositories. Many publishers are developing business models to accommodate Open Access – some examples are BioMed Central [12] and Springer Open Choice [13]. Moreover, various funding agencies – like the FAO at the international level and ICAR at national level promoting the open access movement in science of agriculture. Therefore, access to free full-text scientific articles in agriculture science will likely continue to improve.

Agricultural Libraries Role

The role of the agricultural libraries remains same "connecting users to his/her information" even in a changing information landscape, but on different magnitude and methods. Despite of well stacks of bound issues of scientific journals and books users have little inclination to come over the library to make use of library information resources rather they want to use it on their desktop, laptop. Now users want everything online digital. Therefore one of the main roles of the agricultural library today is to provide a digital portal to scientific information. On a library's website, multiple search engines can usually be accessed, including commercial search engines to which users would have no free access otherwise. Often, libraries provide a direct link from search engines to the full-text articles in journals to which the library subscribes. Apart from offering access to databases and full-text journal articles, the agricultural libraries can also play an important role in educating the users in information literacy skills, enabling them to search the scientific literature effectively. Almost all SAUs and deemed agricultural universities and institutions conducts one credit course in library usage, scientific writing etc at the PG and Doctoral level to educate and increase the efficiency in literature search and retrieval.

Conclusions

The ability to carry out an efficient literature search is a key skill for researchers faced with an abundance of online scientific information. Future developments in text mining techniques, as well as the Open Access movement are expected to further increase the amount of scientific information that will be available to end users. Agricultural libraries continue to play an essential role in the dissemination of scientific literature and the training/educating of information literacy

skills. These developments are definitely going to enhance the empowerment of users in information access and use of scientific information they need.

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Potato DUS Descriptors, Molecular Markers for Varietal Identification and Plant Variety & Farmer's Right

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Protection of plant varieties, rights of farmer's and plant breeder's is required under the preview of agricultural globalization as well as an encouragement for further development of new improved plant varieties. Government of India had sought for establishment of an effective system for this and enacted "The Protection of Plant Varieties and Farmers' Rights (PPV&FR) Act, 2001" adopting *sui generis* system. This act is in compliance with International Union for the Protection of New Varieties of Plants (UPOV), 1978 and also has provisions to protect the interests of plant breeders, individual farmers and of farming community. The legislation provides to implement Trade Related Intellectual Property Rights that supports the specific socio-economic interests of all the stakeholders including private, public sectors and research institutions, and of farmers. For the implementation of the provisions of the Act the Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture and Farmers Welfare established the Protection of Plant Varieties and Farmers' Rights Authority on 11th November, 2005 headquartered at NAS Complex, New Delhi. The authority is headed by The Chairperson who is the Chief Executive of the Authority. The authority comprise of fifteen members in addition to Chairperson, as notified by the Government of India. Eight of them are exofficio members on behalf of various Departments/ Ministries, three from State Agricultural Universities and the State Governments, one representative each for farmers, tribal organization, seed industry and women organization associated with agricultural activities nominated by the Central Government. The Registrar General is the ex-officio Member Secretary of the Authority.
PPV&FR Act caters the rights of both the plant breeders and farmers. It asserts the necessity and importance of recognizing and protecting the rights of farmers with respect to the their contribution in conserving, developing and selecting varieties, improving and making available Plant Genetic Resources for the breeding new plant varieties. The Act not only protects the new farmers variety developed by them but also to the value added to wild species or traditional varieties/ landraces through selection and identification for their economic traits. The farmer's rights considered their roles as users, conservers and breeders. Farmers are granted nine specific rights, which are as following:

1. **Access to seed (Section 39(1)**
   Farmers can save, use, sow, re-sow, exchange, share or sell their farm produce, including seed of protected varieties. However, they are not entitled to sell the branded seed of a variety protected under the Act.
2. **Benefit sharing (Section 26)**
   Farmers who provide Plant Genetic Resources to breeders for developing new varieties shall have right to receive a fair share of benefit from the commercial gains of the registered varieties. The Act combines the provisions for access and benefit-sharing along with Plant Breeder's Rights. Biological Diversity Act, 2002 permits use of genetic resources in breeding activities. PPV&FR Act necessities breeders to mention the geographical origin and nomenclature of the genetic resources used in the parentage of their new variety.
3. **Compensation (Section 39(2)**
   Seed material of the registered varieties must be sold with the full description of its agronomic performance under recommended management practices. When such seed fails to perform at farmer's field under standard cultivation condition, he/she is eligible to claim compensation from the breeder through the intervention of the PPV&FR Authority.
4. **Reasonable seed price (Section 47)**
   Farmers have the right to access seed of registered varieties at a reasonable price. When such condition is not met, the breeder's exclusive right over the variety is withheld under the

provision of compulsory licensing, and is obligated to license the seed production, distribution and sales of the variety to a competent legal entity. Compulsory licensing of protected varieties ensures adequate seed supply to farmers at fair price.

5. **Farmer's recognition and reward for contributing to conservation (Section 39(i)(iii) & Section 45(2)(C)**
   Farmers engaged in conservation and improvement and made significant contributions in providing genetic resources for breeding, get recognition and rewards from the national gene fund. The recognition and rewards are in form of Plant Genome Saviour Community awards given to farming communities and individual farmers for their contribution to *in-situ* and on farm conservation. Every year five Plant Genome Saviour Community Awards of Rupees ten lakhs each along with citation and memento to be conferred to the farming communities for their contribution in the conservation of Plant Genetic Resources. The Authority also gives ten Plant Genome Saviour Farmer Reward of Rupees one Lakh fifty thousand each with citation and memento and twenty Plant Genome Saviour Farmer Recognition of one lakh to the farmers engaged in the conservation of the Genetic Resources of the landraces and wild relatives of economics plants and their improvement through selection and conservation.
6. **Registration of farmers' varieties (Section 39(1)(iii)**
   The PPV&FR Act permits registration of farmer's varieties that fulfill the criteria for distinctness, uniformity, stability and has proper denomination/nomenclture. Farmer's varieties do not require the novelty trait. The registered farmer's varieties are entitled to all Plant Breeders Rights.
7. **Prior authorization for the commercialization of essentially derived varieties (Section 28 (6)**
   Farmers need to provide prior authorization for the commercialization of any essentially derived variety where farmers varieties are used by a third party as source material for the development of such EDVs. This facilitates farmers to negotiate for authorization with the breeder with respect to royalties and benefit-sharing.
8. **Exemption from registration fees and payments for farmers (Section 44)**
   Farmers have the privilege of complete exemption of any kind of fees or other payments. This includes fees of tests for distinctness, uniformity and stability, annual and renewal fee for registered farmer's variety and other services rendered by the PPV&FR Authority as well as for legal proceedings related to infringement or other cases in courts and tribunals.
9. **Protection from innocent infringement (Section 42)**
   The act considers of the old unrestrained rights that the farmers had over the seed of all varieties and the poor legal literacy among the farming community. In case of any infringement of the act if a farmer proves before court that he or she was not aware of the existence of any such act he or she will not be punished.
   In addition to farmer's right, breeders have exclusive rights to produce, sell, market, distribute, import or export the protected variety and can appoint agent/ licensee and may exercise for civil remedy in case of infringement of rights. Researcher can use any of the registered variety under the Act for conduct of experimental research as parental line for developing new variety. However repeated use needs prior permission of the registered breeder.

Potato DUS Descriptors

Potato DUS guidelines were based on 51 descriptors on all plant characters including:

a) Canopy (2 characters): Plant- Foliage structure; Time of maturity (days)
b) Stem (8 characters): Solidity; Cross Section; Height of main stem (cm); Predominant colour; Secondary colouration; Distribution of secondary colour; Plant Wing; Wing type
c) Leaf (9 characters): Leaf Structure; Anthocyanin colouration of rachis; Anthocyanin colouration of midrib; Leaf Length; Leaf width; Leaflet (lateral) shape; Leaflet waviness of margin; Leaflet glossiness of upper side; Pubescence of blade at apical rosette
d) Flower (17 characters): Anthocyanin colouration of bud; Anthocyanin colouration of floral stalk; Anthocyanin colouration of pedicel articulation; Pedicel articulation position; Corolla colour; Corolla size (diameter); Inflorescence size; Anthocyanin colouration of outer side in

white flowers; Intensity of anthocyanins colouration of corolla on inner side; Anther colour; Anther cone type; Pistil type; Stylar length; Stigma shape; Stigma lobe; Premature bud dropping; Intensity of flowering

e) Tuber (9 characters): Predominant skin colour; Secondary skin colour; Distribution of secondary skin colour; Skin type; Shape; Depth of eye; Predominant colour of flesh; Secondary colour of flesh; Distribution of secondary colour of flesh
f) Lightsprout (6 characters): Predominant colour; Shape; Intensity of anthocyanins colouration at base of sprout; Intensity of anthocyanins colouration at sprout tip; Pubescence base; Length of apical sprout

DUS Descriptors testing site

DUS testing of potato are carried out at hill location CPRS, Kufri for floral characters and plain regions CPRIC, Meerut and CPRS, Jalandhar for sprouts, vegetative and tubers characters. The standard bed size of DUS test plot is 4.8 m^2 with 4 rows of 2 m length and 60×20 cm row to row and plant to plant distance in 3 replications of 120 plants each. The distinctiveness and stability observations are recorded in 30 plants or 30 plant parts ie 10 plants per replication excluding those in the border rows. Uniformity of characteristics is recorded on the plot as a whole by visual assessment in a single observation on group of plants or parts of plants. For uniformity in the sample size of 120 plants, the number of off-types must not be more than two.

Grouping characters

Grouping of varieties facilitate the assessment of distinctiveness. Traits that do not vary, or vary only slightly, within a variety with its various states are fairly evenly distributed across all the varieties in the collection are suitable for grouping. In potato predominant colour of lightsprout, predominant colour of stem, floral corolla colour and predominant colour of tuber skin are the four grouping characters.

DUS testing material (quality and quantity)

Exotic test planting material must be free of any quarantine requirements. The minimum quantity of planting material to be supplied by the applicant must be 300 fully matured, skin cured tubers immediately (not later than 15 days) after harvest for each year of testing. Medium sized tubers of 3.5-5 cm diameter, disease and pest free, healthy, no mechanically damaged tuber material without any chemical or bio-physical treatment is optimum for DUS testing trial.

Stages of observation:

1. 30 days after withdrawal from cold storage: Light sprout parameters are recorded at this stage.
2. Full foliage growth (50 days after planting): Canopy (compactness), stem and leaf parameters are recorded at this stage.
3. Full flowering: about 50% of flowers open, main period of flowering: Floral parameters are recorded at this stage.
4. Ripening stage (foliage turns yellow, after 90 days of planting): Canopy (time of maturity) is recorded at this stage.
5. Harvest maturity (115 days after planting): All tubers parameters are recorded at this stage.

Molecular markers for potato variety identification:

Distinctness, uniformity and stability test involves significant observations at each crop stage, time-consuming, based on mainly polygenically controlled traits and is influenced by environmental and location factors. This led to search for other robust techniques for variety identification/distinction. Biochemical markers have the disadvantages of protein interactions during different development stages and causes inaccurate results. Molecular markers have the advantages of reproducibility, less influenced by the environment, non stage or tissue specific, lesser cost etc.

RAPD markers identify polymorphism created due to insertions, deletions, translocations and inversions. In potato RAPD markers OPA-03 and OPC-04 led to identification and formation of distinct band pattern and identification of eighteen commercial potato varieties (Chakrabarti *et al*., 1999). Simple Sequence Repeats (SSR)/ Microsatellites are short tandem repeats flanked by

conserved DNA sequences. SSRs are ideal fingerprinting markers that detect multiple loci, co-dominant, cheaper, hypervariable, generate better quality amplicons, suitable of multiplexing, low template DNA requirement and higher reproducibility. Ghislain *et al*., 2009 developed potato genetic identity kit based on 24 SSR markers (Table 1) ie two markers for each chromosome separated by 10 centiMorgan distance except for markers on chromosome VII at 3 centiMorgan.

Table 1. SSR markers of Potato genetic identity kit and their chromosomal location

Chromosome Number	**Marker**	**Chromosome Number**	**Marker**
Chromosome I	STM5127, STG0016	Chromosome VII	STM0031, STI0033
Chromosome II	STM1064, STM5114	Chromosome VIII	STM1104, STI0003
Chromosome III	STM1053, STG0010	Chromosome IX	STM1052, STI0014
Chromosome IV	STI0001, STI0012	Chromosome X	STG0025, STM1106
Chromosome V	STI0032, STPoAc58	Chromosome XI	STG0001, STM0037
Chromosome VI	STM0019, STI0004	Chromosome XII	STM5121, STI0030

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Potato seed certification

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The purpose of seed certification is to maintain and make available to the public, through certification, high quality seeds and propagating materials of notified kind and varieties so grown and distributed as to ensure genetic identity and genetic purity. Seed certification is also designed to achieve prescribed standards. Certification shall be conducted by the certification agency notified under section 8 of the seed Acts, 1966. seed of only those varieties which are notified under section 5 of the seed act,1966 shall be eligible for certification.In the national policy of seed multiplication, the multiplication phases of seed have been grouped into three categories i.e. i) breeder or basic seed; ii) foundation I and II and iii) certified seed.

Breeder seed: breeder seed is seed or vegetatively propagating material directly controlled by the originating or sponsoring plant breeder of the breeding progamme or institute and/ or seed whose production is personally supervised by a qualified plant breeder and which provides the source for the initial and recurring increase of foundation seed.

Breeder seed shall be genetically so pure as to guarantee that in the subsequent generation i.e certified foundation seed class shall conform to the prescribed standards of genetic purity. The other quality factors of breeder seed such as physical purity,inert matter, germination etc. shall be indicated on the lable on actual basis. Breeder seeds are considered to be pure, disease free and no tolerance limit is fixed while for foundation (FS I and FS II) and certified seed the tolerance limits for viruses off-type, tuber-borne diseases and grades have been fixed by Government of India.

Foundation Seed: Foundation seed shall be the progeny of breeder seed, or be produced from foundation seed which can be clearly traced to breeder seed. Foundation seed produced directly from breeder seed shall be designated as foundation seed stage –I. thus foundation seed produced from foundation seed stage-I shall be designated as foundation seed stage –II.

Certified Seed: Certified seed shall be the progeny of foundation seed and its production shall be so handled as to maintain specific genetic identity and purity.

Breeder seed is only being monitored by the certification agency but it is not certified by them. In India, there are nineteen state seed certification agencies in different states certifying the seed of different crops. The states in which potato seed certification is in operation are H.P, J&K, Punjab, Haryana, U.P, Bihar, W.B, Assam, M.P, Gujarat, Orissa, Karnataka and Tamil Nadu.

CERTIFICATION STANDARDS OF POTATO: *(Solanum tuberosum l.)*

I. Application and Amplification of General Seed Certification Standards

A.The General Seed Certification Standards are basic and, together with the following specific standards constitute the standards for certification of seed potato.

B. Classification of seed potato on the basis of area of production: There shall be two types of seed potatoes, namely the Hills and plains-grown and shall be designated as Hill seed (HS) and plains seed (PS) respectively. Hill Seed (HS) shall be grown in the high hills generally 2500 meters above the mean sea level or in situations declared technically suitable for seed production plains seed (PS) shall be grown in such areas where aphid infestation is low during the crop growing season and which are technically suitable for seed production.

II. Land Requirements:

A crop of seed potato shall not be eligible for certification if grown on land infested with: -wart (*Synchytrium endobioticum* (Schilb.) Perc. and or cyst forming nematodes; -brown rot (*Pseudomonas solanacearum* (E.f. Sm.) E.F. Sm. or on-cyst forming nematodes within the previous three years; -common scab (*Streptomyces scabies* (Thaxt.) Waks. & Henrici).

III. Field Inspection:

A minimum of four inspections shall be made as follows:

1. The first inspection shall be made about 45 days after planting in the hills and about 35 days after planting in the plains to verify isolation, offtypes and the extent of disease infection with specific reference to mild and severe mosaics, leaf roll, yellows, brown rot and other relevant factors;

2. The second inspection shall be made about 60-65 days after planting for early varieties and about 70-75 days after planting for late varieties or at appropriate growth stage depending on the crop duration of the variety concerned to check isolation, offtypes and extent of disease infection with specific reference to mild and severe mosaics, leaf roll, yellows, brown rot and other relevant factors;

3. The third inspection shall be made immediately after haulms cutting/destruction in order to verify that haulms have been cut/destroyed by the prescribed date and in proper manner;

4. The fourth inspection shall be made about 10 days after haulms cutting/destruction and before harvesting in order to verify that no re-growth of haulms has taken place.

IV. Field Standards:

A. General Requirements

1. Isolation

The fields of seed potato shall be isolated from the contaminants shown in the column 1 of the Table below by the distances specified in columns 2,3 and 4 of the said Table

		Minimum distance (meters)	
	Foundation seed		Certified seed
Contaminants	Stage-I	Stage-II	
1	2	3	4
Fields of other varieties	5	5	5
Fields of the same variety not conforming to varietal purity requirements for certification	5	5	5

B. Specific requirements

Factor		Maximum permissible limits		
		Foundation seed		Certified
	Stage -I		Stage-II	
1	2	3	4	5
Off-types	I & II Inspection	0.050%	0.050%	0.10%
Plants showing symptoms of : -Mild mosaic	I & II Inspection	1.0%	2.0%	3.0%
-Severe mosaic, leaf roll and yellows	I & II Inspection	0.50%	0.750%	1.0%
*Total virus	-	1.0%	2.0 %	3.0%

**Plants infected by brown rot (syn. Bacterial wilt) (*Pseudomonas* (E.F. Sm.) *solanacearum* E.F. Sm.)	I & II Inspection	None	None	3 plants per hectare
***Re-growth of plants after destruction of haulms	IV Inspection	0.50%	0.50%	0.50%

*Of the two inspections, the higher virus percentage will be considered for the purpose of the specified limits of tolerance.

**The presence of brown rot infected plants within the specified limits of tolerance shall be permitted in the areas known to be infected with this disease. In case of plants suspected to be infected with brown rot, the neighbouring plants, one on either side should also be rogued along with tubers.

***Standards for re-growth after destruction of haulms shall be met at fourth inspection to be conducted about 10 days after haulms cutting.

Note: 1. All Off-types and diseased plants should be rogued out along with the tubers and destroyed. 2. Gaps in the seed plot should not be more than 10.0% 3. Haulms must be destroyed as close to the ground as possible before the date specified by the Certification agency. Failure to destroy haulms in time shall render the crop liable for rejection.

V. Seed Standards

A. Specification in respect of size and weight of seed material for foundation stage-I, foundation stage-II and certified class shall be as under

Size	Mean length and two widths at the middle of tuber	Corresponding weight
(a) Hill seed (HS) Seed size Large size	 30 mm-60mm above 60mm	 25-150 gm above 150gm
(b) Plains seed (PS) Seed size large size	 30 mm- 55 mm above 55 mm	 25-125 gm above 125 gm

Note: 1.The size of tuber will be decided either on the basis of mean of two widths of a tuber at the middle and that of length or on the basis of corresponding weight of tuber. 2. In a seed lot, tubers not conforming to specific size of seed shall not exceed more than 5.0% (by number) 3. (a) The seed material shall be reasonably clean healthy firm and shall conform to the characteristics of the variety. The tubers not conforming to the varietal characteristics shall not exceed 0.050% and 0.10% (by number) for foundation and certified seed classes respectively. (b) Cut, bruised, unshapy, cracked tubers or those damaged by insects, slugs or worms shall not exceed more than 1.0% (by weight.) (c) Greenish pigmentation on tubers will not be a disqualification for certification.

B. Maximum tolerance limit of tubers showing visible symptoms caused by the diseases mentioned below will be as follows:

Diseases (Contaminants)		Maximum permissible limits	
	Foundation		Certified
	Stage-I	Stage-II	
1	2	3	4
Late blight (*Phytophthora infestans* (Mont.) de Bary), dry rot (*Fusarium caeruleum* (Lib) Sacc.) or Charcoal rot (*Macrophomina phaseoli* (Tassi) G. Goidanich)	1.0% (by number)	1.0% (by number)	1.0% (by number)
Wet rot (*Sclerotium rolfsii*) Sacc.)	None	None	None

*Common scab (*Streptomyces scabies*(Thaxt)Waks. & Henrici)	3.0% (by number)	3.0% (by number)	5.0% (by number)
**Black scurf (*Rhizoctonia solani* Kuchn.)	5.0% (by number)	5.0% (by number)	5.0% (by number)
***Total diseases	5.0% (by number)	5.0% (by number)	5.0% (by number)

*Even if a single tuber infected with common scab is detected in a seed lot, the entire seed lot shall be treated with approved fungicide before seed lot is declared fit for certification. Seed lots having infected tubers more than the prescribed limits will not be certified even after treatment.
**(a) A tuber carrying 10.0% or above scurfed surface will be considered as one infected unit. (b) Seed lots having black scurf infection more than the prescribed limits could be certified after treatment with approved chemical/fungicide.
***For all diseases, the higher disease percentage will be considered for the purpose of the specified limits of tolerance.
Tagging : There are three types of tags with two specifications for different classes of seed.

S. No	Seed class	Tag colour	Size (cm)
1.	Breeder seed	Golden	12 X 6
2.	Foundation seed	White	15 X 7.5
3.	Certified seed	Blue	15 X 7.5

Quality control: Quality control test is being done to determine the genetic purity of a given seed lot of released cultivars and the extent to which the given sample confirm to the prescribed standards.
Sampling: The samples for grow out test is to be drawn simultaneously by the seed supplier and the seed recipient by following the standard procedure. In a lot of 100 quintal, 250 seed potato tubers are drawn by each party along with tag having details of source of seed, variety and lot number.
Procedure of grow out test: Grow out test is performed by growing the sample seed tubers in the next crop season as per standard package of practices. During this test, observation on germination, morphological characters and incidence of viruses are recorded to ascertain their genetic purity.

B. CERTIFICATION STANDARDS OF POTATO TISSUE CULTURE RAISED MINITUBER (PTCMT) STANDARDS

I. Application and Amplification of General Seed Certification Standards.

A. The General Seed Certifications Standards are basic and, together with the following specific standards constitute the standards for certification of PTCMT. As the name implies, these standards are applicable to tissue culture raised mini tubers multiplied under laboratory and greenhouse conditions as laid here.

B. The General Standards are amplified as follows to apply specifically to the PTCMT:

1. *Eligibility requirements for certification*
The PTCMT to be eligible for certification shall be from a source meeting the following standards for laboratory and greenhouse facilities.
i. Laboratory and greenhouse facilities used for production of plantlets/microtubers or minitubers shall be maintained free of potato insects or vectors of potato pathogens. Failure to keep such insects under control may cause rejection of all lots maintained in the facility. All potting or growth media shall be sterile. Clean water shall be used in a laboratory or greenhouse operation.

ii. Hygienic conditions should be maintained strictly during micro-propagation, potting, planting, irrigating, movement and use of equipment and other laboratory and greenhouse practices to guard against the spread of diseases or insects in the facilities used for seed multiplication.

iii. All micro propagation and greenhouse facilities must be approved, as per the standard/guidelines.

iv. The greenhouse (protected environment) must be insect proof and be equipped with a double-door entrance, provision for footwear disinfection prior to entering the protected environment and insect proof ventilation screening on intakes and exhaust openings. The persons entering the protected environment should use Wellington boots (Plastic boots)and change lab-coat in the changing area to reduce the chances of inadvertent introduction of vector or insects clinging to clothes.

v. The material being initiated for producing PTCMT must be of notified variety[1] and confirmed identity. It must be duly documented with respect to origin.

vi. The plants of a potato varieties being initiated for tissue culture should be tested in an accredited laboratory[2] for freedom from the following:

PVA, PVS, PVM, PVY, PYX, PLRV, PALCV, PSTVd and endophytic or epiphytic bacteria and fungi. Tests must be carried on a minimum of ten plantlets of each variety. For virus testing ELISA or an equivalent method should be used, for viroid RT-PCR should be used, and for fungi and bacteria light microscopy and culturing on media should be used.

2. *Classes and Sources of seed:*

i. The facility should use recognized aseptic initiation and propagation procedures' (i.e. follow procedures and use equipment, which will maintain sterile conditions as per standard tissue culture norms.)

ii. The initiating facility must maintain following information on each variety for review and audit by the Competent Authority once in a year: variety identification, date of initiation, origin and testing results from accredited laboratory.

iii. Tests must be carried out on a minimum often plantlets selected at random, for each variety by an accredited laboratory. No plant should contain PVA, PVS, PVM, PVY, PYX, PLRV, P ALCV, PSTVd and other endophytic or epiphytic bacteria and fungi.

iv. Valid pathogen testing results are required prior to the initiation of micro tuber production cycle or planting of test tube plantlets in the greenhouse.

v. PTCMT shall be produced and multiplied from certified *in-vitro* plants or microtubers, as per the requirements

vi. PTCMT shall conform to the same Minimum Seed Standards as Specified for breeder's seed.
vii. If required PTCMT may be used for producing Foundation stage-I and Foundation stage-II, which can be certified using existing potato seed certification procedure.

3. Controlled Environment Requirements:

i. All micropropagation and greenhouse facilities must meet the standards given above under eligibility requirements.

ii. The soil used for PTCMT production should not be infested with pathogen and pests of potato, particularly the following:

• wart *(Synchytrium endobioticum* (Schilb.) Perc.) or cyst forming nematodes;

• brown rot *(Pseudomonas solanacearum* (E.F. Sm.) E.F. Sm.) or non-cyst forming nematodes within the previous three years;
common scab *(Streptomyces scabies* (Thaxt.) Waks. & Henrici).

4. Inspection of Controlled Environment Facility Used For Products of PTCMT

a. The grower must notify the Competent Authority of his production plans well in advance of the planting.

b. The crop must be grown from certified basic *in-vitro* plants or micro tubers, which were produced, in an aseptic environment.

c. A minimum of three inspections shall be made as follows:

i. The first inspection shall be made 35 days and 45 days after planting for plains and hillsrespectively to verify growing conditions, extent of disease infection and off-types and also to confirm isolation requirement;

ii. The second inspections shall be made at 60-65 days after planting to verify off-types, disease infection if any and pathogen testing, on a representative sample, comprising of 1% of the plants with a minimum of 5 and a maximum of 25 plants sampled for each variety;

iii. The third inspection shall be made immediately after haulmcutting/ destruction in order to verify that haulms have been cut/destroyed by the prescribed date and proper manner.

iv. Effective sanitation practices including insect and disease monitoring and prevention must be adhered to.

v. Basic Stock can be planted in commercially available medium, which has not been recycled. If nursery beds are used, the substrate should be properly sterilized before planting.

vi. The greenhouse must be free from all potato and solanaceous plant debris before planting.

vii. No field-produced seed potatoes (including pathogen tested clonal selections), non-seed potatoes, nor any other solanaceous species of plants can be grown in the protected environment while used to produce Basic Stock.

viii. Varieties must be separated by appropriate partitioning of greenhouse to prevent varietal mixture.

ix. If testing performed by an accredited laboratory reveals the presence of banned virus (es), fungus or bacteria all the crops in the protected environment will be non-eligible for certification and the entire material will be destroyed.

x. In the eventuality of detection of insect (particularly aphids, thrips and white flies) vectors (for which yellow sticky traps should be put at least at three places in a greenhouse) by Competent Authority, the grower must provide post harvest test results to this Authority. A representative sample, representing each variety grown in the protected environment must be post harvest tested and if the results are negative for PVA, PVS, PVM, PVY, PYX, PLRV and PALCV and PSTVd, the crop will be assigned Basic Stock status or otherwise rejected.

5. Field Standards:

Field Standards for direct use of PTCMT as seed

A. General requirements

1. Isolation: Not applicable as plants are grown in greenhouse.

2. All micropropagation and greenhouse facilities must be notified by Department of Agriculture and Cooperation, as per the standards given above under eligibility requirements.

B. Specific requirements

Maximum permissible limits Factor	Maximum permitted (%)*
*Off -types	0.05
** Plants showing symptoms of: - Mild mosaic (Maximum) - Severe mosaic, leaf roll, yellows and apicalleaf curl (Maximum)	 0.05 0.05

* * Plants infected by brown rot (syn. Bacterial wilt) *(Ralstonia solanacearum)* *Maximum permitted before dehaulming **Maximum permitted at final inspection, though the diseases mentioned above are not expected to be present in tissue culture raised plants but it is essential to maintain a good crop hygiene.

C. Seed Standards for PTCMT

Factor	Permissible limit for certified class
Weight of mini tuber (Minimum)	1.0 gm
Physical purity(Minimum)	98.0 %
Germination/Sprouting(Minimum)	90 %
Varietal Purity (Minimum)	99 %
Seed-borne virus (Maximum)	0.01 %

Marketing and export of potato in India

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Fruits and vegetables are more difficult to market than to produce. Perishable and bulky nature of the produce makes marketing process more difficult and vulnerable to uncertainties to a higher extent. India has experienced silent potato revolution during last 65 years. In 1949 (when CPRI was established), India produced 1.54 million tonnes potato out of 0.234 million ha with an average yield of 6.58 t/ha. However, the country produced 45 million tonnes of potatoes from 1.9 million ha with an average yield of 23.68 t/ha showing an increase of 8.1, 29 and 3.6 times in area, production and yield of potato, respectively. At present India is facing a problem of plenty as far as potato is concerned. However, CPRI believes to take this production to 125 million tonnes by the year 2050 (Singh *et al*., 2014).

Potato production is not a problem in India and planners need to think from marketing end for further improvement. The reported post harvest losses for potato are 16% of the annual production (FAOSTAT). The export performance of Indian potato is quite dismal as we have rarely been able to export more than 0.5% of our domestic production (Kumar, 2005). Potato processing in India is growing fast, yet the proportion of processed potato is still low i.e. nearly 7.5% of the production against up to 60% in developed countries (Rana, 2011 and Singh *et al*., 2014). Other marketing functions such as grading, packing, storage and transportation etc. also need transformations and special attentions as for as potato is concerned (Pandit *et al*., 2003).

POTATO MARKETING SYSTEM

Conventional (APMC act)

Conventionally potatoes are taken to wholesale markets through different marketing channels and then go to consuming markets or consuming centres from these markets. A chain of market intermediaries such as field procurement/ assembling agents, forwarding agents, commission agents, wholesalers, and retailers are operating to complete the marketing process. However, some of these commission agents and wholesalers may act at more than one point. Due to lesser quantity of produce flowing through a particular marketing chain, the marketing efficiency of the process is generally low. For example, most of the small retailers have a target of earning Rs 400 to 800 a day and they accordingly adjust their marketing margins which may be up to 100%, or even more, of their purchase price.

Model Act: With the promulgation of Model Act in 2003 a new era of agricultural marketing was promised. The provision of creating private markets in various parts of the country resulted in varied performances in different states. Some of the states were quick in creating state of the art private markets having all modern amenities while the others showed lack-lustre interest. With the results big corporate houses to came and joined the business of procurement and retailing in agriculture. Since marketing margins under conventional marketing system were huge the new business model was assessed to be profitable for big companies.

Retail Chains: At present a large number of big and reputed corporate houses have shown interest in this sector. Very high level of apprehensions was built among the conventional retailers regarding the dangers to their existence. However, Dr P Chengappa, present Vice Chancellor at the University of Agricultural Sciences, Bangalore opined very early that fresh fruits and vegetables are a small part of this retail juggernaut and even if retail chains do very well, they will capture only 10 per cent of the market in another 10 years. Actually the retail chains have failed to corner a significant share of sales of fruits and vegetables in India. It is in spite of strong theoretical explanation that the new marketing system will eliminates market intermediaries by directly purchasing from the farmers and selling to the retailers, providing cheaper and better quality produce. Now the estimated 1.2 crore street vendors and small retailers in our country, out of which nearly 30 lac retailers and vendors sell fruit and vegetables,

are much assured about their livelihood sustenance even in every part of every urban area in India.

Future trading: Futures trading is an agreement between a buyer and a seller obligating the seller to deliver a specified asset of specified quality and quantity to the buyer on a specified date at a specified place and the buyer, in turn, is obligated to pay to the seller a pre-negotiated price in exchange of the delivery. However, for speculators futures trading is a process under which sellers make promises to deliver something they don't have; and buyers promise to accept delivery of something they don't want- and both legally break their promises. Here profit maximization is the prime objective of the speculator while loss minimization is hedger's aim. Both speculators and hedgers seldom allow future contracts to mature, by nullifying the contract with reverse sale or purchase of contracts. In India MCX, NCDEX and NMCE are the three major commodity future exchanges while the first two trade in potato. Major part of potato future trades take place in MCX and some details of the potato contacts are given in table 1. Price discovery, hedging and speculation are the main functions of future markets. For further details on the subject the readers may refer to Rana *et al*. (2008).

Table 1: Contract details (MCX Potato)	
Lot size	30 MT
Margin	6% of value
Leverage	16.67 times (Pay for 1.8 MT to buy a contract of 30 MT)

Contract farming: Contract farming is a written or verbal contract between buyer and seller of purchase/ sale of an agricultural commodity at pre defined terms and conditions. These terms and conditions are generally related to price, quantity and quality of the produce. Contract farming has been an important tool to cut risk in the procurement process of processing quality potatoes, seed potatoes and specialty potatoes. There are many contract farming models prevalent in various Indian states and some of them are exploitative too. The concept is still maturing in India and its popularization is required because from farmers' point of view contract farming can reduce price risk associated with excessive price volatility of farm produce. Potato is a preferred crop for the industrial contractors due to the importance of potatoes as suitable raw material in the processing industry. It is worth mentioning here that industry needs potato tubers having high dry matter and low reducing sugars contents. Seed supply chain of specialized processing varieties is another important issue in this business. Hence, the processing industry opts contract farming in order to exercise better control over quality and quantity of the required raw material. For further details on the subject the readers may refer to Singh *et al*. (2011).

IMPORTANT POTATO MARKETING FUNCTIONS

Grading: Grading is very important function and offers a convenience of choosing a product according to customers' taste and preference(s). In Indian potato markets grades differ from market to market. Since very high proportion of grading is done by human beings hence size under the same grade may differ from person to person. CPRI has developed mechanical graders, which are used by large farmers only. Much needs to be done on popularizing grading practices adopted in international potato markets. Due to non-adoption of such grading practices our potato exporters are facing a lot of problems. Now Indian markets have started rewarding well graded and sorted produce with the higher prices.

Transportation: The transportation cost in India is very high due to very slow effective speed of goods movement. Rail transport is quite cheap but railway's very low priority for potato transport creates problems for our domestic as well as export marketing. Frequent thefts in rail transport add to the problems. Bad condition of existing roads, absence of roads in rural areas and scarcity of express highways make our cargo movement very slow while frequent interruptions of cargo on inter state borders and traffic corruption aggravate the situation. Potatoes transported both using rail and road are liable to large scale spoilage due to hot weather and prolonged exposure to the sun. It creates wide price gap between potato producing and consuming area.

Cold Storage: Shortage of cold storage space in many states of India, erratic electricity supply, lack of cold stores on and near ports, inadequate cold chain and expensive cold storage or cold chain facilities create problems for potato marketing in India. We know that about 90% of potato in India is produced in Rabi season hence storage plays an important role in distributing supply over the remaining months of the year. Cold store operators also indulge in different malpractices such as changing/ stealing stored material. Farmers do not get any compensation even if the produce is spoiled due to the negligence of cold store operators. Except for few states where electricity supply is too much interrupted of obstructed, the cold storage facilities have been adequate. However, India needs to do a lot on the front of modernization of existing cold storage facilities. In order to cater to the needs of processing industry and consumers who dislike sweetness of conventionally cold stored (2-4 ^{0}C) potatoes, the popularization of potato storage at elevated temperature (10 to 12 ^{0}C) after CIPC treatment has proved to be a boon.

Processing: Food processing is the sunrise sector of India. The importance of this industry is not limited only to the contribution to the GDP, but it also provides many other desirable socio-economic benefits such as increased employment opportunities; improvement in income and lifestyle of the rural people leading to reduction of migration of rural masses to cities; and mitigation of huge post harvest and storage losses, specially, in fruits and vegetables. Tremendous potential of Indian potato processing industry and its rapid growth during recent past has been widely discussed (Chengappa, 2004; Rana *et al*., 2004; Pandey *et al*., 2006; Rana and Pandey, 2007; Singh, 2010; Rana, 2011; Singh and Rana, 2012). India possesses wide agro-climatic conditions and areas suitable for adequate and round the year supply of processing quality potatoes. In addition, now we have potato varieties that are bred according to the requirements of the processing industry. The increasing proportion of potato processing in India shall not only avoid glut like situations but also carry forward the potato revolution in India. However, estimation of the requirement of raw material (processing quality potatoes) for potato processing industry is of utmost importance.

McCain Foods is popularizing French-fries in India in anticipation of a strong demand for convenience and variety in food products. Changing eating habits, growing number of working women and acceptance of western life style, specially in large cities has encouraged setting up of a large number of fast food restaurants in India that serve French-fries too. However, McCain Foods experienced constraints in the availability of raw material for French-fries in addition to infrastructural bottlenecks in the country. The company set up 30000 tonnes French fries plant in Gujarat during 2006 which has now been upgraded to more than double its original capacity. French fries is the fastest growing potato product in our country. With the results other French fries unit have started processing in Gujarat during the recent years. Potato chips, potato sticks, and potato 'bhujia' are one of the most common other processed products available in markets. Besides our traditional potato products like dehydrated chips, papads, waries and Laccha etc. are also finding increasing popularity not only in India but among NRIs abroad also. Potato powder/ flakes is another rapidly growing segment of potato processing industry in India. New players are establishing facilities and the existing ones are expanding aggressively. The raw material requirement of potato processing industry and the corresponding demand for processed potato products can be viewed from table 2 and 3, respectively.

Table 2: Raw material demand of potato processing industry (million t)			
Product(s)	2010	ACGRs	2050
Potato chips	2.45	4.5	14.22
Potato flakes/ powder	0.29	7.6	5.44
Frozen potato products	0.06	11.6	5.40
Total	2.80	5.61	25.06
Source: Singh *et al*. (2014)			

Table 3: Demand of potato products (thousand t)				
Product(s)	2010		2050	
	Thousand t	Per capita g	Thousand t	Per capita g
Potato chips	612	506	3555	2194
Potato flakes/ powder	52	43	979	604
Frozen potato products	34	28	2808	1733
Total	698	577	7342	4532
Source: Singh *et al*. (2014)				

Exports: Although, Indian potato is free from major prohibitive diseases like wart, ring rot, black leg, and nematodes etc., yet due to the want of appropriate certification our seed is badly affected under sanitary and phyto-sanitary sanctions. Opinion on genetically modified potatoes and anti-dumping duties are potential trade barriers of the future for Indian potato exports. Very high levels of farm subsidies by developed countries distort international potato trade too. Unfair means adopted by Dutch seed-potato multinationals who have been reported giving expensive gifts and bribes to control their business in Bangladesh and Sri Lanka. Lack of long term and consistent potato exports policy is one of lacking initiatives at our part. Absence of required market intelligence i.e. information on international prices and preference and non-seriousness on quality assurance are costing us a lot. Time of product arrival in the international market plays a crucial role in earning profits.

Variety is a most important consideration in the mind of farmers going for buying seed-potato. A number of Indian potato varieties and hybrids developed by the CPRI are already grown in different countries including Afghanistan, Nepal, Bhutan, Bangladesh, Sri Lanka, Philippines, Madagascar, Bolivia and Vietnam either under Indian names or modified local names e.g. Kufri Chandramukhi (in Afghanistan), Kufri Jyoti (in Nepal & Bhutan) and Kufri Sindhuri (in Bangladesh & Nepal). Five Indian hybrids, I-654 (as CCM-69.1 in Mexico); I-822 (as cv. Krushi in Sri Lanka); I-1035 (as cvs. Montanosa in Philippines and Mailaka in Madagaskar); I-1039 (as cvs. India in Bolivia and Red Skin in Vietnam); and I-1085 (as cvs. Sita in Sri Lanka and BSUP-04 in Philippines) are being officially grown. Many countries are growing Indian potato varieties/ hybrids unofficially too.

Since many countries out of these are our actual or potential seed-potato importers, we have an important opportunity of increasing our exports to them. In most of our neighbouring countries potato in grown under short day conditions like India and the varieties as well as physiological age of seed produced under long day (temperate) conditions of Europe and Canada etc. are not suitable here. India is the only country in South Asia that has its own potato seed production programme, which further increases our seed potato export potential to these countries. The annual seed potato market in SAARC countries alone is 616,000 tonnes. Our exporters are getting inquiries even from the countries like Egypt and Turkey.

Bulk of Indian potato is harvested during January to March when fresh potato is not available in most parts of the Northern Hemisphere. It creates an important export opportunity for Indian table potato to many EU and other countries. Sri Lanka, Bangladesh, UAE, Nepal, Mauritius, Pakistan, Maldives, Singapore, Japan, Malaysia and Saudi Arabia are our existing or

potential table potato importers. Our table potato of Kufri Sindhuri and Kufri Jyoti varieties is in great demand in Nepal and Bangladesh while Kufri Chandarmukhi has high preference in Afghanistan (Singh and Rana, 2005). Iran, although an important exporter of table potato in one part of year, imports sizeable potato during the other part. India should target this potential market too.

India is gradually becoming an important supplier of French-Fries to countries like Mauritius, UAE, Sri Lanka, Malaysia, Bangladesh, Nepal and Singapore etc. MNCs are showing increasing interest to make India an outsourcing destination for their global French-Fries need. McCain Foods, a leading MNC in French-Fries has started large scale contractual potato growing for French fries with the Indian farmers specially in Deesa area of Gujarat. This year a lot of such potato has been exported by McDonald's, another MNC in instant potato processing, after buying from the McCain Foods. This is an example that needs to be emulated by other corporates in India. Besides this India has been exporting potato chips to Bangladesh, Oman, Sri Lanka, Maldives, Canada, Kuwait, UAE, UK and USA etc.

Dried potato products like potato flour, starch, dehydrated chips and flakes etc. are exported from India to Sri Lanka, UAE Maldives, South Africa and UK. This is an area where Indian exporters should also concentrate. There is an untapped potential of exporting canned or baby potatoes from India. Presently we are canning potatoes for the requirements of our military and para-military forces. Processing quality potato from India is getting demand from Middle East, especially Dubai. Countries like Japan, China, Korea, Malaysia and Singapore etc. have great demand for processing potato. We have three varieties suitable for processing namely Kufri Jyoti, Kufri Chandramukhi and Kufri Lavkar,; and four specially bred for processing, namely Kufri Chipsona -1, Kufri Chipsona-2 and Kufri Chipsona -3 for chipping purpose and Kufri Surya for making French fries. In India we have a lot of scope for growing potato suitable of processing under warmer conditions. This is a great opportunity we should exploit for the betterment of our national economy and potato farming.

Gluts

Glut is a market situation where supply of a commodity exceeds its demand and with the result prices of that commodity crash. This term is more commonly associated with the agricultural goods and perhaps the most with the potato. The years 1975, 1979, 1982, 1985, 1987, 1988, 1997, 2000 and 2003 were the glut years for Indian potato. Although now potato is not thrown on the roads yet farmers face low prices after every 3-4 years. Acreage adjustment to the last year's potato prices; seasonal and regional production of potatoes; release of many short duration and high yielding potato varieties; inelastic price and income elasticities of potato demand; inadequate cold storage capacity; under-developed potato processing industry; very low exports of potato as well as its products; seed being the most important cost factor of potato cultivation, farmers use last year's unsold (cold stored) potato as seed; market manipulations; transportation bottlenecks; farmers' financial commitments against the crop to be harvested; majority of small farmers going for early potato crop with the objective of taking three crops a year; unawareness of general masses on potato nutrition; and non-adoption of low-cost/ input technology are the main reasons for potato gluts in India.

Large scale technical expertise and huge investments in mechanisation restricts regular potato growers from switching over to other crops however, higher profits in some years tempt new farmers to jump into potato business increasing crop area significantly. There are some other biotic and abiotic factors that contribute their share to make it very difficult to avoid potato gluts. As a result of easy availability of loans in agriculture sector over the recent past, more and more farmers are falling under the burden of debts. Many potato farmers fear loss of their land and occurrence of suicide stories like cotton growers under the influence of more frequent and violent price fluctuations of the concerned produce. However, prudent behaviour at farmers' level with the adequate support at government and research level can go a long way in mitigating the impact of gluts on farmers and their families. Potato being capital intensive crop, gluts have

far reaching socio-economic adverse impact on potato farming community and it is very important to identify control measures for such gluts as well as to take concerted steps towards their proper implementation. Fortunately, the current market conditions indicate lower or even no incidence of gluts in agricultural commodities in general and potato in particular.

PROJECTED SCENARIO

Potato has been found assuming an important role of food security option in India (Singh and Rana, 2013). Rapid urbanization, rising number of working women, elevated tendency of eating outside and increasing number of fast food restaurants in India is increasing per capita demand of potato in India at an annual compound growth rate of 2.04%. The population of India is estimated to rise at an annual compound growth rate of 0.78%. The component wise analysis of this huge rise in estimated demand (including potato demand in processing and seed sectors in addition to the wastage on account of post harvest losses) has been given in table 4. The marketing of fresh, processing quality and seed potatoes will bring new challenges in the future.

Table 4: Component wise potato demand (million t)

Year	Demand$	Fresh	Processing	Seed	Waste
2010	35.10 (100.00)	23.94 (68.21)	2.67 (7.61)	2.96 (8.43)	5.53 (15.75)
2050	121.81 (100.00)	78.47 (64.42)	25.06 (20.57)	6.10 (5.01)	12.18 (10.00)

Note: Figures in parenthesis are percentages; $: net domestic demand
Source: Singh et al. (2014)

CONCLUSION

India is the second largest producer of potatoes after China however, cultivation of majority of potatoes in India under sub-tropical conditions pose highly area specific problems for us. More than 90% of our potato production faces hot summer soon after its harvest creating very strong need for cold storage in the country. Similarly more than 85% potato production takes place in North India states which emphasise the need of efficient transportation. Majority of potato growers being small and marginal are subjected to exploitation in the markets. Due to smaller volumes flowing in many marketing channels the marketing efficiency in India is low. Smaller holdings and marketable surpluses create conditions adverse for proper marketing especially grading, sorting and selling in the markets at the right time. The huge estimated rise in potato production in the future will further make the marketing process complicated and challenging.

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Economics of seed potato production in India

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The potato is one of the most widely cultivated horticultural crops in India. It is the number one vegetable crop in the country. During 1999-2000, it accounted for about 28 percent of the total 90 million tonnes of vegetable production in the country. The contribution of potato to the national agricultural economy is manifold. The potato crop is cultivated on 0.6 percent of the total cropped area but it contributes about 3.5 times more than both wheat and paddy per unit area to the national economy in agricultural sub-sector. For a developing country like India, where labour is surplus and capital limited, high yielding and labour intensive crops like potato, have added advantage in increasing food production and employment generation. Being a short duration crop, it fits well in relay cropping systems. Returns from investment on research and development are an important yardstick to judge the importance of a particular crop in the national economy. Outlay on potato research and development in 1998-99 was approximately Rs. 100 million, which was only 0.17 percent of the estimated monetary contribution of Rs. 58,750 million to the national economy.

The choice of a commercial crop enterprise like potato mainly depends on its profitability relative to other potential crop enterprises. This holds good strongly when the farm holdings are dwindling fast and if we take into account the fact that potato is cultivated by farmers belonging to all size-class of holdings. The farmer is motivated by various factors such as risk and uncertainty, his family needs and other socio-economic considerations in the formulation of his farm plans. But, in the ultimate analysis, the net returns per rupee of investment are the key factor for allocation of the farm resources. In this perspective, an understanding of the methodology to investigate costs and returns of crop enterprises and the economics of potato production assumes added importance for the farmers, researchers, policy makers etc. It helps particularly the growers in taking decisions on alternate efficient use of production inputs, allocating resources to different enterprises/crops for maximizing production, selecting crop combinations and deciding the levels of input use so as to economize expenditure and maximize returns.

Concepts, methods and estimation of costs:

The economics of potato production is determined by input prices, resource use efficiency, technology, management, fixed costs, productivity, price policy, product prices and external market conditions. The items of cost of cultivation cover the variable costs and the fixed costs, which are paid by the farmer only once for a period of time.

The variable costs incurred to produce a crop consist of those costs which can be directly allocated to the crop and fixed costs are those costs which would not be allocated to a particular farm enterprise and have to be incurred whether the crop is grown or not. This classification of costs into two categories is essential because a farmer like another entrepreneur will continue to grow the crop if he covers the variable costs in the short run and both the costs (total cost) plus a sufficient margin in the long run. The items covered under these costs are as follows.

Paid-out costs:

(i) Hired labour (human, animal and machinery (ii) Maintenance expenses on owned animals and machinery (iii) Expenses on material inputs such as seed (home-grown and purchased), fertilizer and manure (own and purchased), pesticides and irrigation.(iv) Depreciation on implements and farm buildings (such as cattle sheds, machine sheds, storage sheds(v) Land revenue and (vi) Rent paid for leased-in land

Imputed costs: Assessed value of family labour, rent of owned land, and interest on owned fixed capital, for which the farmer does not incur any cash expenses.

Cost concepts:

The comprehensive scheme for studying the cost of cultivation of principal crops was initiated in 1970-71 in the country. The cost concepts such as cost A_1, cost A_2, cost B and cost C and procedure for evaluation and allocation of joint costs were laid down (Kapre, 1974). These have been further improved and redefined (Govt. of India, 1990). Different cost concepts have their own implications in policy formulation. Accordingly, the items of costs and these concepts of costs are as follows:

Cost A_1 **(i)** Value of hired human labour, **(ii)** Value of hired bullock labour, **(iii)** Value of owned bullock labour, **(iv)** Value of owned machinery labour, **(v)**Hired machinery charges, **(vi)** Value of seed (farm produced and purchased), **(vii)** Value of insecticides and pesticides, **(viii)** Value of manure (owned & purchased), **(ix)** Value of fertilizers **(x)** Depreciation on implements and farm buildings, **(xi)** Irrigation charges, **(xii)** Land revenue, cesses and other taxes, **(xiii)** Interest on working capital, and **(xiv)** Miscellaneous expenses.

Cost A_2 = Cost A_1 + rent paid for leased-in land.

Cost B = Cost A_2 + imputed value of own land + imputed interest on fixed capital

Cost C = Cost B + imputed value of family labour.

Cost of cultivation and cost of production:

These two concepts are, more often than not, misunderstood and need clarification. Cost of cultivation refers to the economic valuation of variable inputs and fixed inputs per unit area say per hectare, while the cost of production for the crop is computed in terms of output per unit of weight say per quintal as follows:

$$\text{Cost of production (Rs/q)} = \frac{\text{Cost C (Rs/ha)} - \text{value of by- product, if any, (Rs/ha)}}{\text{Yield per hectare (in quintals)}}$$

Precautions in costing of input:

The following main considerations should be taken into account while costing the inputs:

i) If an input has been purchased, its value should be taken as such.
ii) The home-produced inputs like seed, manure etc are evaluated at the rates prevalent in the village/cluster of villages at the time of planting of the crop
iii) Transportation charges incurred for the inputs have to be included under the appropriate cost component as miscellaneous costs.
iv) Owned tractor labour cost includes (a) cost of fuel (b) depreciation of tractor and sheds (c) cost on repairs and (d) wages of driver.
v) Family labour cost is imputed at the wage rates paid to the attached farm servant, subject to minimum wages fixed by state government for farm labour.
vi) Interest on working capital is calculated on the value of the purchased items of inputs for half the crop period at the bank rate of interest for crop loans.
vii) Interest on owned fixed capital is charged for the crop period.
viii) Rent on owned land. It is taken as per the prevailing rate in the village, subject to ceiling for fair rent fixed in land legislation of the concerned state.
ix) Main product and by-product are evaluated at the post-harvest rates prevalent in the village concerned.
x) Joint costs are to be allocated according to area under each crop.

Methods for Costing:

Cost of cultivation can be calculated by following the survey method or cost accounting method. Normally, the cost of cultivation is estimated by using the survey method in the country. The cost accounting is used when the researcher wants to generate data for a number of years. This method is also useful while assessing the income from agricultural products and in determining the fixation of prices of the agricultural products. Cost accounting involves detailed enquiry entailing stay of the investigator in the village/cluster for longer time and frequent visits to the sampled farmers.

Economics of ware potato production

a) Costs and Returns from potato in the hills: The economics of potato in Shimla hills of Himachal Pradesh is presented in Tables 1. The total cost of cultivation (cost C) was Rs. 18,475 per hectare in 1989-90 in Himachal Pradesh, while the net returns were Rs 2998 and output-input ratio equal to 1.16. Category-wise analysis of economics of potato indicates that the large farmers earned the highest net returns of Rs 6903/ha, while the medium farmers earned the lowest i.e. Rs 1932/ha. The better returns accrued to the large and marginal farmers mainly due to higher productivity per unit area. Component-wise analysis of cost of cultivation shows that the seed input accounted for the highest average cost of 28.6%, followed by human labour 25.8%, rental value of land 23.2%, bullock labour 8.6%, fertilizers and manure 7.7%. The other cost factors such as plant protection, interest on working capital, interest on fixed capital and depreciation together contributed 6.1% towards the cost of cultivation of Rs. 18,475/ha. On the basis of the survey conducted on farmers fields in Shimla district during 1997-98, the cost of potato cultivation was estimated Rs. 36,950/ha, with net returns of Rs. 5990 /ha.

b) Costs and Returns from potato in plains:

The plains, particularly in the Gangetic region are the potato bowl of India. It accounts for about 80% of potato production. Uttar Pradesh is the leading potato – growing state of the country contributing about 33 % area and 40 % production during 2000-2001 to 2004-05. A CPRI study in different potato growing states of the country showed that the total cost of cultivation was Rs. 67395, 51229, 41524, 42274, 41530 and 42207 for Gujarat, West Bengal, Bihar, Punjab, MP and UP (Table 2). Component-wise cost of cultivation revealed that seed, human labour, fertilizer and manure cost were the major items of cost.

West Bengal is the second largest potato growing state in the country accounting for 25 and 32 percent of potato area and production during 2000-2001 to 2004-05. Component-wise cost analysis in West Bengal showed that seed, human labour, manures and fertilizers and bullock, machineries were the major items of the total cost of cultivation (cost C) of potato.

Economics of Seed Potato Production:

It is recognized that the informal seed potato production system meets the major requirement of seed potatoes in India. The input-output relationship, profitability and resource use efficiency in seed potato production was examined in Jalandhar district during 2003-04. The overall cost of seed potato production was estimated at Rs. 250/q. Expenditure towards seed was highest (32.84%) followed by manures and fertilizers (13.73%), farm machineries (11.29%), hired human labour (9.01%). Net income was worked out to be Rs. 11405/ha. Further, cost of production decreases with the increase in technology adoption due to increase in yield. Moreover it was found that seed potato production in Punjab was under decreasing return to scale.

Conclusion:

Although potato production is a very remunerative crop enterprise yet its profitability is dented by the periodical gluts and price crashes. Being a vegetatively propagated crop, seed quality and adoption of the Seed Plot Technique (SPT) are critical for improvement of potato

productivity and profitability. To sustain crop production at desired levels the government should develop a horticultural price policy. Since production and marketing are inter-related, the multifarious marketing problems need to be resolved with the help of a package of measures ensuring better returns to the growers especially small and marginal farmers. This can be achieved through promoting higher potato consumption for augmenting internal disposal and also boosting adoption of value addition techniques and strategies for encouraging the exports.

Component wise breakup of cost of production in leading potato growing States (Rs./ha) for the Year 2009-10

Component	Punjab	Haryana	Uttar Pradesh	Bihar	West Bengal
Seed	22309.5	22345.5	29274	25570.5	13863
Farm Machinery	4144.5	2728.5	5611.5	5626.5	4722
Hired human labour	7785	8529	10642.5	13698	13536
Irrigation charges	432	861	976.5	847.5	4065
Plant protection	1189.5	1363.5	1185	1134	2116.5
Manure and Fertilizer	9868.5	9184.5	10977	867	13093.5
Miscellaneous expenses	18630	15549	8667	4077	17985
Total working capital	64359	60561	67333.5	59620.5	69381
Interest on working capital	3217.5	3028.5	2892	2980.5	2082
Value of family labour	282	1191	859.5	1905	1458
Total variable cost	67858.5	64780.5	71085	64506	72921
Yield (q/ha)	246.7	211.3	205	202.2	235
Variable cost of production (Rs/kg)	2.75	3.07	3.47	3.19	3.10
Transportation cost (field to cold store + Handling charges) (Rs/kg)	0.6	0.5	0.4	0.3	0.4
Cost of Gunny bag (Rs/kg)	0.5	0.4	0.3	0.4	0.4
Storage cost (Rs./kg)	1.5	1.4	1.2	1.5	1.3
Total cost of production (Rs./kg)	**5.35**	**5.37**	**5.37**	**5.39**	**5.20**

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PRACTICALS

Transgenics and Gene expression analysis

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With the completion of genome sequencing of more and more organisms, research focus has now been shifted from sequencing to delineating the biological functions of all genes coded within the genome of a particular organism. Methodologies of biological research are evolving from "one gene in one experiment" to "multiple genes in one experiment" paradigm.

Introduction to Microarray

Molecular Biology research evolves through the development of the technologies used for carrying them out. It is not possible to research on a large number of genes using traditional methods. DNA Microarray is one such technology which enables the researchers to investigate and address issues which were once thought to be non traceable. One can analyze the expression of many genes in a single reaction quickly and in an efficient manner. DNA Microarray technology has empowered the scientific community to understand the fundamental aspects underlining the growth and development of life as well as to explore the genetic causes of anomalies occurring in the functioning of the human body.

A typical microarray experiment involves the hybridization of an mRNA molecule to the the DNA template from which it is originated. Many DNA samples are used to construct an array. The amount of mRNA bound to each site on the array indicates the expression level of the various genes. This number may run in thousands. All the data is collected and a profile is generated for gene expression in the cell.

Microarray Technique

An array is an orderly arrangement of samples where matching of known and unknown DNA samples is done based on base pairing rules. An array experiment makes use of common assay systems such as micro plates or standard blotting membranes. The sample spot sizes are typically less than 200 microns in diameter usually contain thousands of spots.

Thousands of spotted samples known as probes (with known identity) are immobilized on a solid support (a microscope glass slides or silicon chips or nylon membrane). The spots can be DNA, cDNA, or oligonucleotides. These are used to determine complementary binding of the unknown sequences thus allowing parallel analysis for gene expression and gene discovery. An experiment with a single DNA chip can provide information on thousands of genes simultaneously. An orderly arrangement of the probes on the support is important as the location of each spot on the array is used for the identification of a gene.

Types of Microarrays

Depending upon the kind of immobilized sample used construct arrays and the information fetched, the Microarray experiments can be categorized in three ways:

1. **Microarray expression analysis:** In this experimental setup, the cDNA derived from the mRNA of known genes is immobilized. The sample has genes from both the normal as well as the diseased tissues. Spots with more intensity are obtained for diseased tissue gene if the gene is over expressed in the diseased condition. This expression pattern is then compared to the expression pattern of a gene responsible for a disease.
2. **Microarray for mutation analysis:** For this analysis, the researchers use gDNA. The genes might differ from each other by as less as a single nucleotide base.

A single base difference between two sequences is known as Single Nucleotide Polymorphism (SNP) and detecting them is known as SNP detection.

3. **Comparative Genomic Hybridization:** It is used for the identification in the increase or decrease of the important chromosomal fragments harboring genes involved in a disease.

Applications of Microarrays

Gene discovery: DNA Microarray technology helps in the identification of new genes, know about their functioning and expression levels under different conditions.

Disease diagnosis: DNA Microarray technology helps researchers learn more about different diseases such as plant (Viral, Bacterial, Fungal and phytoplasm diseases) and animal diseases (heart diseases, mental illness, infectious disease and especially the study of cancer). Now, with the evolution of microarray technology, it will be possible for the researchers to further classify the types of diseases on the basis of the patterns of gene activity in the cells. This will tremendously help the scientific community to develop more effective drugs as the treatment strategies will be targeted directly to the specific type of diseases.

Drug discovery: Microarray technology has extensive application in *Pharmacogenomics.* Pharmacogenomics is the study of correlations between therapeutic responses to drugs and the genetic profiles of the patients. Comparative analysis of the genes from a diseased and a normal cell will help the identification of the biochemical constitution of the proteins synthesized by the diseased genes. The researchers can use this information to synthesize drugs which combat with these proteins and reduce their effect.

Toxicological research: Microarray technology provides a robust platform for the research of the impact of toxins on the cells and their passing on to the progeny. Toxicogenomics establishes correlation between responses to toxicants and the changes in the genetic profiles of the cells exposed to such toxicants. Eg. Mycotoxin resistant of *Phytophthora infestans* strain.

GEO

In the recent past, microarray technology has been extensively used by the scientific community. Consequently, over the years, there has been a lot of generation of data related to gene expression. This data is scattered and is not easily available for public use. For easing the accessibility to this data, the **National Center for Biotechnology Information** (**NCBI**) has formulated the **Gene Expression Omnibus** or **GEO**. It is a data repository facility which includes data on gene expression from varied sources.

Microarray probe design parameters

For 25-35 mers

Parameter	Minimum Value	Maximum Value	Default Value	Unit
Probe Length	10	99	30	bases
Probe Length tolerance	0	15	3	
Probe Target Tm	40	99	63	°C
Probe Tm Tolerance (+)	0.1	99	5	
Hairpin Max ÄG	0.1	99.9	4	Kcal/mol
Self Dimer ÄG	0.1	99.9	7	Kcal/mol
Run/Repeat	2	99	4	bases

For 35-45 mers

Parameter	Minimum Value	Maximum Value	Default Value	Unit
Probe Length	10	99	40	bases
Probe Length tolerance	0	15	3	
Probe Target Tm	40	99	70	°C
Probe Tm Tolerance (+)	0.1	99	5	
Hairpin Max ÄG	0.1	99.9	6	Kcal/mol
Self Dimer ÄG	0.1	99.9	8	Kcal/mol
Run/Repeat	2	99	5	bases

For 65-75 mers

Parameter	Minimum Value	Maximum Value	Default Value	Unit
Probe Length	10	99	70	bases
Probe Length tolerance	0	15	3	
Probe Target Tm	40	99	75	°C
Probe Tm Tolerance (+/- above)	0.1	99	5	
Hairpin Max ÄG	0.1	99.9	6	Kcal/mol
Self Dimer ÄG	0.1	99.9	8	Kcal/mol
Run/Repeat	2	99	6	bases

Usefullness of the Microarray technology:

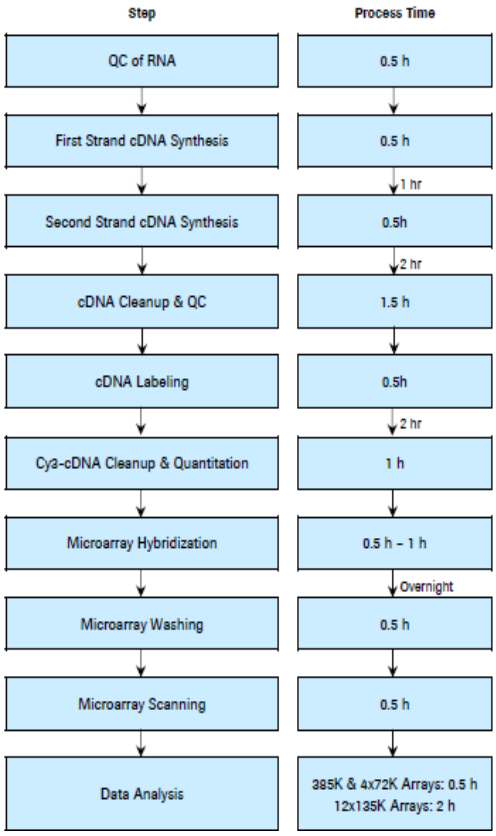
- Whole genome gene expression profiling
- Pathway/Function specific gene expression profiling
- Pathogenic mechanism
- Pathogen responses to environmental stimuli
- Host pathogen interactions
- DNA-Protein binding sites

Drawback:

- The result may be too complex to interpret
- The results are not always quantitative enough

The technology is still too expensive

Microarray Work flow



Isolation and Quantification of RNA

Spectrophotometric QC of cDNA

Quantitate each cDNA sample according to the following formula:
A Concentration (µg/ml) = A260 x 50 x Dilution Factor

Verify that all samples meet the following requirements:
• Concentration ≥ 100 ng/µl
• A260/A280 ≥ 1.8
• A260/A230 ≥ 1.8

Bioanalyzer/Gel QC of cDNA

1. Transfer 250ng of cDNA to a sterile microcentrifuge tube. Store the remainder of your sample on ice or at -15 to -25°C.

2. Analyze the samples using the Agilent Bioanalyzer and RNA 6000 Nano Kit.
3. Compare the Bioanalyzer traces to those traces in Figure 2 and Figure 3. Degraded samples appear as significantly lower intensity traces with the main peak area shifted to the left and typically exhibit much more noise in the trace.

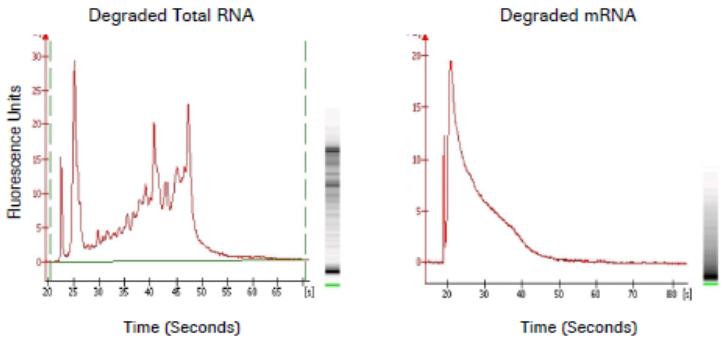


Figure 2: Example Traces for Degraded RNA Samples from a Eukaryotic Organism

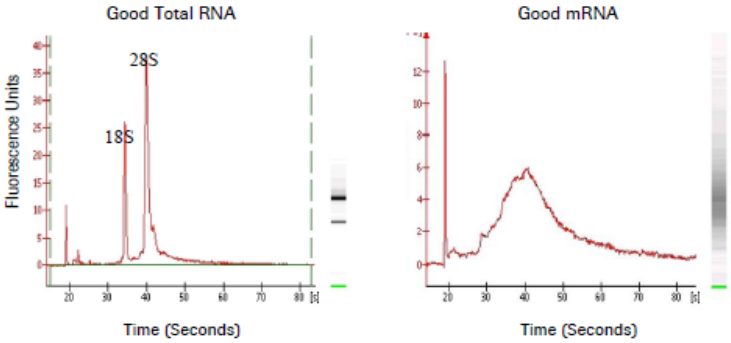


Figure 3: Example Traces for Non-degraded RNA Samples from a Eukaryotic Organism

Step 1. Preparation of double-stranded cDNA

1. Thaw all necessary components and place them on ice.
2. Pipet the following into a sterile 0.2 ml reaction tube:

Component	**1 µg Total RNA**	**5 or 10 µg Total RNA**
200 µM Oligo (dT)15 (vial 5)	1 µl	2 µl
Water, redist. (vial 12)	__ µl*	__ µl*
Total RNA	__ µl*	__ µl*

Total	**10.5 µl**	**21 µl**
*Add the required amount of Total RNA and bring the rest of the sample up to the total volume with Water (vial 12)		

3. Place tubes at 70°C for 10 minutes and chill in an ice water bath for 5 minutes.
4. Prepare first strand Master Mix in a 1.5 ml reaction tube.

Component	**1 µg Total RNA**	**5 or 10 µg Total RNA**
5x RT-Buffer AMV (vial 1)	4 µl	8 µl
100 mM DTT (vial 3)	2 µl	4 µl
AMV 25 U/µl (vial 2)	1 µl	2 µl
Protector RNase Inhibitor 25 U/µl (vial 4)	0.5 µl	1 µl
10 mM dNTP mix (1mM)f (vial 7)	2 µl	4 µl
Total	**9.5 µl**	**19 µl**

5. Gently mix the master mix from step 1.4. Quickly spin in a microcentrifuge and place on ice.
6. Add the master mix from step 1.5 to the sample from step 1.3. Mix gently and quickly spin in a microcentrifuge.
7. Incubate samples from step 1.6 at 42°C for 1 hour.
8. Place the tube on ice to terminate the reaction. Samples can be stored at -15 to -25°C overnight or proceed to step 1.9 for second strand synthesis.
9. Pipet the following components into the first strand reaction tube from step 1.8 on ice.

Component	**1 µg Total RNA**	**5 or 10 µg Total RNA**
cDNA from RT-reaction from step 1.8	20 µl	40 µl
5x 2nd strand synthesis buffer (vial 9)	15 µl	30 µl
10mM dNTP mix (vial 7)	0.75 µl	1.5 µl
2nd strand enzyme blend (vial 10)	3.25 µl	6.5 µl
Water, redist. (vial 12)	36 µl	72 µl
Total	**75 µl**	**150 µl**

10. Add the following to the reaction from step 1.15 and pipette up and down to mix:

Component	**1 µg Total RNA**	**5 or10 µg Total RNA**
cDNA from RT-reaction from step 1.15	94.25 µl	188.5 µl
Proteinase K (vial 14)	2.5 µl	5 µl
Total	**96.75 µl**	**193.5 µl**

11. Centrifuge for 1 minute at 13,000 x g and discard the flowthrough.
12. Centrifuge for 1 minute at 13,000 x g to dry the Filter Tube
13. Transfer the Filter Tube to a clean 1.5 ml tube.
14. Add 50 µl of pre-warmed (55°C) Elution Buffer.
15. Centrifuge for 2 minute at maximum speed.
16. Carefully transfer the supernatant to a fresh 1.5 ml tube to avoid residual silica fleece in the sample.
17. Samples can be stored overnight at -15 to -25°C.

18. Perform a spectrophotometic assessment of cDNA concentration.
    Concentration ≥ 100 ng/µl
    260/A280 ≥ 1.8
    260/A230 ≥ 1.8
19. Optional: Assessment of cDNA size distribution using the Bioanalyzer RNA Pico Kit.

Step 2: Sample Labelling cDNA labelling:

Be aware of the following when using the NimbleGen One-Color DNA Labeling Kits:
- Aliquot dNTPs and Cy primer into single-use amounts.
- Stop Solution could precipitate. Vortex or heat if necessary.

1. Prepare the following solution in a 15 ml tube:

Random Primer Buffer	
Random Primer Buffer (vial 2)	1,100 µl
β-Mercaptoethanol*	2 µl
Total	**1,102 µl**

Prepare fresh buffer each time primers are resuspended.
*Do not use bottles of β-Mercaptoethanol that have been opened for more than 6 months

2. Briefly centrifuge Cy3 Random Nonamers (vial 3) because some of the product could have dislodged during shipping. Dilute the primer in 1,050 µl of Random Primer Buffer with β-Mercaptoethanol. Aliquot 40 µl individual reaction volumes in 0.2 ml thin-walled PCR tubes and store at -15 to -25°C, protected from light.

Assemble the following components in separate 0.2 ml thin-walled PCR tubes:

Component	**All array formats**
cDNA	1µg
Diluted Cy3 Random Nanomers from step 2	40µl
PCR Grade water (Vial 1)	To volume
Total Volume	80 µl

3. Heat-denature samples in a thermocycler at +98°C for 10 minutes. Quick-chill in an ice-water bath for 2 minutes.

Note: Quick-chilling after denaturation is critical for high-efficiency labeling.

4. Prepare the following dNTP/Klenow master mix for each sample prepared in step 4. Note: Keep all reagents and dNTP/Klenow master mix on ice. Do not vortex after addition of Klenow.

dNTP/Klenow Master Mix: Recipe per Sample formats	**All Array**
dNTP Mix (10 mM each dNTP) (vial 5) PCR Grade Water (Vial 1) Klenow Fragment (3'> 5' exo) – 50 U/ µl (Vial 4)	10 µl 8 µl 2 µl

5. Add 20 µl of the dNTP/Klenow master mix prepared in step 5 to each of the denatured samples prepared in step 4. Keep on ice.

Component	**All array formats**
Reaction volume from step 4	80 µl
dNTP/Klenow master mix	20 µl
Total	**100 µl**

6. Mix well by pipetting up and down 10 times.

Do not vortex after addition of Klenow.

7. Quick spin to collect contents in bottom of the tube
8. Incubate for 2 hours at 37 in a thermocycler with heated lid, protected from light
9. Stop the reaction by addition of the Stop Solution.

Component	**All array formats**
Reaction volume from step 5	100 µl
Stop Solution (Vial 6)	21.5 µl
Total	**121.5 µl**

10. Vortex briefly, spin, and transfer the entire contents to a 1.5 ml tube containing isopropanol.

Component	**All array formats**
Reaction volume from step 9	121.5 µl
Isopropanol	110.0µl
Total	**231.5 µl**

11. Vortex well. Incubate for 10 minutes at +15 to +25°C, protected from light.
12. Centrifuge at 12,000 x g for 10 minutes. Remove supernatant with a pipette. Pellet should be pink.
13. Rinse pellet with 500 µl 80% ice-cold ethanol. Dislodge pellet from tube wall by pipetting a few times.
14. Centrifuge at 12,000 x g for 2 minutes. Remove supernatant with a pipette.
15. Dry contents in a DNA vacuum concentrator on low heat until dry (approximately 5 minutes), protected from light.
16. STOP POINT: Proceed to step 18, or store labeled samples at -15 to -25°C (up to 1 month), protected from light.
17. Spin tubes briefly prior to opening. Rehydrate each pellet in 25 µl PCR Grade Water (vial 1).
18. Vortex for 30 seconds and quick-spin to collect contents in bottom of the tube. Continue to vortex or let sit at +15 to +25°C, protected from light, for approximately 5 minutes or until the pellet is completely rehydrated, then vortex again and quick-spin.
19. Quantitate each sample by using these following formula
    centration (µg/ml) = A260 x 50 x Dilution Factor
20. Based on the concentration, calculate the volume of Cy3-labeled cDNA sample required for each hybridization per the following table and aliquot in a 1.5 ml tube:

Sample requirements	**385k Array**	**4x 72 Karray**	**12x 135k array**
Cy3 labelled cDNA Sample	Prok: 3µg Euk: 6µg	Prok: 2µg Euk: 4µg	Prok: 2µg Euk: 4µg

21. Dry contents in a DNA vacuum concentrator on low heat, protected from light.
22. STOP POINT: Proceed to *Next step*, or store labeled samples at -15 to -25°C (up to 1 month), protected from light.

Step 3: Hybridization and Washing

1. This step describes the NimbleGen protocol for sample hybridization and washing
2. Set the Hybridization System to +42°C. With the cover closed, allow at least 3 hours for the temperature to stabilize
3. Using components from a NimbleGen Hybridization Kit, prepare the hybridization solution master mix according to the following table. For 4x72K and 12x135K arrays, the amount listed is sufficient to hybridize all arrays on one slide. To hybridize multiple slides, adjust the amounts accordingly.

Hybridisation solution master	**4x72K**	**12x135K Array**

mix to hybridise a single slide Array	**385K**	**Array**	
2x Hybridisation Buffer	11.8 µl	29.5 µl	88.5 µl
Hybridisation component A	4.7 µl	11.8 µl	35.4 µl
Alignment Oligo	0.5 µl	1.2 µl	3.6 µl
Total	**17 µl**	**42.5 µl**	**127.5 µl**

4. Add the appropriate amount of hybridization solution to each sample according to the following table:

Components 385K Array	**Each Sample for a 4x72K Array**	**Each Sample for a 12x135K Array**
Resuspension sample from step 2 5 µl	3.3 µl	3.3 µl
Hybridisation solution from step 13 µl	8.7 µl	8.7 µl
18 µl	**12.0 µl**	**12.0 µl**

5. Vortex well (approximately 15 seconds) and spin to collect contents in bottom of the tube. Incubate at +95°C for 5 minutes, protected from light.
6. Place tubes at +42°C (in the Hybridization System sample block or heat block) for at least 5 minutes or until ready for sample loading. Vortex and spin prior to loading.
7. **Prepare Mixers**
8. Locate the appropriate mixer. Remove from its package and follow the Manufacturer instructions manual
9. **Load & Hybridize Samples** Refer to the appropriate provided by the Manufacturer instructions manual when loading sample.
10. Hybridize sample at +42°C to the array(s) for 16 - 20 hours.

Wash Hybridized Arrays

1. To ensure high quality data, it is important to proceed through all the washing and drying steps without interruption. The NimbleGen Microarray Dryer dries up to 24 slides at a time. If using a microarray dryer that dries one slide at a time, wash only one slide at a time.
2. Locate the components of the NimbleGen Wash Buffer Kit and NimbleGen Array Processing Accessories (refer Manufacturere instruction manual (Nimblegen)).
3. Before removing the mixer-slide assemblies from the Hybridization System, prepare Washes I, II, and III according to the following tables. Note that you prepare two containers of Wash I.

Washing Multiple Slides	Wash I (user-supplied dish[1])	Washes I, II, and III (wash tank[2])
VWR Water	243 ml	243 ml
10X Wash Buffer I, II, or III (vial 1, 2, or 3)	27 ml	27 ml
1 M DTT solution from step 1	27 µl	27 µl
Total	**270 ml**	**270 ml**

Washing One Slide	Wash I (user-supplied dish[1])	Washes I, II, and III (slide container[2])
VWR Water	243 ml	24.3 ml
10X Wash Buffer I, II, or III (vial 1, 2, or 3)	27 ml	2.7 ml
1 M DTT solution from step 1	27 µl	2.7 µl
Total	**270 ml**	**27 ml**

4. To facilitate the removal of the mixer, heat the shallow dish containing Wash I to +42°C. Roche NimbleGen recommends measuring the temperature of Wash I at every use. Keep the remaining three wash solutions at +15 to +25°C.
5. Insert the Mixer Disassembly Tool into the shallow dish containing warm Wash I. If you will be washing multiple slides, insert a slide rack into the wash tank containing Wash I at +15 to +25°C.
6. Remove a mixer-slide assembly from the Hybridization System and load it into the Mixer Disassembly Tool immersed in the shallow dish containing warm Wash I.
   Note: Do not allow the mixer-slide assembly to cool before removing the mixer. Keep power on to the Hybridization System's heat block and mixer system during mixer-slide disassembly, and transfer each mixer-slide assembly one at a time to Wash I for immediate removal of the mixer.
7. With the mixer-slide assembly submerged, carefully peel the mixer off the slide. It is important to hold the Mixer Disassembly Tool flat while removing the mixer and to avoid any horizontal movement or scraping with the mixer across the slide. Do not touch the array surface of the slide.
8. Working quickly, discard the mixer and remove the slide from the Mixer Disassembly Tool.
9. Gently agitate the slide for 10 - 15 seconds in the shallow dish containing warm Wash I to quickly remove the hybridization buffer.
10. If washing multiple slides, transfer the slide with the barcode at the top into a slide rack (Figure 10) in the wash tank that contains Wash I. If washing one slide, transfer the slide into a slide container that contains Wash I. Agitate vigorously for 10 - 15 seconds.
11. Wash for an additional 2 minutes in Wash I with vigorous, constant agitation. If washing multiple slides, move the rack up and down with enough agitation to make foam appear. If washing one slide, shake the slide container at least 20 times every 10 seconds.
12. Quickly blot the rack, or edges of the slide if only washing one slide, several times using paper towels to minimize buffer carryover. Transfer the slide(s) to Wash II and wash for 1 minute with vigorous, constant agitation. If washing multiple slides, rock the wash tank so the wash solution covers and cleans the tops of the slide(s).
13. Transfer the slide(s) to Wash III and wash for 15 seconds with vigorous, constant agitation. If washing multiple slides using the slide rack, rock the wash tank so the wash solution covers and cleans the tops of the slide(s).

14. Remove the slide(s) from Wash III. Spin dry in a NimbleGen Microarray Dryer or other microarray dryer per the manufacturer's recommendation. For a NimbleGen Microarray Dryer, the recommended drying time is 2 minutes (120 seconds).
15. Remove the slide(s) from the NimbleGen Microarray Dryer or other microarray dryer. Blot dry the edges with lint-free paper to remove any residual moisture
16. Proceed immediately to the steps for scanning the array(s)

Step 4: Scanning

Scannning the hybridised Array slide by using Nimblegen Scanner software
Refer the Nimblegen Mannual

Step 5: Nimblegen Microarray Data Analysis

Step 1. Burst Multiplex Image (4x72K & 12x135K Arrays Only)
Step 2. Import Image
Step 3. Extract Image
Step 4. Confirm Experimental Integrity (4x72K & 12x135K Arrays Only)
Step 5. Generate an Experimental Metrics Report
Step 6. Analyze Data using Array star Software

Refer to the *Nimblegen Scan Software User's Guide*

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DNA fingerprinting/Genetic fidelity

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The analysis is performed by extracting genomic DNA from the leaf material using standard Plant DNA isolation Kit. The technique involves amplifying specific polymorphic regions of DNA by Polymerase Chain Amplification (PCR). The amplified DNA fragments are then resolved through capillary electrophoresis.

Requirements:

1. Taq DNA polymerase (1U/reaction)
2. Taq DNA polymerase buffer (1X)
3. Primers (Forward & Reverse) (10 pmol / reaction each)
4. Deoxynucleotide triphosphate (dNTPs) (2.5 mM)
5. $MgCl_2$ (if not included in Taq DNA polymerase buffer) (2.5 mM)
6. Template DNA (100 ng / reaction)
7. Polymer POP7
8. Cathode buffer
9. Anode buffer
10. Capillary array

Protocol: The reaction mixture calculated below is on the basis of reaction volume of 16 □l and for 5 reactions.

Water	20.0 µl
Taq pol. buffer (10X)	10.0 µl
$Mgcl_2$ (25 mM)	08.0 µl
dNTPs (2.5 mM)	10.0 µl
Primer (F)	**05.0 µl**
Primer (R)	**05.0 µl**
Taq DNA poly. (5U)	02.0 µl

Mix gently and dispense 12 µl each of reaction mixture to PCR tubes containing the template 100.0 ng (4 µl / reaction) DNA (@25 ng / µl).

Carry out the PCR in the thermal cycler with following temperature regime

Cycle	Temperature	Time
1	95^O C	12 min
	94^O C	15 sec
10	50^O C	15 sec
	72^O C	30 sec
	89^O C	15 sec
20	50^O C	15 sec
	72^O C	30 sec
1	72^O C	30 min

Prepare mixture of Hi-Di (Highly deionized) Formamide and internal marker ROX. Take 12 µl of HiDi Formamide and 0.5 µl of ROX for one sample. Dispense 12.5 µl of mix into 5 separate PCR tubes. Add 4 µl of PCR amplified samples to each tube and denature the samples at 95^O C for 5 minutes. Snap chill the samples on ice and load the samples into the '*ABI* 3500 - Genetic analyzer' system. Run the samples using '*ABI* 3500 Data Collection' software and analyze the data through '*ABI'* genotyping software package 'GeneMapper'.

Soil and plant tests for fertilizer recommendation

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With the ever-increasing population of India, the demand for food also increasing. It is estimated that cultivated area can be increased at the most by 11% whereas the demand for food is likely to increase by over 35% by 2025 in India. This will put pressure on the land and the capacity of soil to supply nutrients to the crops, will diminish with time. In order to sustain productivity, the principle 'what we take from soil, we must return to it' can be accomplished by scientific nutrient management. However, their indiscriminate use can result either in their under utilization, leaching, toxicity and pollution problems.

More than 75% potato growing area in the country falls under alluvial Indo-Gangetic plains and the rest falls in the northern and eastern Himalayas, Nilgiri hills in south and peninsular India. Because of high crop intensity, the removal of macro, secondary and micronutrients is far higher than their addition from fertilizers and manures. Likewise, in acidic soils, the P dose has to be kept at higher level in view of P fixing problem. Under such a situation, it is imperative to judiciously use the available fertilizer nutrients and organic manures on the basis of soil and plant tests values. The soil tests helps in selecting the proper manuring schedule for various crops in a sequence by knowing about the nutrient build up or depletion in the soil profile. Plant tests on the other hand identify the nutrient deficiency in standing crop and in the event of deficiency of a particular nutrient, help in taking corrective measures.

Soil testing

Nutrients need of potato depends upon the tuber yield response to applied fertilizers, agro-climatic conditions, variety and nutrient availability from the soil pool. The nutrient status of the soil further depends upon the quantity of total and available nutrients present in the soil pool. Several methods are available for assessing the suitability of soil for potato cultivation.

Tests for physico-chemical properties

pH, texture, water holding capacity, pore space and conductivity exert a great influence on nutrient availability to potato.

Soil pH: Potato can be grown on wide range of soils from acidic (pH 4.5) to alkaline (pH 8.5), however, soils of neutral pH are preferred. The soils of extreme pH and EC hinder plant growth while acidic soils suffer from P stress because of high P fixation. The saline soils are not suitable for potato as it inhibit the potato growth.

Soil texture: It is a good index for assessing nutrients and water supplying capacity and suitability of soil for potato. Sandy loam and loamy soils having high organic matter are considered best for potato. The light textured alluvial soils from Indo-Gangetic plains with low amount of organic matter and water retention capacity require heavy dose of fertilizers and water for getting optimum yields. Likewise, heavy textured soils i.e. clay loam to clayey soils of peninsular India, suffer from the water logging and cracking, and obstruct the tuber growth beside tuber greening and tuber moth infestation. The international pipette method is standard procedure used for categorizing the soil.

Soil testing methodology

Several methods involving use of selective extractants are available for testing each nutrient. The choice of method is judged by the availability of nutrients in soluble form for its absorption by the plant.

Organic matter: The contribution of organic matter towards availability of N, P, K and micronutrients i.e. Zn, Cu, Fe and Mn is of great importance in acidic hill soils having high organic matter content. It has a special significance in North western and eastern hills where the crop is rainfed and its capacity to hold water and nutrients helps the crop to withstand drought during early stages of crop growth. It is the earliest test used for estimating the N availability in

soils. The test for estimation of organic matter is based on oxidation of organic matter with chromic acid.

Major nutrients: Major part of soil N is in the organic form and is closely related to organic matter. It has to undergo mineralization to inorganic forms i.e. nitrate and ammonium before absorption by the roots. Chemical methods based on oxidation of organic matter are available for assessing the soil N availability. Among these, $KMnO_4$-oxidisable N, total N, $K_2Cr_2O_7$-oxidisable N and organic matter are generally employed to judge the N content of soil. $KMnO_4$-oxidisable N and $K_2Cr_2O_7$-oxidisable N are based on oxidation of organic matter under alkaline and acidic conditions, respectively. Although, total N gives a true picture of soil N but is seldom used for potato. $K_2Cr_2O_7$-oxidisable N is quite effective in judging N needs of potato, both in hills and plains. Among the inorganic tests used for N estimation, nitrate test fairs better in years of normal rainfall but not in years of high rainfall.

Phosphorus is retained in soil as insoluble P compounds of Fe, Al and Ca in acidic soils and Ca and Mg in alkaline soils, hence choice of method for estimating P depends upon the quantity of each fraction present in the soil. Bray P method employing 0.03 N NH_4F in 0.25 N HCl is useful in soils with high amount of Al and Fe compounds while Olsen's method using 0.5 M $NaHCO_3$ (pH 8.3) is a good index of P availability in alluvial and black soils containing Ca and Mg forms of P. Although, Indian soils are rich in total K, yet only a fraction of total K is available in exchangeable form. Both exchangeable and non-exchangeable K tests are good for judging K needs of potato. Exchangeable- K determined by 1N NH_4OAc method gives a fair index of K needs of potato. Among other tests for available K, 3N cold H_2SO_4 is equally good.

Secondary and micronutrients: Soil tests for secondary and micro- nutrients hve become necessary in view of their widespread deficiency resulting from intensive cropping and use of high analysis fertilizers. For secondary elements i.e. calcium and magnesium, ammonium acetate method is normally employed whereas 0.15% $CaCl_2$ method gives fairly good results for sulfur in potato. The DTPA test for micronutrients viz. Zn, Fe, Mn and Cu has found wide application in potato.

Interpretation of soil test results

The soil tests interpretation involves an economic evaluation of the relationship between soil test and potato response to the applied fertilizer nutrient. The soil test values obtained are of little use unless some fixed value is determined below which the fertilizer application to potato will be economical. These estimates are called critical limits and are discussed below:

Percent yield: It is control yield/ yield with optimum nutrient dose and multiplied by 100. It is calculated from the agronomical trials conducted on different soils varying in nutrient content within a particular soil group. Simultaneously, soil is tested for the nutrient content by a number of methods. The method giving highest correlation with percent yield is adopted for that group of soil. The critical limit obtained from Cate and Nelson's graphical technique categorizes the soils into responsive and non-responsive groups. Likewise, in statistical technique, the soils can be classified into more than two class viz. low, medium and high range of nutrient level.

Organic Carbon: In *Tarai* and hill regions in northwestern Himalayas, the soils with 1.5 and 2.5 per cent organic carbon, respectively are considered to be deficient in N, P and K.

Major nutrients: It is not feasible to estimate the critical limits for N where crop is grown under rainfed conditions as soil tests for N are affected by moisture, temperature and C/N ratio. In irrigated crop, the leaching loss influences the suitability of N estimates. Still the response to N has a direct relationship with soil test value (Fig. 1). The critical limit for $K_2Cr_2O_7$- oxidisable N in acidic hill and alluvial soils is 1357 ppm and 375 ppm, respectively.

The critical limits for available P estimated by Bray P1 method is frequently used for predicting potato response to P application (Table 1). In acidic hill soils, soils with less than 32, 32-89 and more than 89 ppm are rated as low, medium and high, respectively. In alluvial soils of northern plains, the critical limit for P by Olsen method for autumn /winter and spring potato is 10 and 13 ppm, respectively.

Table 1. Critical limits of P and K for potato

Soil Test	Acidic	Alluvial
	Phosphorus	
Olsen (ppm)	16	10
Bray (ppm)	35	-
Organic C (%)	1.30	-
	Potassium	
NH_4OAc-K (ppm)	120	105
3N cold H_2SO_4 (ppm)	112	-
Organic C (%)	2.0	-

The ammonium acid test is used for determining potato response to K, both in hills and plains. The critical limit for K by this method for acidic, light alluvial and heavy alluvial are 120, 105 and 130 ppm, respectively. A rapid test based on oxidation of organic matter by chromic acid is also useful for estimating of organic carbon, N, P and K in a single extract.

Secondary and micronutrients: For laterite soils, the critical limits for exchangeable Ca and Mg by neutral NH_4OAc method are 1.5 and 0.3 m.e. /100g. Though no critical limits have been fixed for S in potato, yet soils containing less than 10 ppm S extracted by 0.15 per cent $CaCl_2$ are considered to be deficient. Among micronutrients, the critical limits of Zn with DTPA reagent for acidic hill and alluvial soils are 0.55 and 0.62 ppm, whereas for Cu, Mn and Fe, the critical limits by DTPA are 0.20, 1.00 and 4.5 ppm, respectively.

Crop response to fertilizers

Although the critical nutrient approach classifies the soils into two or more groups, yet for quantification of potato response to applied fertilizers, it is necessary to have additional field data for obtaining optimum yield.

The optimum dose of fertilizer is calculated from the regression equation $Y= a + bX – cX^2$ and X is calculated from another equation $X = 1/2c[p/q-b]$ where b and c are the constants obtained from regression curve, p and q are the prices of one kg nutrient and commodity under study. The optimum dose obtained holds good for a specific crop in that soil group.

Major nutrients: Lower the soil N, higher is the response to N application. For example, the alluvial soils with < 288 ppm $K_2Cr_2O_7$ -N will respond more than the soils with> 500 ppm N. In organic matter rich acidic soils, the average response at recommended N dose will of higher magnitude in soils containing <700 ppm K_2 Cr_2 O_7-N thereby showing that potato response to applied N depends upon the N status of the soil.

The response to P is high in P fixing acidic soils of northern and eastern Himalayas and red soil of southern peninsula. The increase in potato yield is more in acidic and alluvial soils than in black soils because of high K supplying capacity of later.

Secondary and micronutrients: Soil test values for secondary and micronutrients also affect potato response to applied nutrients. The response ranging from 2.7 to 4.5 t/ha have been observed with S application in soils containing less than 10 ppm S. In magnesium deficient soils with less than 15 ppm Mg, 10-17 percent higher yields have been obtained with Mg application. Likewise, in alluvial soils with DTPA- extractable 0.30 ppm Zn and 1.5 ppm Fe, application of $ZnSO_4$ and $FeSO_4$ is beneficial. $CuSO_4$ application can increase the potato yield in soils with < 0.48 ppm Cu.

Plant testing

The plant testing may be non-destructive, consisting of leaf or petiole analysis or destructive, in which whole plant is removed and analyzed for nutrient concentration or uptake studies/fertilizer recovery.

Three stages are critical for tuber yield in potato, i.e. stolon formation, tuber initiation and tuber bulking. The first two stages are ideal for tissue testing because in case of deficiency of a particular nutrient, corrective measures can be taken up. Petioles or leaves from 3rd fully

expanded leaf from top of the main shoot are collected (when the plant is not under stress), washed, and dried at 80^{O} C to stop enzymatic reactions. The dried and powdered tissue is used for chemical analysis.

Major nutrients: Both NO_3-N and total N tests are good indices for judging N needs of potato because of their strong association with tuber yield. However, petiole's nitrate-N has an edge over leaf- total N as increase in nitrate-N concentration in petioles is proportional to N supply at a given time. Estimation of NO_3-N is simple and involves mercerization of fresh petiole in 2 per cent acetic acid and estimation of NO_3-N. Estimation of total N estimation requires acid digestion for converting the organic N to NH4+ form.

As most of P within plant is present in organic combination, therefore, the leaf or petioles are analyzed for total P content by tri-acid. Its close association with tuber yield is well established and can serve as a good index for P assimilation in potato. Most of the K present in petioles is acid soluble hence petiole-K is better than leaf K.

The critical concentration for N, P and K in petiole or leaf depends upon the cultivar, growth stage and season (Table 2). It is high at early stages and tend to decrease with time and is higher for late maturing cultivars. Recently, critical nutrient range (CNR) depicting the range of nutrient concentration in the plant tissue at a specific growth state has proved to be a better index than the critical nutrient concentration (CNC) concept.

Table 2. Critical limits of N, P and K in potato leaf and petioles at different growth stages

Plant parts	K. Jyoti		K. Chandramukhi		K. Sindhuri	
	S.F	T.I.	S.F	T.I.	S.F	T.I.
	Nitrogen					
Leaf	6.25	5.85	5.87	4.87	-	-
Petiole	2.85	2.27	1.50	0.96	1.65	0.93
	Phosphorus					
Leaf	-	-	-	-	-	-
Petiole			0.35	0.29	0.38	0.25
	Potassium					
Leaf	3.56	3.05	4.69	2.55	-	-
Petiole	11.85	10.70	11.10	7.90	-	-

S.F. Stolon formation; T.I. Tuber initiation

Secondary and micronutrients: Analysis for S alone may not he useful in foliar diagnosis. Therefore, N: S and P: S ratios in petioles or leaflets are used frequently for predicting deficiency or sufficiency of S. Likewise, P: Mg and P: Zn ratios in leaf are widely used for predicting the deficiency of Mg and Zn in potato.

In alluvial soils, N : S ratio of more than 21:1 in leaf indicates severe S deficiency whereas in acidic soils, N: S ratio of < 19:1 is sufficient for optimum yields. Similarly P: Zn ratio of ≤ 240 indicate severe Zn deficiency, whereas >70 P: Zn ratio reflects severe P deficiency. Likewise, the critical deficiency limits for Fe and Cu, are 80 and 8.5 ppm, respectively. The toxicity of Zn and Cu in plant will occur if these exceed 450 and 34 ppm, respectively.

Plant tests for modifying fertilizer recommendations

Tissue tests for N and K have been employed in correcting nutrient deficiency by applying corrective dose to the standing crop at early stages of crop growth. Early sampling for leaf petioles i.e. 40-45 days in hills and 20-30 days in plains is best for tissue testing and taking corrective measurer (Table 3). For micro-nutrients deficiency, the foliar application is most appropriate method.

Table 3. Corrective dose of N and K for top dressing on the basis of tissue tests

Location	Petiole N (%)	Dose (kg N/ha)	Response (t/ha)	Petiole K (%)	Dose (kg K_2O/ha)	Response (t/ha)
	Kufri Jyoti					
Hills	<1.50	118	12.2	<10.0	75	4.2
	1.50-2.00	96	10.8	10.0-11.0	54	3.2
	2.0-2.5	29	3.9	11.0-12.0	40	2.3
	>2.5	-	-	>12.0	30	1.8
Plains	**Kufri Chandramukhi**					
	<0.51	150	9.3	8.9	70	-
	1.02	150	9.1	-	-	-
	1.52	92	4.4	-	-	-
	>2.34	37	0.9	-	-	-

Plant tests for assessing fertilizer recovery

Plant tests have been used for working out fertilizer use efficiency, nutrient recovery, checking the depletion of nutrients in a cropping system and planning precise fertilization schedule for the crop sequence so as to maintain soil fertility and productivity. For nutrient recovery and hence fertilizer use efficiency, whole plant i.e. haulms, tubers and often roots are taken from the check plots and fertilized plots.

Suggested reading

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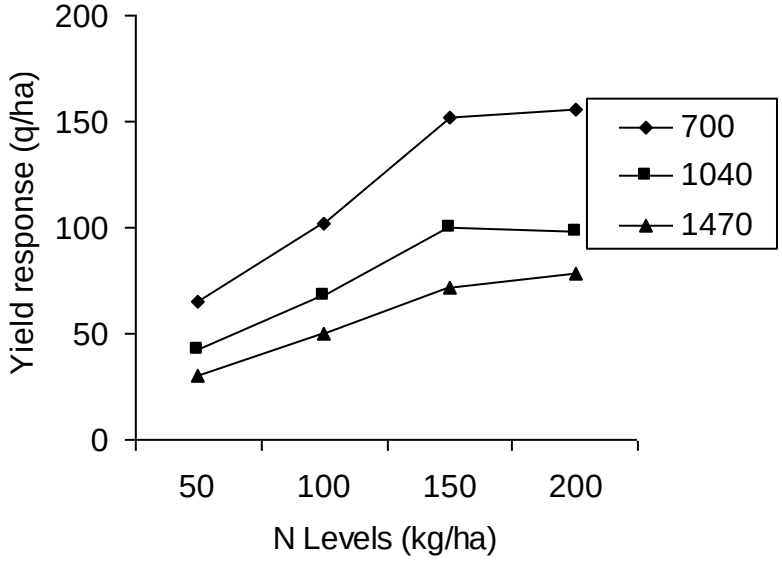

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Potato response to N in relation to soil test value ($K_2Cr_2O_7$-N

Screening techniques for late blight resistance

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Production of *P. infestans* inoculum

P. infestans inoculum is multiplied using tuber slice method. Tubers of any susceptible potato cultivar are surface sterilized with ethyl alcohol and cut into 1 cm thick slices using a sharp sterilized knife. Slices are placed in plastic petri dishes and inoculated Potato is a high value food crop contributing directly to hunger reduction as well as increasing income, especially in the developing countries. Potato cultivation is affected worldwide by late blight, the most destructive potato disease caused by an oomycete *Phytopthora infestans,* affecting the quality of tuber and yield as well. Resistance in plants is mainly of two types; vertical which is race specific, monogenic and expressed in the form of hypersensitive response while horizontal resistance is polygenic, race-non-specific, durable or quantitative. However, pathogen develops matching virulences and leads to breakdown of resistance in potato varieties. Therefore, instigating new resistance sources remains the ultimate objective to combat this notorious pathogen and for which robust screening methods are needed.

Production of *P. infestans* inoculum

P. infestans inoculum is multiplied using tuber slice method. Tubers of any susceptible potato cultivar are surface sterilized with ethyl alcohol and cut into 1 cm thick slices using a sharp sterilized knife. Slices are placed in plastic petri dishes and inoculated with *P. infestans* by scratching the mycelium on slice surface with sterilized needle and incubated in air tight plastic boxes lines with moist foam sheet at 18±1^{o}C for a week in the dark. A thick white growth of sporangiophore with plenty of *P. infestans* sporangia would cover the tuber slices surface within 5-6 days.

Zoosporangial germination

Tuber slices containing *P. infestans* zoosporangia are gently dipped in sterilized distilled water to dislodge the zoosporangia from tuber slice surface. This zoosporangial suspension is then kept at 12±2^{o}C for 60-90 min for releasing zoospores. The zoospore suspension is calibrated to a desired level ($6x10^4$ zoospores/ml) using haemocytometer for each isolates. This zoospore suspension is utilized for inoculating the leaves or tubers for screening the material for foliage/tuber resistance.

Screening for Foliage Resistance

Detach leaf method: Plants of genetic material to be screened are raised under glass house in the pots. On the 45th days after planting, fourth leaves from the top are plucked from each clone and placed in plastic trays on perforated plastic separators (Umaerus and Lihnell, 1976). Five leaves of each clone are collected and placed in the tray. Leaves of a susceptible variety are kept to serve as control. These leaves are inoculated with 20 ul zoospore suspension using an autopipette. *P. infestans* zoospores suspension is prepared and calibrated as described above. The trays are incubated at 18±1^{o}C for 5 days. High humidity in the trays is maintained with the help of moist foam sheet. Observations are recorded on the 6th day of inoculation measuring the leaf area infected. The accessions are categorized into highly resistant, resistant, moderately resistant and susceptible on the basis of following scale.

Lesion area (cm2)	**Grade**
Up to 1.0	Highly resistant (HR)
1.1 to 2.5	Resistant (R)
2.5 to 6.0	Moderately resistant (MR)
>6.0	Susceptible (S)

Whole plant method: Whole plant screening is done in the screening chambers having controlled temperature, humidity and fluorescent light. Plants of genetic material to be tested along with a susceptible cultivar are raised in pots in the glass house. Forty days after planting, the pots are transferred to the screening chamber. Close the chamber and put on the humidifier for 1-2 hrs. so that leaf surface is completely wet. Prepare zoospore suspension of most complex races and spray the plants with inoculums. Keep the humidifier off for a night and then incubate the plants at 18±1°C with 90% humidity. After 6 days, score the plants using the following scale:

% Area infected	Score
Trace of infection	9
10	8
11-25	7
26-40	6
41-60	5
61-70	4
71-80	3
81-90	2
Collapsed	1

(Malcolmson, 1976)

Tuber resistance

Tuber slice method: In this method 3 tubers of each accessions to be tested, are surface sterilized with ethyl alcohol and cut into 1 cm thick slices aseptically by a sterilized knife. One slice from each tuber is kept in a sterilized petri dish. Three slices of a susceptible variety are also placed in a petri dish to serve as control. Tuber slices are inoculated with zoospore suspension using filter paper discs (0.3 cm^2) dipped in the zoospore suspension prepared and concentration adjusted as per methods given above. The petri dishes are kept in plastic trays lined with moist foam sheet and incubated at 18±1°C for 5 days in dark. Length and breadth of the lesions are measured and lesion area calculated as per following formula:

Lesion area = π/4 x ab, where a= length and b= breadth of the lesion

The accessions are categorised in 4 groups as per scale given under foliage resistance by detach leaf method.

Whole tuber test: After 7-16 days of harvest, give superficial injury to the tubers. After 24 hrs of injury place the tubers on wet filter paper in seed boxes. Incubate the tuber (i) By spraying the zoospore suspension of *P. infestans* (Lapwood, 1967 and Bjor (1987); (ii) By placing the soaked filter paper discs on the lanticels or eyes (Lacey, 1967). After inoculation, close the box to avoid drying and incubate at 18±1°C. After two weeks score the tubers individually on the following scale (Bjor, 1987).

Score	0	1	2	3	4	5
% of the surface with symptoms	0	5	5-10	10-25	25-30	>50

Field Screening: Field screening is generally done on a large population of plants. For this purpose disease should be recorded in each cultivar right from its appearance till the maximum build up of the disease at regular (weekly) intervals. This data may be used to calculate the Vander Plank 'r' (apparent infection ratio Van der Plank, 1963), AUDPC (Area under disease progress curve, (Wilcoxon, 1975, Shaner and Finney, 1977) or mean values (Simmonds and Wastie, 1987). However, out of them AUDPC has been considered more reliable for categorizing the cultivars according to their resistance grades.

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Isolation, identification and inoculation techniques for *Ralstonia solanacearum* and *Streptomyces* species

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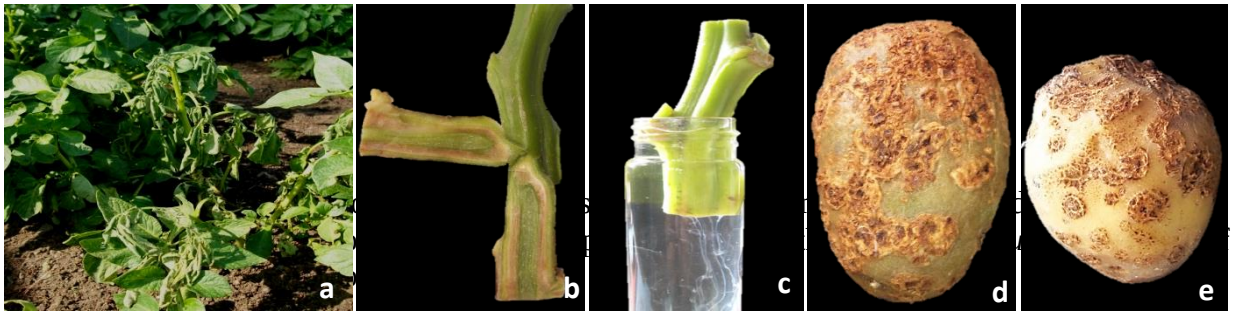
Collection of bacterial wilt/ scab infected plants/ tubers

Potato plants/ tubers infected with typical symptoms of bacterial wilt/ common scab disease (Fig 1, 2a) are obtained from various potato growing regions. At each collection site, tubers (brown rot/scab infected) or stem pieces (6 to 10 cm) of wilt infected plants showing typical brown rot/scab/wilt symptoms are collected, labelled properly and brought to laboratory immediately for isolations.



Isolation and maintenance of *Ralstonia solanacearum* isolates

Potato tubers/ stem pieces are washed thoroughly in running tap water for 5 to 7 minutes to remove soil adhered to their surfaces and air dried. The samples (stem pieces and tubers) are then surface disinfected with 70% ethanol, peeled, subsampled and macerated in sterile distilled water. Macerates are streaked on Kelman's triphenyltetrazolium chloride (TZC) agar medium (Kelman, 1954) with slight modification (Peptone, 10 g litre^{-1}; glucose/ glycerol, 2.5 g/ 5 ml litter $^{-1}$; Casamino Acids, 1 g litre^{-1}; yeast extract, 1 g litre^{-1}; agar, 15 g litre^{-1}; TZC, 50 mg litre^{-1}; pH 7.0-7.1). Plates are incubated at 28±2 ºC for 48 to 72 hrs. This medium is useful for distinguishing *R. solanacearum* among other bacteria during isolation, and for distinguishing virulent (wild type) colonies from avirulent mutant ones during purification of cultures. Bacterial colonies developing the typical irregular mucoid colonies are again transferred to fresh TZC medium for further purification. Well separated typical wild type *R. solanacerarum* colonies (Fig. 2b) are further transferred to medium modified by exclusion of TZC for multiplication of inoculum free of formazan pigment (which is slightly bacteriostatic).Two loop full of bacterial culture are then transferred in 2 ml of double distilled sterile water and the cultures stored at 20±2 ºC.

TZC stock solution: One gram of 2,3,5 triphenyltetrazolium chloride is dissolved in 100 ml of distilled water, placed in a light proof capped bottle, autoclaved for only 8 minutes or sterilized by filtration and stored refrigerated. To each 200 ml of melted, somewhat cooled Kelman's basal medium i.e. Casamino Acid Peptone Glucose/Glycerol Agar (CPG) medium, one ml of TZC stock solution is added to give a final concentration of 50 mg litre^{-1}

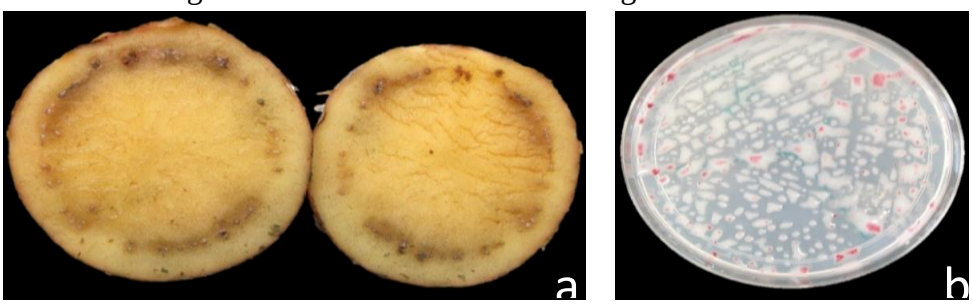


Fig 2: Grey-brown discoloration of vascular tissues and bacterial ooze in potato tuber infected by *R. solanacearum* (a); typical *R. solnacearum* colonies on TZC agar medium (b)

Characterization of *R. solanacearum* isolates into biovars

Biovars of *R. solanacearum* strains are determined by standard procedure (Hayward 1964). The following basal medium is used for biovar identification: $NH_4H_2PO_4$, 1.0 g; KCl,0.2 g; $MgSO_4.7H_2O$, 0.2 g; Peptone, 1.0 g; 1% (wv^{-1}) aqueous solution of bromothymol blue, 0.3 ml; agar, 1.5 g; distilled water, 1litre.

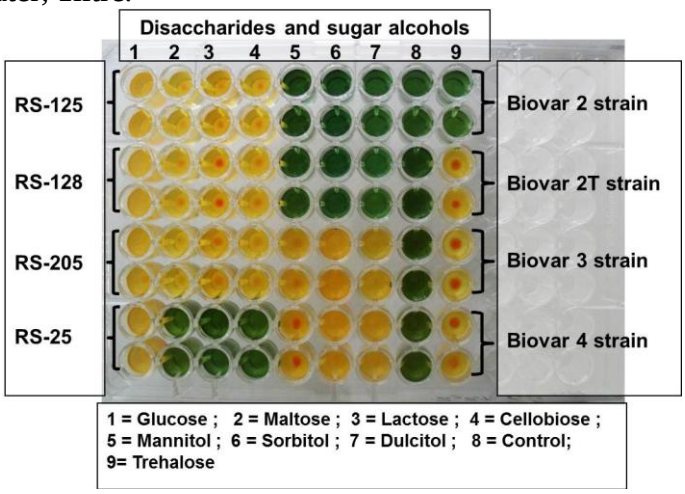


Fig 3: Micro-titre plates showing results of biovar test with *R. solanacearum* strains

The pH of the medium is adjusted to 7.1 with 40% (wv^{-1}) NaOH solution before addition of the agar. Five millilitres of a 10% (wv^{-1}) pre-sterilized solution of the sugars (lactose, maltose, cellobiose) and sugar alcohols (mannitol, sorbitol and dulcitol) are added to 45 ml of molten cooled basal medium separately. 200µl of these media were then dispensed into each well of 96-well micro-titre plates. Hayward's medium without a carbon source and un-inoculated wells serves as control. Each well is inoculated with 3 µl of a 2 x 10^9 CFU ml^{-1} cell suspension prepared from overnight CPG broth culture. The cultures are incubated at 28±1 ºC and examined at 3, 7 and 14 days for change of pH (yellow colour; Fig 3). Positive cultures change the culture medium from green to yellow. Each test is replicated three times. The biovar classification is done as described in Table 1.

Isolation of genomic DNA

Bacterial growth from a well separated colony on TZC agar is used to inoculate 1.5 ml of CPG broth in 2.0 ml Eppendorf tubes. The cultures are grown at 28ºC for 48 hours with vigorous shaking. Total genomic DNA is extracted by rapid method as described by Chen and Kuo (1993). 1.5 ml of saturated culture is harvested with centrifugation for 3 min at 12,000 rpm. The cell pellet is re-suspended and lysed in 300 µl

Table 1: Differentiation of *R. solanacearum* strains into biovars based on the ability to utilize disaccharides and oxidize hexose alcohols producing acid when positive (+)

Biochemical Test	**Biovar**				
	1	2	3	4	5
Utilization of					
Cellobiose	-	+	+	-	+
Lactose	-	+	+	-	+
Maltose	-	+	+	-	+
Oxidation of					
Dulcitol	-	-	+	+	-
Mannitol	-	-	+	+	+

Sorbitol	-	-	+	+	-

of lysis buffer (40 mM Tris-acetate pH 7.8, 20 mM sodium-acetate, 1 mM EDTA, 1% SDS) by vigorous pipetting. To remove most proteins and cell debris, 100 µl of 5M NaCl solution is added and mixed well, and then the viscous mixture is centrifuged at 12,000 rpm for 10 min at 4°C. After transferring the clear supernatant into a new vial, an equal volume of chloroform is added, and the tube is gently inverted at least 50 times when a milky solution is completely formed. Following centrifugation at 12,000 rpm for 3 min, the extracted supernatant is transferred to another vial and the DNA is precipitated with 100% ethanol, washed twice with 70% ethanol, dried in speed-vacuum, and re-dissolved in 50 µl of TE buffer. If required, RNA could be removed by adding RNAase in the lysis step for 30 min at 37°C.

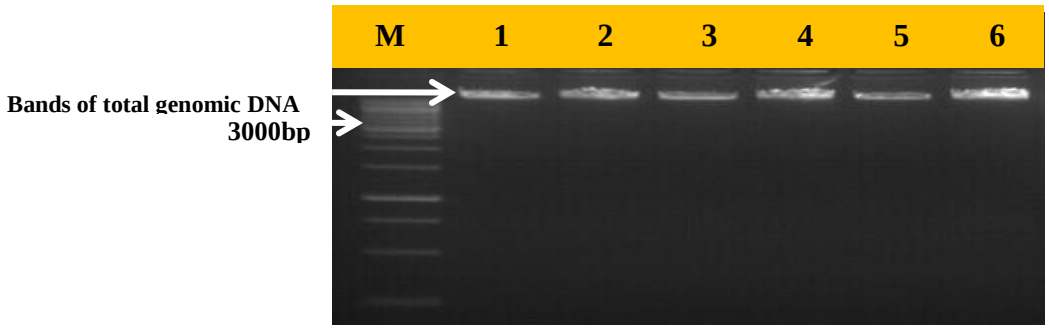


Table 2: Stock solutions for preparation of lysis buffer for DNA extraction

Sr. No.	Name of stock solution	Required concentration	Volume (µl) required for 10 samples @ 300µl lysis buffer per sample
1.	Tris-acetate (1.0M)	40mM	120
2.	Sodium acetate (1.0M)	20mM	60
3.	EDTA (0.1M)	1mM	30
4.	SDS (10%)	1%	300
5.	Distilled water	-	2490
	Total		3000
6.	NaCl (5.0M)	100µl (added after lysis to remove most proteins and cell debris)	

Purity check and quantification of DNA

For purity check and quantification, 5µl template DNA is loaded with 2µl of 6X loading dye on 1.0% agarose gel in Tris–Acetate EDTA buffer with 0.04 µl ml^{-1} of ethidium bromide at 100V for 1hr and visualized under ultraviolet (UV) light. DNA samples showing intact bands are selected for PCR amplification.

Characterization of *R. solanacearum* isolates into phylotypes

Phylotype identification of each isolate is done as described (Fegan and Prior 2005; Prior and Fegan 2005). Multiplex PCR (Pmx-PCR) is carried out in 25 ìl final volume of reaction mixture, containing 1×Taq Master Mix (PCR buffer, 1.5 mM MgCl2, 200 ìM of each dNTP, 50 mM KCl, 10 mM Tris-HCl and 1.25U of Taq DNA polymerase.), 6 pmoles of the primers Nmult:21:1F, Nmult:21:2F, Nmult:22:InF, 18 pmoles of the primer Nmult:23:AF and 4 pmoles of the primers 759 and 760 (Opina *et al*., 1997). The following cycling programme is used in a thermal cycler: 96°C for 5 min and then cycled through 30 cycles of 94°C for 15 s, 59°Cfor 30 s and 72°C for 30 s, followed by a final extension period of 10 min at 72°C.

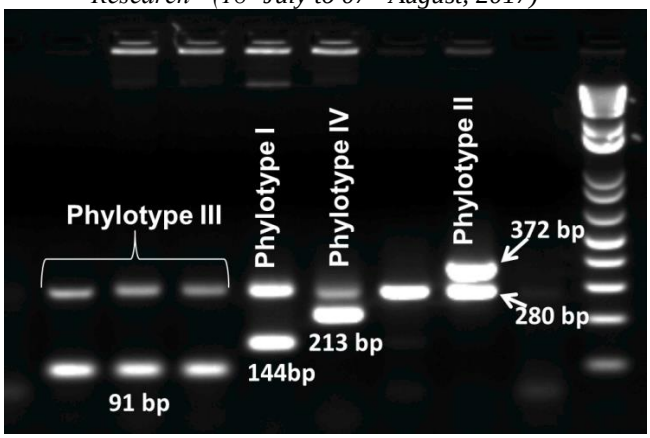


Fig 5: Pmx-PCR using phylotype-specific primers showing PCR products of 280bp (i.e. *R. solanacearum*) amplicons for all isolates, 91bp (Phylotype III, lane 1-3), 144bp (phylotype I, lane 4), 213bp (Phylotype IV, lane 5) and 372bp (phylotype II, lane 7) amplicons

A 5 ìl aliquot of each amplified PCR product is then subject to electrophoresis on 1% agarose gel, stained with ethidium bromide and visualized on a UV-trans-illuminator. This Pmx-PCR produces the following phylotype-specific PCR products: a 144-bp amplicon from phylotype I strains; a 372- bp amplicon from phylotype II strains; a 91-bp amplicon from phylotype III strains; and a 213-bp amplicon from phylotype IV strains (Fig 5) and *R. solanacearum* specific PCR product of 280-bp.

Table 3: List of primers used for multiplex PCR

S No.	Primer	Primer sequence	Expected band size	Remarks
Primers for multiplex PCR				
1.	Nmult:21:1F	5′-CGTTGATGAGGCGCGCAATTT-3′	144 bp	Phylotype I (Asiaticum)
2.	Nmult:21:2F	5′-AAGTTA TGGACGGTGGAAGTC-3′	372 bp	Phylotype II (Americanum)
3.	Nmult:22:InF	5′-ATTGCCAAGACGAGAGAAGTA-3	213 bp	Phylotype IV (Tropical)
4.	Nmult:23:AF	5′-ATTACGAGAGCAATC GAAAGATT-3′	91 bp	Phylotype II (African)
5.	Nmult:22:RR	5′-TCGCTTGACCCTATAACGAGTA-3		Amorce reverse unique
6.	759R	5'-GTCGCCGTCAACTCACTTTCC-3'	280 bp	Universal *R. solanacearum* specific primers
7.	760F	5'-GTCGCCGTCAGCAATGCGGAATCG-3'		

Inoculation

Ralstonia solanacearum isolates are inoculated to susceptible cultivars of potato (cv. K. Jyoti), tomato (cv. Pusa Rubi), brinjal (Pusa Purple Long) etc. for pathogenicity test or screening of varieties. The pathogenicity test or screening of varieties is conducted on potted plants (healthy plants grown in sterilized soil) in glass/ green house (temp. 20-30ºC) using 3-5 replicates for each isolate/ cultivar. A bacterial suspension of an isolate is prepared in distilled sterilized water using

48 h old growth on casamino acid peptone glucose (CPG) agar medium. The plants are inoculated by stem stab method when they are 15-20 cm tall. Usually 3rd of 4th axil bud from the top is inoculated by injecting/ placing a droplet of suspension (15-20 µl) on injury. Plants stabbed with sterilized distilled water serve as control. The inoculated plants are incubated at 20-30ºC temperature and observed for wilt appearance till 40 days of inoculation.

Isolation and maintenance of *Streptomyces* isolates

Potato tubers are washed thoroughly in running tap water for 5 to 7 minutes to remove soil adhered to their surface and air dried. The tubers are surface disinfected with 70% ethanol and small tuber pieces containing scab lesions are cut out and surface disinfected for one minute in 1% sodium hypochlorite solution. After several rinses in sterile distilled water, the tissues (about 1g) are ground in a pestle mortar in 5 ml of sterile water. Serial dilutions are plated onto starch casein agar (SCA) medium (Soluble starch 10g, casein 0.3g, potassium nitrate (KNO3) 2g, di-potassium hydrogen ortho-phosphate (K2HPO4) 2g, magnesium sulphate (MgSO4*7H2O) 0.05g, calcium carbonate (CaCO3) 0.02g, ferrous sulphate (FeSO4*7H2O) 0.01g, agar 20g, distilled water to make the final volume 1 litre, pH 7.2). Plates are incubated at 28±1 °C, and individual characteristic powdery (mycelial) bacterial colonies are picked and serially transferred until a pure culture is obtained. Potato dextrose agar medium is used for routine sub culturing of *Streptomyces* strains. Strains are maintained as spore suspension in 20% glycerol at –20°C.

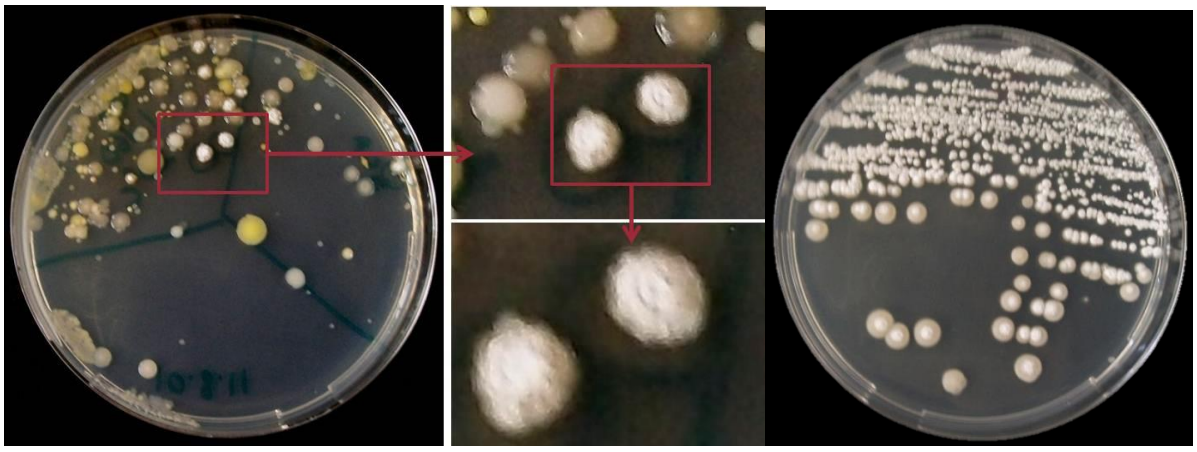


Fig 6: Typical leathery colonies of *Streptomyces* on starch casein agar giving powdery appearance and pure culture of *Streptomyces* (right)

Extraction of genomic DNA

Bacterial growth from a well purified culture on SCA medium is used to inoculate 1.5 ml of starch casein broth in 2.0 ml Eppendorf tubes. The cultures are grown at 28±1ºC for 48-72 hours with vigorous shaking (200 rpm). Genomic DNA is isolated by a versatile quick-prep method for genomic DNA of Gram-positive bacteria with some modifications. Mycelia (1–2 ml) grown in a starch casein broth shake culture are centrifuged, rinsed with TE and re-suspended in 0.4 ml SET buffer (75 mM NaCl, 25 mM EDTA, 20 mM Tris, pH 7.5) (Table 4). Lysozyme is added to a concentration of 1 mg ml^{-1} and incubated at 37ºC for 0.5–1 hour. Then 0.1 volume 10% SDS and 0.5 mg Proteinase K ml^{-1} are added and incubated at 55ºC with occasional inversion for 2 hours. One-third volume 5 M NaCl and 1 volume chloroform are added and incubated at room temperature for 0.5 hour with frequent inversion. The mixture is centrifuged at 4500 g for 15 minutes and the aqueous phase is transferred to a new tube using a blunt-ended pipette tip. Chromosomal DNA was precipitated by the addition of one volume 2-propanol with gentle inversion, washed twice with 70% ethanol, dried in speed-vacuum, and re-dissolved in 100 µl of distilled water. The dissolved DNA is treated with 20 mg RNase A ml^{-1} at 37ºC for 1 hour. Samples are extracted in the same volume of phenol/chloroform/isoamyl alcohol (25:24:1) and precipitated with 2.5 volume cold ethanol and 0.1 volumes 3 M sodium acetate. The pellets are washed with 70% ethanol, dried and dissolved in 50 µl TE or distilled water.

Table 4: Stock solutions used for preparation of lysis buffer for DNA extraction

Sr. No.	Name of stock solution	Required concentration	Volume required for 10 ml of SET buffer
1.	NaCl (5.0M)	75 mM	150 µl
2.	EDTA (0.1M)	25 mM	2500 µl
3.	Tris (2.0 M)	20 mM	100 µl
4.	Distilled water	-	7250 µl
	Total		10,000 µl

Purity check and quantification of DNA

For purity check and quantification, 5µl template DNA was loaded with 2µl of 6X loading dye on 1.0% agarose gel in Tris–Acetate EDTA buffer with 0.04 µl ml^{-1} of ethidium bromide at 100V for 1hr and visualized under ultraviolet (UV) light. DNA samples showing intact bands were selected for PCR amplification.

Sequencing of the 16S rRNA gene

The 16S rRNA gene was amplified from DNA of *Streptomyces* isolates by PCR using the primers 16S-1F (5'-CATTCACGGAGAGTTTGATCC-3') and 16S-1R (5'AGAAAGGAGGT GATCCAGCC-3') (Takeuchi *et al*., 1996). Amplification is carried out in 50 µl of reaction volume containing 10 mM Tris HCl (pH 9.0), 50 mM KCl, 0.1% Triton X100, 1.0 to 2.0 mM MgCl , 250 µM each dNTP, 10 pmol each primer, *Taq* DNA polymerase at 5 U/µl, 20 to 40ng DNA template, and MilliQ water to bring the volume to 50 µl. The thermal cycler is set to the following conditions: initial denaturation at 95°C for 3 min; followed by 40 cycles of denaturing at 95°C for 20 s, annealing at 60°C for 30 s, and extension at 72°C for 2 min; and ending with a 4°C hold.

PCR products of 1,500 bp (expected size) are electrophoreses in 1% agarose gel in Tris–Acetate EDTA buffer with 0.5 µl ml^{-1} of ethidium bromide at 105V for 1 hour and visualised and photographed under ultraviolet (UV) light.

For sequencing of PCR products, amplified products are further purified using Qiagen Minielute PCR purification kit following manufacturer's guidelines. The concentration of each product is estimated by gel electrophoresis with a low DNA mass ladder (Fermentas 1 Kb) and diluted with ultra-pure water to give a final concentration of 10-20 ng μl^{-1} for sequencing. PCR products are then cycle sequenced using forward primer in thermal cycler (Gen-AmpR PCR System 9700 of M/S Applied Biosystem). The cycle sequenced products are further purified. DNA samples then sequenced by an Applied Biosystem (Foster City, CA, USA) Model, AB310 Genetic Analyzer).

The 16S rRNA gene sequences analysis are performed using off-line software CLUSTAL X, Org. CLUSTAL W or X is a multiple sequence alignment program which calculates the best match for the selected sequences, and lines them up so that similarities and differences can be seen. Sequence data of all the 48 strains are manually edited and compared with the sequences from NCBI through BLAST programme. Sequences are aligned with the reference sequences from NCBI and phylogenetic analysis is done using CLUSTAL X software (Thompson *et al.*, 1994).

Pathogenicity test

Pathogenicity of *Streptomyces* strains is examined through Tuber Slice Assay as described by Hao *et al*., (2009). Potato tubers (cv. Kufri Chandermukhi) free from surface diseases are washed and sterilized for 1 min with 10% bleach (0.0625% NaClO). A tissue disk (20 mm in diameter by 7 mm in height) is bored from the tuber and placed on moist filter paper in a petri plate (100mm x 15mm). Pathogenicity of *Streptomyces* strains is examined as described by Loria *et al*. (1995). *Streptomyces* sp. inoculum is prepared from the cultures grown on oatmeal agar medium for 5 to 7 days. Agar plugs from *Streptomyces* cultures (3 mm-diameters) are placed at the centre of the potato tuber disks replicating thrice. Agar plugs from oat meal agar placed on potato tuber slices served as control. The disks were incubated in a moist closed container at 28±1°C in the dark and observed for necrosis of tuber slices.

Fig 7: Pathogenicity assay on tuber slices of cv. Kufri Chandermukhi: necrotic halos produced by the plugs from 5 days old oat meal agar cultures of *Streptomyces* strains, slices without halo are control.

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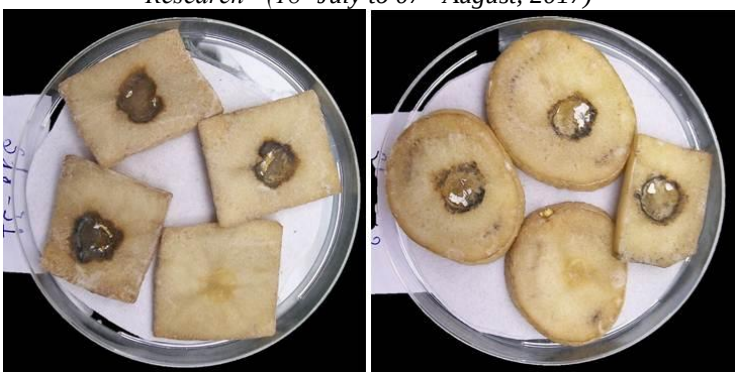

Fig 7: Pathogenicity assay on tuber slices of cv. KufriChandermukhi: necrotic halos produced by the plugs from 5 days old oat meal agar cultures of *Streptomyces* strains, slices without halo are control.

Elisa for detection of potato pathogens

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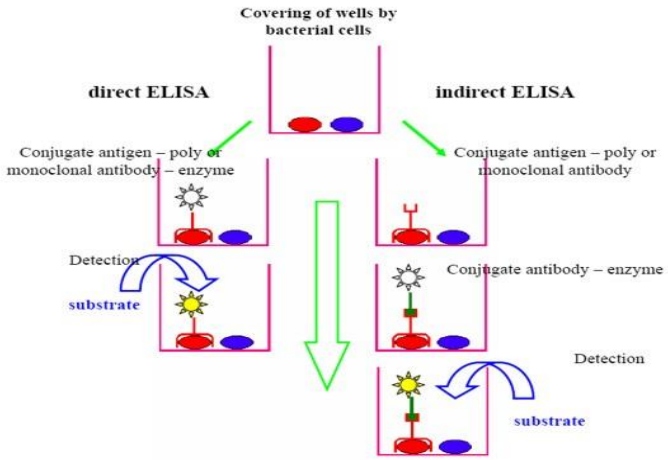
Serological methods: Serological methods are most often applicable tests for detection and identification of bacterial/viral plant pathogens. These types of methods based on interaction between antigen-antibody. Usually, the detection of plant viruses by serological techniques is based on the capacity of the antigens (virus particles) to react *in vitro* with their specific antibodies. Antibodies, specific for a particular virus are adsorbed to a carrier. In case of plant viruses 'trapping' IgG is usually bound to a solid phase (polystyrene homologous antigen is detected by an antibody linked with an enzyme). ELISA (Enzyme-linked Immunosorbent assay) is simple to perform, highly sensitive, reliable assay (delectability varies from 0.01 ng/ml to 500 ng/ml of virus depending on the virus), and rapid when automated. It is suitable for testing a large number of samples at a time without requiring large quantities of expensive and difficult to produce antisera. Of the several variants of ELISA, the most commonly used ones is double antibody sandwich-(DAS) (Clark and Adams, 1977; Goodwin and Banttari, 1984). ELISA has the added advantage of its ability to detect ultra low concentration viruses, like PLRV and PALCV in crude extracts. The common direct double antibody antibody sandwich format of ELISA in polystyrene microtitre plates (Clark and Adams, 1977) is the classical one and is widely used in routine testing of viruses. The reaction of colour intensities can be ready using an ELISA reader to quantify the relative antigen titres. It is recommended that at least two wells/sample be used and compared with A405 nm values obtained either through alkaline phosphatase-p-nitro-phenol) phosphate or A620nm values for penicillinase-bromothymol blue systems for the healthy samples. A sample may be considered infected only if its mean absorbance values are greater than that of mean plus 2SD of the mean of healthy controls.



Direct and indirect ELISA

Another effective variant is indirect ELISA. In this case, antibodies from two different animal species are required for trapping and detection, respectively. It helps to cut down the background. It is probably the best ELISA format for establishing the seroaffinity among strains of related viruses or even different viruses (Koening and Paul, 1982). Besides, a single (universal) conjugate can be used in this format for detection of different viruses (Koenig and Paul, 1982). Adams and Barbara (1982) used in indirect ELISA based on $F(ab)^2$ fragments for trapping of antigens and the enzyme labelled protein-A for the detection to help in assessing serological relationship among virus strains but C 1q-indirect ELISA was found less sensitive for serologically different virus strains.

Buffers and other solutions PBS (Phosphate-buffered saline), pH 7.4, NaCl-8.0g, KH_2PO_4 - 0.2g, Na_2HPO_4-2.9g, KCl-0.2g, Adjust pH with either NaOH or HCL and make upto 1L.

Coating or bicarbonate buffer, pH 9.6**,** Na_2CO_3-1.59g, $NaHCO_3$- 2.93g make up to 1L with distilled water

Sample Buffer pH 7.4, Na_2HPO_4 - 0.02M, NaH_2PO_4 -0.02M, Adjust the pH and add 0.15M NaCl

Extraction buffer/ Conjugate buffer pH 7.4, Sample buffer+0.05% Tween 20 +2% polyvinl pyrrolidon (PVP) + 0.2%, Egg albumin + 1M Urea.

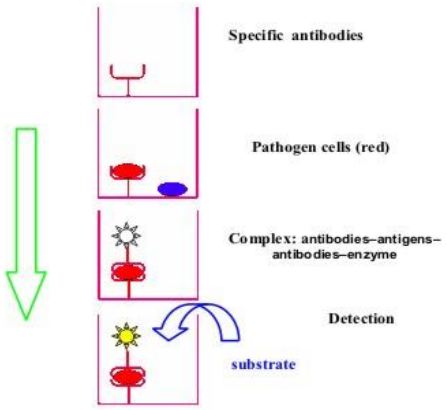
Substrate buffer pH 9.8, Diethanolamine, 97 ml, $MgCl_2$-100mg, Adjust pH 9.8 (with 6N Hcl) and make up to 1L with distilled water.

Alkaline phosphate (AP) and p-nitrophenly phosphatase system: It was standardized by Clark and Adams (1977) and is the most common. It may be adopted with slight modifications. Therefore, the procedures involving das-ELISA with alkaline phosphatase, horse radish peroxidase and penicillinase are detailed hereunder:

DAS-ELISA: In DAS-ELISA, in first step, the specific antibodies are covered the wells of micoplate. Antibodies are attached to the bottom of each well and then the samples with bacterial cells are added. The plate is then gently rinsed to remove unbound bacteria cells. After washing, there are only cells which antigens reacted with antibodies. Next, the antibodies conjugated with enzyme are added (detection of pathogen can be possible when complex antibodies-antigens-antibodies-enzyme is obtained). In the last step, the substrate is added and then the plates can be read visually or by machine.

The test is performed in micro-ELISA plates as follows:

1. Add 100-200ul purified diluted r-globulin in coating buffer to each well of the microtitre plate. Incubate 3 at 37°C.
2. Wash by flooding wells with PBS- Tween-20. Leave at least 4-5 min. Repeat washing five times.
3. Add 100-200 ul aliquots of the test sample to well. Leave 3 h at 37°C or overnight at +4°C.
4. Wash plate 5 times as in 2 above.



DAS-ELISA (direct)

5. Add 100-200ul aliquots of enzyme labelled r-globulin conjugate to each well. Incubate 3 at 37°C.
6. Wash plate 5 times as in 2 above.
7. Add 100-200 ul freshly prepared substrate (p-nitro-phenol phosphate) @ 0.6 mg/ml in substrate buffer. Incubate at room temperature 10 min to 1 h.
8. Stop reaction by adding 20 ul 3M NaOH to each well.
9. Assess results by (a) Visual observation (b) measurement of absorbance at 405'nm.

Protocols for Electron Microscopic detection of plant viruses

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Viral diseases can be detected or diagnosed by visual symptoms viz., mosaic patterns on leaves, stunting of the plant, leaf malformations, and tuber malformations. The other method of detection is by variants of electron microscopy (Garg et al., 2001; 2003), protein based (ELISA) and nucleic acid based polymerase chain reaction (Raigond et al., 2013). But all the above diagnostic techniques other than electron microscopy gives an indirect evidence of the causal agent (etiology) whereas, Transmission Electron Microscope (TEM) gives a direct access to see the causal agent. It is one of the most powerful scientific tools for carrying out detail structural studies of biological materials. In general, the purpose of any microscope is to form magnified image of a specimen so as to observe its maximum structural details (resolution). In TEM studies, negative staining makes it easy for detection of viruses from liquid samples. This led to the widespread application of TEM in the field of basics of virology and rapid diagnosis of viruses (Brenner and Horne, 1959).

Material required:

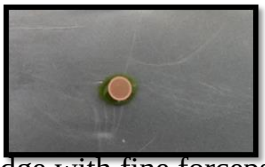
Clean glass Petridis, Para film or dental wax, filter paper, copper grids of 400 mesh coated with carbon, Sorenen's phosphate buffer, micropipette with disposable tips, clean and dry tweezers with very tips, negative stain for example: 2% UA in DDW, eppendorf tubes of 0.5ml capacity, wooden tooth picks and antiserum etc.

1) **Leaf dip method-** This method was originally described by Brandis (1957) and this method is mainly used for the detection of viruses particularly for those viruses that remain in very high concentration in leaf tissues.

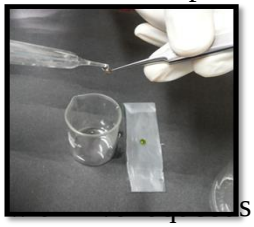
Protocol

- Grind infected leaf of about 2-3mm diameter in phosphate buffer 0.07M, pH 6.5.

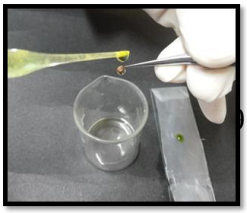
- Place 10 µl of homogenate on paraffin or waxed slide in a wet Petriplate.
- Place carbon coated copper grids (film side downward) on the surface of the droplet, ensuring that the grid surface is wet and allow it for 2-5 minutes.



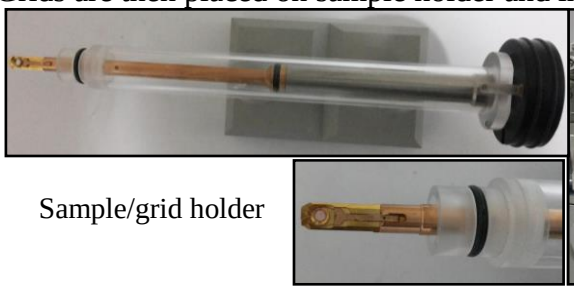
- Pick up the grid by its edge with fine forceps and wash the grid with 10-15 drops of double distilled water (DDW) to remove the sap.



- The grids are stained [illegible]s uranyl acetate (UA). The excess stain is immediately drained off by using Whatman filter paper.



- Grids are then placed on sample holder and inserted in TEM.



Sample/grid holder

120kV TEM

- The grids are observed under TEM for its morphology.

2) Immunosorbent electron microscopy (ISEM):

It is a procedure in which EM support film is first coated with a layer of antibody which serves to trap the virus preparation. ISEM is used for two main purposes as follows,

1) To trap increased number of particles on grid, by reducing the amount of host material.
2) To estimate degree of serological relationship between viruses by making use of trapping response.

Trapping- Float the carbon coated grids (carbon side down) over drop of diluted antiserum (1:1000) in phosphate buffer (pH 6.5, 0.07 M). The drop can be put on a piece of parafilm and leave for1 h at 37°C.

- Wash the grid for 10-15 n phosphate buffer.
- Drain briefly and then place them over a drop of 10µl leaf extract and leave for 30 min. at room temperature.
- Remove the grid and wash with approximately 10-15 drops of DDW.
- Stain with 2-4 drops of 2% freshly prepared uranyl acetate solution.
- Dry the grid by draining.
- Examine the grid under the TEM at 20,000 magnification.
- Count the number of particles from 10 different areas of treated and control grids.

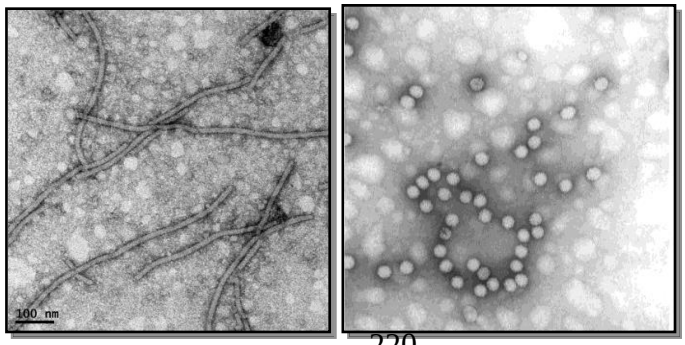
Decoration:

It is a process whereby virus particles are first individually observed to grid and grids are floated over specific antibody, layer of specific antibody halo is seen to have absorbed the stain and became electron dense. Milne and Luisoni (1977) first introduced the decoration method to the Plant Virology.

- Virus particles are adsorbed to the grid.
- Grid is carefully washed with phosphate buffer.
- Put the grid over a drop of diluted antiserum at room temperature for 30 minutes.
- Wash the grid with 10-15 drops of DDW & stain with 2% UA and allow to dry.

Expected results

1. We can see the size and shape (morphology) of the virus particles.



Elongated (PVA) and isometric (PLRV) *Potato virus*

Potato processing

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Potato is an important crop not only of the developed countries but also of the developing countries. For table purpose about 68% of potato production is consumed, while for processing operations share is 7.5%. Chips, dehydrated items, mainly instant mashed potatoes, frozen fries and other frozen products are gaining importance due to changing lifestyles and urbanisation. Potato processing (chips and French fry) should have certain minimum requirements such as shape, size, texture, dry matter and low susceptibility to accumulation of reducing sugars. Potatoes of high solid content/specific gravity are more efficient for processing. Dry matter or solid content of tuber is one of the prime characters used by the potato processors. For potato chips making, it is desirable to have potato varieties with round or oval tubers, dry matter should be more than 20% and the reducing sugar content should be less than 150mg/100g Fresh Weight. Dry matter from processing point of view is particularly significant when frying as the greater the dry matter the less fat uptake because there is less water for it to replace. Not only this, high dry matter content increases chip yield and crispy-consistency. The reducing sugar (glucose and fructose) content is critical in the quality processing potatoes, as it negatively affect the colour and flavor of finished product due to Malliard reaction (condensation of free amino acids and reducing sugars at higher temperature). Besides this, low reducing sugars and phenol contents are required to avoid dark color and bitter taste of processed products, which too negatively affect consumer acceptance. Keeping into consideration of morphological and biochemical parameters following varieties released by CPRI have been found suitable for processing parameters.

Characteristics of Indian potato varieties for processing

Variety	**Shape/size**	**Dry matter (%)**	**Reducing sugars (mg/100g FW)**
Kufri Chipsona-1	Oval/Large	21-24	45-100
Kufri Chipsona-2	Round/Large	21-25	44-93
Kufri Chandramukhi	Oval/Large	18-20	250-324
Kufr Jyoti	Oval/Large	18-21	106-275
Kufri Lauvkar	Round/Large	18-20	200-250

Besides the above shown 5 varieties, which are used by the processing industries, 3 more varieties have been released by the Institute for processing purpose, these are Kufri Chipsona -3, Kufri Surya and Kufri Himsona. Recently, Kufri Frysona has released by the institute for making French fries. Its tubers are oblong to long-oval preferably more than 75 mm size with shallow eyes to yield desirable long sticks of international standard and yield minimum peeling losses.

Potato chips

Chip colour and especially the taste determine the quality of potato chips. Light golden yellow color is preferred while dark brown coloured chips are unacceptable. Preferred size for making chips is 40-60mm. Potato varieties *viz.* Kufri Chipsona-1, Kufri Chipsona-2 Kufri Jyoti and Kufri Lauvkar contains high dry matter and accumulate low sugars.

Potato chips

Take potatoes and wash

↓

Peel the potatoes

↓

Make thin slices

↓

Blanch these slices in hot water (80° C)

↓

Cool in cold water

↓

Surface dry the slices

↓

Frying (at 180°C)

Potato flour

Potato flour can be used in combination with other cereal and pulses flour for preparation of number of products like *biscuits, cake, bhujia prantha* etc.

Potato flour

↓

Take 1.0kg of peeled potatoes in 2Litre of water in a cooker

↓

Add 5.0g of potassium metabisulphite and boil

↓

Mash the boiled potatoes and spread on tray

↓

Dry it in oven at 60-80°C till fully dry, turn upside down in between for proper drying

↓

Grind the dried mash and sieve it to get flour

↓

Store in air-tight containers and use when required

1kg of potatoes will give approximately 200g flour

Potato starch

Potato starch may be in several industries for preparation of soups, gravy, puddings. As softening agents starch is used in cakes, breads, biscuits and cookies. In paper industry it is useful in coatings smooth white paper because of strong binding power. Other uses include for the preparation of dextrins, adhesives, glues, drugs and binder etc.

Preparation of starch

Take 1kg of potatoes and peel it

↓

Cut into small pieces

↓

Add 1Litre of water containing 2.5g of potassium metabisulphite to the pieces

↓

Grind the pieces in a mixer-grinder

↓

With the help of water, sieve it in a tub through muslin cloth

↓

Let the starch settle on the surface of tub

↓

After the starch settle down, rinse it with fresh water for at least 2-3 times till the water is clear

↓

Collect the starch in a container and dry it in oven at 60°C/sunlight till fully dry

↓

Grind the starch to make fine powder and sieve

↓

Store the starch in airtight container and use when required

1kg of potatoes will give approximately 100g of starch

Potato flakes

Potato can be preserved by drying or half frying that minimizes the chances of microbial growth and lessens moisture content. Potato flakes/sreds are used generally in ready to use products in making of aaloo paranth, aaloo tikki and smosa etc.

Potato flakes

Take potatoes

↓

Wash and peel the potatoes

↓

Make slices

↓

Wash the slices

↓

Half fry the slices and cool it

↓

Complete drying

↓

Mash the slices

↓

Drying in drum dryer

↓

Remove the dry layer from drum

↓

Pack and store

Potato shreds

Take large sized potatoes and wash

↓

Peel the potatoes

↓

Shred the potatoes

↓

Treat these sticks with a solution containing salt and potassium metabisulphite for 2minutes

↓

Dry the sticks in solar drier or 3-4 days till they become brittle

↓

Store in airtight container

↓

Shallow –fry these dried sticks and mix spices before serving

Tissue culture media preparation

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Plant tissue culture media is prepared mainly using five different stock solutions. A general protocol for the preparation of nutrient stock solutions based on MS medium is given below:

1. Preparation of Plant tissue Culture Stock Solutions

A. Preparation of macro stock Murashige and Skoog (MS-I)

Macro stock Murashige and Skoog (MS-I) in 1000 ml

S. No.	Name of the chemical	Molecular formula	Quantity (g)	
			(10X)	(40X)
1.	Potassium nitrate	KNO_3	19.0	76.0
2.	Ammonium nitrate	NH_4NO_3	16.5	66.0
3.	Potassium di-hydrogen phosphate	KH_2PO_4	1.7	6.8
4.	Magnesium sulphate hepta- hydrate	$MgSO_4.7H_20$	3.7	14.8

Take 500 ml double distilled water in a 2.0 liter beaker, weigh, add and keep on dissolving each salt sequentially in a descending order as listed above (dissolve by magnetic stirring), and finally make up the volume to 1000 ml by distilled water. Store at 4°C. Always check for precipitation in macro stock before using it for medium preparation.

B. Preparation of calcium stock Murashige and Skoog (MS-II)

Calcium stock Murashige and Skoog (MS-II) in 1000 ml

S. No.	Name of the chemical	Molecular formula	Quantity (g)	
			(10X)	(40X)
1.	Calcium chloride dihydrate	$CaC1_2.2H_2O$	4.4	17.6

Take 500 ml double distilled water in a 2.0 liter beaker, weigh and add $CaCl_2.2H_20$, dissolve by magnetic stirring, and finally make up the volume to 1000 ml by distilled water. Store at 4°C.

C. Preparation of micro stock Murashige and Skoog (MS-III)

Micro stock Murashige and Skoog (MS-III) in 1000 ml

S. No.	Name of the chemical	Molecular formula	Quantity (mg)	
			(10X)	(40X)
1.	Boric acid	H_3BO_3	62.0	248.0
2.	Manganese sulphate mono-hydrate	$MnSO_4.H_20$	169.0	676.0
3.	Zinc sulphate hepta-hydrate	$ZnSO_4.7H_20$	86.0	344.0
4.	Potassium iodide	KI	8.3	33.2
5.	*Sodium molybdate di-hydrate	$Na_2MoO_4.2H_2O$	2.5	10.0
6.	**Copper sulphate penta-hydrate	$CuSO_4.5H_20$	0.25	1.0
7.	**Cobalt chloride hexa-hydrate	$CoCl_2.6H_20$	0.25	1.0

* Should be dissolved separately and then added to the stock solution

** Since it is difficult to weigh small quantities, prepare separate stocks of these salts @1.0 mg ml^{-1} and then add required quantity. Take 500 ml of double distilled water in a 2.0 liter beaker, weigh, add and keep on dissolving each salt sequentially in descending order as listed above

(dissolve by magnetic stirring), and finally make up the volume to 1000 ml by distilled water. Store at 4°C.

Note: $Na_2MoO_4.2H_20$ should be dissolved separately in approximately 20 ml of distilled water and added to the stock and $MnSO_4.H_2O$ takes time to dissolve.

D. Preparation of iron stock Murashige and Skoog (MS-IV)

Iron-EDTA stock (MS-IV) in 1000 ml (10 X)

S. No.	Name of the chemical	Molecular formula	Quantity (mg)	
			(10X)	(40X)
1.	Sodium EDTA di-hydarte	$Na_2EDTA.2H_20$	373.0	1492.0
2.	Ferrous sulphate hepta-hydrate	$FeSO_4.7H_20$	278.0	1112.0

Take 1000 ml double distilled water in a 2.0 liter amber colored bottle, and warm the water near boiling. Now weigh and add $Na_2EDTA.2H_20$ while stirring under a magnetic stirrer; after $Na_2EDTA.2H_2O$ has been dissolved, add gradually $FeSO_4.7H_20$ while still under mild magnetic stirring. This will yield a clear yellow solution. Immediately after adding $FeSO_4.7H_20$ close the bottle, keep on stirring at least for an hour and store at 4°C.

E. Preparation of vitamin stock Murashige and Skoog (MS-V)

Vitamin stock (MS-V) in 1000ml

No.	Name of the chemical	Quantity (mg)	
		(10X)	(40X)
1.	myo-Inositol	1000.0	4000.0
2.	Glycine	20.0	80.0
3.	*Thiamine HCI	1.0	4.0
4.	*Nicotinic acid	5.0	20.0
5.	*Pyridoxine HCI	5.0	20.0

* Since it is difficult to weigh small quantities, prepare separate stocks of these salts @1.0 mg ml^{-1} and then add required quantity.

Take 500 ml double distilled water in a 2.0 liter beaker, keep on adding and dissolving the vitamins sequentially in descending order as listed above, and finally make up the volume to 1000 ml by adding distilled water. Store at 0-4°C. Vitamin stock is very prone to microbial contamination in storage. Therefore, always check the stock before using.

2. Media preparation

Routine preparation of culture media using stock solutions is simple and involves following steps. The method has exemplified for preparing 1.0 liter of MS basal medium.

Murashige and Skoog (MS) media for 1000ml

S. No.	Stock solution	Quantity (ml)	
		10X	40X
1.	MS-I	100.0	25.0
2.	MS-II	100.0	25.0
3.	MS-III	100.0	25.0
4.	MS-IV	100.0	25.0
5.	MS-V	100.0	25.0

Mixing of stock solutions

For the preparation of 1.0 liter MS medium, the above stock solutions should be added sequentially in about 500 ml of double distilled water at the following rate:

1. In general, for potato micropropagation the Murashige and Skoog medium is supplemented with 0.1 M sucrose/sugar (20 or 30 g) and 4.19 µM (1.0 mg/ml) D-calcium pantothenate.

2. Weigh and add required quantities of sucrose and dissolve by magnetic stirring.
3. According to the purpose of the medium, other medium conjugates/ additives are added, and the volume of the medium is made up to 1000 ml by distilled water.
4. Adjust the pH of the medium to 5.8 using 0.1 N NaOH or 0.1 N HCl before autoclaving. Note that the pH meter should be calibrated by standard buffers (pH 4.0 and 7.0) immediately before adjusting the medium pH.
5. For preparing semisolid medium, heat the medium until near boiling in a microwave oven or gas oven with intermittent stirring and add agar at the rate of 6.0-8.0 gl^{-1} or gelrite at the rate of 2.0 gl^{-1}.
6. Mix thoroughly and dispense measured volume into culture tubes/containers/vessel using automatic media dispenser.
7. For preparing liquid medium, pH adjusted media are directly poured in suitable containers.
8. Plant tissue culture media are usually autoclaved at 121°C for 20 min (15 Ib in^2 or 1.05 kg cm^2).
9. Autoclaving is generally done in a horizontal or vertical autoclave.
10. Minimum time necessary for steam sterilization of media is dependent on volume of medium per vessel
11. Autoclaved media are kept in ambient temperature for a day and then transferred in a dust-free closed cabinet for subsequent use. Semisolid medium starts drying up, and therefore should be used within a fortnight after its preparation.

3. Sterilization Techniques

3.1 Steam sterilization

1. Plant tissue culture media are generally sterilized by autoclaving at 121°C (15 psi). The time required for sterilization depends on the volume of the medium in the vessel.
2. Always dispense medium in small aliquots whenever possible because many media components are broken down on prolonged exposure to heat and pressure.
3. Media exposed to temperatures in excess of 121°C may not properly gel or may result in poor culture growth.
4. Thermolabile components are prepared and filter-sterilized through a 0.2 µ filter in a sterile container.
5. The filtered chemicals is aseptically added to the culture medium, which has been autoclaved and allowed to cool to approximately 35-45°C.
6. The medium is then dispensed under aseptic conditions in the laminar flow.

3.2 Filter sterilization:

For sterilization of small volume (50 ml), use syringe filters. Generally syringe filters are available in 4, 13 and 25 mm diameters. Readymade PTFE or cellulose ester membranes (0.2µ) are convenient to use since they are packed sterile, and manufactured with female luer-lok™ inlet and male outlet as a standard; the filter housing can easily be connected to a syringe.

3.3 Surface sterilization of explants:

To prevent bacterial and fungal growth, tissue explants are surface sterilized before they are used to establish axenic/*in vitro* cultures. The most common disinfectants are listed below with the concentration and exposure. There is no universal working procedure for disinfecting plant tissue explants; the procedure varies from tissue to tissue and species to species. It is recommended that the experimenter standardize his/ her own protocol based on the following guidelines:

1. Wash the tissue explants with mild detergent (Tween-20) before treatment with the disinfectant solution.
2. Rinse the explants thoroughly under running tap water for 10-30 min.
3. Submerge the explants into the disinfectant solution, seal the bottle and gently agitate.
4. Under sterile conditions, decant the disinfectant solution and rinse the explants several times with sterile distilled water.

Commonly used disinfectants for plant tissue culture

S. No.	Name of the Disinfectants	Concentration (%)	Exposure time (min)
1.	m hypochlorite	9-10	5-30
2.	ım hypochlorite	0.5-5.0	5-30
3.	gen peroxide	3-12	5-15
4.	alcohol	70-95	1-5.0
5.	nitrate	1.0	5-30
6.	ric chloride	0.1-1.0	1-5
7.	lkonium chloride	0.01-0.1	5-20

*Commercial bleach contains about 5% sodium hypochlorite and thus may be used at a concentration of 10-20% which is equivalent to 0.5-1.0% sodium hypochlorite.

Fig. Process for the sterilization of culture media

Protoplast isolation and Fusion

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Protoplast Isolation

1. *In vitro* plant material: Three-week-old *in vitro*-grown microplants, raised from single nodal cuttings are used to isolate mesophyll protoplasts under sterile conditions
2. Microplants growth condition: 16 h photoperiod/40µmol m^{-2} s^{-1}/20°C.
3. Pre-isolation (protoplast) incubation: Cultivate *in vitro* plants at 20°C for 48 h under a 16-h photoperiod in the dark (covered with black muslin cloth) prior to protoplast isolation to integrate cell cycles.
4. Protoplast isolation: Mince young leaf tissues (1.0-2.0 g) in a Petri dish (Ø = 90 × 15 mm) containing **Protoplast Digestion Solution (PDS)**: 10.0 ml digestion solution for 1.0 g leaf tissue.
5. Incubation (for protoplast): 16 h/ dark/ 25°C/ optional: gyratory shaking at 40-50 rpm; not exceeding 50 rpm.
6. Post-isolation handling:
   - Add 0.3 M KCl (filter-sterilize) (FS) to the digestion medium containing released protoplasts (protoplast suspension) in a 1:1 ratio (PDS:KCl).
   - For example, add 15.0 ml of 0.3 M KCL to 15.0 ml digestion medium/ solution followed by gentle shaking of PDS containing released protoplast
   - Filter the suspension through a 40-µm nylon mesh, and collect in 10 ml centrifuge tubes; 60µ can be used, but the debris and/ or undigested tissues will be much.

Protoplast purification

- Centrifuge the filtrate at 50 ×g (60 RCF) for 5 min, and then resuspend the pallet in 10 ml of 0.6 M sucrose (filter sterilized).
- Layer 1.0 ml of 0.3 M KCl onto this protoplast suspension, and centrifuge at 50 g for 5 min.
- Recover the live protoplasts (green) from the sucrose: KCl interface, and dilute with 10.0 ml of 0.3 M KCl.

- Centrifuge at 50 ×g for 5 min to pellet the protoplast. Resuspend the pellet in 0.5 M mannitol (sterile) to a final density of 1×10^6 protoplasts ml^{-1} for electrofusion.

Protoplast Digestion Solution (PDS) Preparation

	Chemicals	Strength	Medium volume (500 ml)
1.	MS Macronutrients		
	KNO_3	0.95 g/L	475.0 mg
	KH_2PO_4	0.085 g/L	42.5=43.0 mg
	$MgSO_4.7H_2O$	0.185 g/L	92.5=93.0 mg
	$CaCl_2.2H_2O$	0.660 g/L	330.0 mg
2.	MS Micronutrients (To be used as half strength)	× 1000	250 µl
3.	MS Fe-EDTA(MS stock 5)	× 100	2.5 ml

	(To be used as full strength)		
4.	MS Vitamins (MS stock 6) (To be used as half strength)	× 1000	0.5 ml
5.	Myo-Inositol	100 mg/L	50.0 mg
6.	Polyvinylpyrrolidone (PVP) [AVM 10,000]	5.0 g/L	2.5 g
7.	MES (FW 195.24 for MES hydrate)	5.0 mM	488.0 mg
8.	D-Glucose (FW 180.16)	0.1 M	9.0 g
9.	D –Mannitol (FW-182.17)	0.4 M	36.434 g
10.	Cellulase 'ONOZUKA' RS or Cellulase ONOZUKA' R-10	1.0 %	Not to be added here
11.	Macerozyme R-10	0.5 %	Not to be added here

Notes

- **pH 5.7** to be adjusted using 0.1/1.0 N NaOH.
- Prepare the above digestion medium without digesting enzymes, adjust the pH and filter-sterilize.
- Store the medium in aliquots (20-25 ml) at -20°C.
- Before using, thaw the medium, add enzymes at required amount to medium aliquots, centrifuge to dissolve and check/adjust the pH (5.7), if required.
- Filter-sterilized (0.2 µm filter) and use.

1.3 Other Solutions

	Chemicals	**Strength**	**Medium volume (500 ml)**
1.	KCl (FW 74.55)	0.3 M	11.183 g
2.	Sucrose (FW 342.30)	0.6 M	102.69 g
3.	D-Mannitol (FW 182.17) (pH 7.0)	0.5 M	45.543 g
4.	**Solution 2 (pH 7)**		
	$CaCl_2.2H_2O$ (MW 147.02 g)	10 mM	220.53 mg
	D-Mannitol (FW 182.17 g) (pH 7)	0.5 M	150 ml
5.	**Solution 3 (pH 7)**		
	$CaCl_2.2H_2O$ (MW 147.02 g)	50 mM	1.1026 g
	D-Mannitol (FW 182.17 g) (pH 7)	0.5 M	150 ml
6.	**Sodium Alginate solution**		
	Na-Alginate (alginic acid sodium salt)	2.8%	2.8 g
	D-Mannitol (FW 182.17 g) (pH 7)	0.5 M	100 ml
7.	**NaOH (FW 40)**	1N	4.0 g/100 ml

Notes:

- Autoclave all solution at 121°C for required duration depending on the volume, and then filter-sterilize using a 0.2 µm filter.
- Use 1N NAOH to prepare 0.1N NAOH and filter-sterilize the solution in small aliquots.
- pH adjustment of Mannitol (pH 7) may take longer time, adjust with 0.1N NaOH.
- Na-Alginate solution may take longer time while dissolving solution.

Protoplast Electrofusion

Protocol:

1. Electrofusion medium: 0.5 M mannitol (FS)/ pH 7.0-7.3/adjust pH with sterile 0.1 N NaOH.
2. Symmetric fusion: 1:1 of each species under laminar work station
3. Electrofusion settings (under laminar):

Method 1:

CHAMBER:	BTX Microslide Model 453/3.2mm gap
Alignment amplitude:	16 V
Alignment time:	25-30 s
Alignment field strength:	5V cm^{-1}
Electroporation amplitude:	260 V
DC pulse width:	60 µs
Number of pulses:	2
Electroporation field strength:	812V/cm

Method 2:

CHAMBER:	BTX Microslide Model 453/3.2mm gap
Alignment amplitude:	32 V
Alignment time:	25-30 s
Alignment field strength:	100V/cm
Electroporation amplitude:	260 V
DC pulse width:	60 µs
Number of pulses:	1
Electroporation field strength:	812 V/cm

Method 3:

CHAMBER:	Disposable cuvette P/N620 (2mm gap)
Alignment amplitude:	16 V
Alignment time:	25-30 s
Alignment field strength:	50 V/cm
Electroporation amplitude:	260 V
DC pulse with:	60 µs
Number of pulse:	2
Electroporation field strength:	812 V/cm

Notes:

- In a disposable cuvette, fuse protoplasts in an aliquot of 400 µl and allow to stand the fusion products for at least 30 min for post-fusion recovery.
- Post-fusion AC pulse can be employed maximum up to 9 s compress the fusion product.

Regeneration of Post-Fusion (Protoplast) Products

Protocol:

1. Dispense 50 µl Na-alginate 2.8%, prepared in 0.5 M mannitol in each box of 'castor rack' and mix well the 50 µl post-fusion products with sodium alginate to form film layer. (1:1 ratio i.e. 50 µl each protoplast and Na-alginate)
2. Add ~5 ml Solution # 3 in each box and incubate at RT inside laminar for 30 min followed by add ~5 ml Solution # 2 and incubate same for 1.0 h
3. Remove Solution # 2 & # 3 by Pasteur pipette and add 5 ml **VKMG (VKM Glucose)** liquid medium and tightly wrapped the 'castor rack' with parafilm.
4. Incubate the castor racks (containing post-fusion products mixed with sodium alginate into VKMG) under dark at 25 °C for regeneration of microcalli (upto 4-6 months, depends upon the species).
5. The first cell division of the protoplast usually occur at 3-4 days after fusion, examine the growth and development of post-fusion products cultures under microscope at regular intervals. Cell aggregates (microcalli) are formed at 4-6 months after the fusion.
6. Before transfer the microcalli onto solid medium, dissolve the sodium alginate film surrounding post-fusion products/microcalli in **Dissolving Solution**.
7. After dissolving sodium alginate, wash microcalli in **Washing Solution**.

8. **Dissolving solution**→ MS_{13K} Medium (Without hormones + Coconut water + Gelrite) + 20 mM or 50mM Na-Citrate dehydrate (pH=7.0)
9. **Washing solution**→ MS_{13K} Medium (Without hormones + Coconut water + Gelrite) (pH=5.8)
10. Finally transfer microcalli onto **MS_{13K} Medium** (solid) in a Petri dish for development of micro-shoots/plants.
11. Transfer of microshoots/plants onto the **MS Medium** for *in vitro* regeneration and multiplication of putative somatic hybrids' plantlets.

VKM Medium Preparation

1. Macroelements

Chemical	Medium volume: 1 lit
KNO_3	1480 mg/L
KH_2PO_4 (FW 136.09)	68 mg/L
$MgSO_4.7H_2O$ (FW 246.48)	984 mg/L
$CaCl_2.2H_2O$ (FW 147.02)	735 mg/L

No need to prepare stock solution, add directly during medium preparation

2. VKM Stocks

Chemical	Medium volume: 1 lit
VKM stock-I	1 ml/L
MS stock-5	10.0 ml/L
VKM stock II	10.0 ml/L
VKM stock III	10.0 ml/L
VKM stock IV	10.0 ml/L
VKM stock-V	10.0 ml/L
VKM stock-VI	400 µl/L

3. Complex additives

Chemical	Medium volume: 1 lit
Casein Hydrolysate	250 mg/L
Coconut water (**store at -20°C**)	20 ml/L

Add directly during medium preparation. Coconut water to be added inside laminar

4. Growth hormone

Chemical	Medium volume: 1 lit
2,4-D (FW 221) (0.2 mg/L) (store at 0°C)*	200 µl/L
α-NAA (1.0 mg/L) (store at 0°C)*	1000 µl/L = 1 ml/L
Zeatin (trans) (FW 219.2) (0.5 mg/L) (store at -20°C)**	500 µl/L

*Separate stock of 1 mg/ml (dissolved in NaOH and water)

**Dissolved Zeatin-vial, supplied by manufacture, in 100 µl KOH and make up water volume to prepare stock of 1 mg/ml. (e.g. 10 mg/10 ml)

5. Sugar: Glucose (0.5M) - 90.1g for 1 litre medium

Note:

- Adjust medium pH: 5.6-5.7 with 0.1 M KOH.
- VKM medium is used for the division and growth of protoplast.
- Please note, that-VKM medium must be filter-sterilized using 0.2µ membrane filter (cellulose acetate/cellulose nitrate + 0.4µ pre filter (glass fiber).
- NO AUTOCLAVING

VKMG (VKM Glucose) medium

- VKM medium must be filter-sterilized using 0.2 µ membrane filter (cellulose acetate) + 0.4 µ prefilter (glass fibre)
- Weigh 9.01 g of 0.5 M Glucose in 100 ml VKM and adjust pH 5.6-5.7 with 0.1 M KOH

VKM STOCKS

Composition of VKM Stock for preparation of VKM Medium

a) VKM Stock-I

MICRO ELEMENTS	**mg/L**	**Stock (×1000)**
H_3BO_3 (FW 61.83)	3.0	300 mg/100 ml
$MnSO_4.H_2O$ (FW 169.02), OR $MnSO_4.4H_2O$, OR $MnSO_4$ (anhyd)	8.0 10.0 7.0	800 mg/100 ml 1000 mg/100 ml 700 mg/100 ml
$ZnSO_4.7H_2O$ (FW 287.54)	2.0	200 mg/100 ml
KI (FW 166.01) (Store in amber colour bottle)	0.75	75 mg/100 ml
$Na_2MoO_4.2H_2O$ (FW 241.95)	0.25	25 mg/100 ml
$CuSO_4.5H_2O$ (FW 249.68)	0.025	2.5 mg/100 ml*
$CoCl_2.6H_2O$ (FW 237.93) (store in amber colour bottle)	0.025	2.5 mg/100 ml*
1.0 ml VKM Stock-I for 1.0 L medium, Store at 2-8ºC		

* Prepare 1 mg/ml separate stock of each and add 2.5 ml to prepare VKM Stock-I

b) MS Stock-5

Chemical	**mg/L**	**Stock (×100)**
$FeSO_4.7H_2O$ (FW 278.01)	27.8	278 mg/100 ml
$Na_2EDTA.2H_2O$ (FW 372.24)	37.2	373 mg/100 ml
10.0 ml MS Stock-5 for 1.0L medium		

c) VKM Stock-II

Sugar & Sugar alcohol	**mg/L**	**Stock (×100)**
d-Mannitol (FW 182.17)	250.0	2.5g/100 ml
d-Sorbitol (FW 182.17)	250.0	2.5g/100 ml
Sucrose (FW 342.3)	250.0	2.5g/100 ml
Fructose (FW 180.2)	250.0	2.5g/100 ml
Ribose (FW 150.1)	250.0	2.5g/100 ml
Xylose (FW 150.1)	250.0	2.5g/100 ml
Mannose (FW 180.2)	250.0	2.5g/100 ml
Rhamnose (FW 182.2)	250.0	2.5g/100 ml
Cellobiose (FW 342.3)	250.0	2.5g/100 ml
m-Inositol (180.16)	100.0	1.0g/100 ml
Glucose*	90,000 (90 g)	9.0 g/100 ml
10 ml VKM Stock-II for 1.0 L medium, Store at 2-8°C		

*Glucose not to be added in the stock; to be added while preparing the medium at the end.

- **Filter-Sterilize (FS)** this stock and to be added inside the laminar work station (while preparing the medium)

d) VKM Stock-III

Organic acid	**mg/L**	**Stock (×100)**
Sodium-pyruvate (FW 110.04)	20.0	200 mg/100ml

(Pyruvic acid-sodium salt)		
Citric acid (FW 192.12)	40.0	400 mg/100ml
Fumaric acid (FW 116.07)	40.0	400 mg/100ml
Malic acid (FW 134.09)	40.0	400 mg/100ml
10ml VKM Stock-III for 1.0 lit medium, Store at 0ºC		

- Filter-Sterilize this stock and to be added inside the LFCA Work station (while preparing the medium

e) VKM Stock-IV

Vitamin	**mg/L**	**Stock (×100)**
Calcium-D-pontothenic acid (FW 283.3)	1.0	10 mg/100ml
Choline chloride (FW 139.63)	1.0	10 mg/100ml
Ascorbic acid (FW 176.12)	2.0	20 mg/100ml
p-Aminobenzoic acid (FW 137.14)	0.02	0.2 mg*
Nicotinamide	1.0	10 mg/100ml
Pyridoxine-HCl (FW 205.6)	1.0	10 mg/100ml
Thiamine-HCl (FW 337.27)	10.0	100 mg/100ml
Biotin (FW 244.3) (Vit H)	0.01	0.10 mg**
10 ml VKM Stock-IV for 1.0 lit medium, Store at 0ºC		

- Filter-Sterilize and to be added inside the LFCA Work sterilize (while preparing the medium)

* 200 µl from 1mg/ml separate stock of p-Aminobenzoic acid.

** 100 µl from 1mg/ml separate stock of biotin in KOH/NaOH.

f) VKM Stock-V

Vitamin	**mg/L**	**Stock (×100)**
Vitamin A	0.01	0.1 mg
10 µl from stock of 10 mg/ ml OR 100 µl from stock of 1 mg/ml for 100 ml VKM Stock-V. Dissolve in 100% ethanol only		
Vitamin B_{12}	0.02	0.2 mg
200.0 µl from 1mg/ml for 100 ml VKM Stock-V		
Vitamin D_3	0.01	0.1 mg
10 µl from stock of 10 mg/ ml OR 100 µl from stock of 1 mg/ml for 100 ml VKM Stock-V. Dissolve in 100% ethanol only		
10ml VKM Stock-V for 1.0 lit medium, Store at -20ºC		

g) VKM Stock-VI

Vitamin	**mg/L**	**Stock**
Folic acid (FW 441.4)	0.4 mg	1 mg/ml
400 µl VKM Stock-VI for 1.0 lit medium, Store at 0°C in amber colour bottle		

- This stock must be kept in an amber colour vial/or wrap Al-foil.

MS_{13K} Medium Preparation

1. Macroelements

Chemical	**mg/L**	**Medium Volume (1000 ml)**

NH_4NO_3	1650	1650 mg/1000 ml
KNO_3	1900	1900 mg/1000 ml
$CaCl_2.2H_2o$	440	440 mg/1000 ml
$MgSO_4.7H_2O$	370	370 mg/1000 ml
KH_2PO_4	170	170 mg/1000 ml

Add directly during medium preparation

2. Microelements

Chemical	**mg/L**	**Stock (×1000)**
H_3BO_3	6.2	620 mg/100 ml
$MnSO_4.H_2O$	16.9	1690 mg/100 ml
$ZnSO_4.7H2O$	10.59	1059 mg/100 ml
KI	0.83	83 mg/100 ml
$Na_2MoO_4.2H_2O$	0.25	25 mg/100 ml
$CuSO_4.5H_2O$	0.025	2.5 mg/100 ml*
$CoCl_2.6H_2O$	0.025	2.5 mg/100 ml*
1 ml for 1.0 lit medium		

* Prepare 1 mg/ml stock and add 2.5 ml to prepare Microelements stock

3. Iron –EDTA (or MS stock-V)

Chemical	**mg/L**	**Stock (×100)**
$Na_2EDTA.2H_2O$	37.2	373 mg/100 ml
$FeSO_4.7H_2O$	27.8	278 mg/100 ml
10.0 ml for 1 lit medium		

4. Vitamins (or MS stock-VI)

Chemical	**mg/L**	**Stock (×1000)**
Nicotinamide	0.5	50 mg/100 ml
Pyridoxine-HCl	0.5	50 mg/100 ml
Thiamine-HCl	0.1	10 mg/100 ml
Glycine	2.0	200 mg/100 ml
1.0 ml for 1 lit medium		

5. Choline chloride

Chemical	**mg/L**	**Stock**
Choline chloride	8.0	1 mg/ml

- 8 ml from stock of 1 mg/ml for 1 lit medium

6. Amino acids

Chemical	**mg/L**	**Stock (×100)**
Arginine-HCl	6.24	62.4 mg/100ml
Asparaginic acid*	4.8	4.8 mg/100ml
Cystein**	1.2	12.0 mg/100ml
Glutamic acid	11.2	112.0 mg/100ml
Histidine	2.08	20.8 mg/100ml
Isoleucin	8.2	82 .0mg/100ml
Leucin	12.48	124.8 mg/100ml
Lysine	12.48	124.8 mg/100ml
Methionine	10.4	104 .0mg/100ml
Phenylalanine	4.0	40 .0mg/100ml

Proline	4.0	40.0 mg/100ml
Threonine	10.4	104.0 mg/100ml
Tryptophan	3.2	32.0 mg/100ml
Valine	10.4	104.0 mg/100ml
10.0 ml for 1 lit medium		

- Asparagine, isoleucine, tryptophan and valine take time to dissolve so use warm water

7. Myo-Inositol: 100 mg for 1 lit medium
8. Sucrose: 30.0 g for 1 lit medium
9. Coconut milk/Water: 50 ml for 1 lit medium
10. Growth Hormones (to be added by sterile - filtration)

Chemical	mg/L	Stock (1mg/ml stock)
IAA	0.1 mg/l	100 µl/ 1 lit
Zeatin-riboside	2.0 mg/l	2 ml/ 1 lit

11. Gelrite: 2.5 g for 1 lit medium

- Prepare MS13K medium by mixing amount/volume of SN 1 to 11
- Adjust pH: 5.8; Autoclave: 121°C for 20 min
- After autoclave, add the required amount of growth hormones by sterile filtration
- This medium is used for Callus regeneration and growth of post-fusion products.

Solubility Parameters of Growth Hormones

SN	Hormones	Solubility	Stock solution	Storage
1.	2,4-D (2,4 dichlorophenoxy acetic acid)	At 60ºC in water bath (ddH_2O)/ 100% EtOH/ 1N KOH or NaOH	20 mg/20 ml	0ºC
2.	α-NAA (Naphthalene acetic acid)	At 60ºC in water bath (ddH_2O)/ 1N KOH or NaOH	20 mg/20 ml	0ºC
3.	Zeatin mix isomer*	1N KOH or NaOH	10 mg/10 ml	-20ºC
4.	Zeatin riboside (ZR)*	1N KOH or NaOH	10 mg/10 ml	-20ºC
5.	BA (N^6Benzyl adenine)	1N KOH or NaOH	20 mg/10 ml	0ºC
6.	GA_3	100% EtOH/1N KOH or NaOH	20 mg/10 ml	0ºC
	*Directly dissolve in the vial supplied by manufactures			

Solubility Parameters of Vitamins

SN	Vitamins	Solubility	Stock solution	Storage
1.	4 (para) amino benzoic acid (PABA) (Vit Bx/Vit H1)	At 60ºC in water bath (ddH_2O)	20 mg/20 ml	2-8 ºC (0ºC)
2.	Foliac acid (Vit M)	1N KOH	20 mg/20 ml	2-8 ºC (0ºC)
3.	Biotin (Vit H/VitB_7)	At 60ºC in water bath (ddH_2O)/ 1N KOH	20 mg/20 ml	2-8 ºC (0ºC)
4.	Vit A (Retinol acetate)*	100% EtOH (@ 25 mg/ml of EtOH)	20 mg/2 ml	-20ºC
5.	Vit B_{12} (Cyanocobalamine)	At 60ºC in water bath (ddH_2O)/ 1N KOH	20 mg/20 ml	2-8 ºC (0ºC)
6.	VitD_3 (Cholcalciferol)*	100% EtOH (@ 10 mg/2ml of 100% EtOH)	20 mg/2 ml	2-8 ºC (0ºC)

7.	Pyrodoxin-HCl (Vit B_6)**	ddH_2O	20 mg/20 ml	2-8 ºC (0ºC)
8.	Thiamine-HCl (Vit B_1)**	ddH_2O	20 mg/20 ml	2-8 ºC (0ºC)
9.	Nicotinamide (Vit B_3)**	ddH_2O	20 mg/20 ml	2-8 ºC (0ºC)
10.	Nicotinic acid (Niacin)** (Vit B_3)	ddH_2O	20 mg/20 ml	2-8 ºC (0ºC)
	*May encounter difficulties in dissolving			
	** Weigh directly while preparing the vitamins stock			

MS Medium Preparation

1. MS Stock- 1

Chemical	**Strength (× 50)**	**100 ml**	**250 ml**	**500 ml**	**1000 ml**
NH_4NO_3 1650 mg/L	20 ml MS Stock 1 for 1L medium	8.250 g	20.625 g	41.250 g	82.500 g
KNO_3 1900 mg/L		9.500 g	23.750 g	47.500 g	95.000 g

2. MS Stock- 2

Chemical	**Strength (× 100)**	**100 ml**	**250 ml**	**500 ml**	**1000 ml**
$MgSO_4.7H_2O$ 370 mg/L	10 ml MS Stock 2 for 1L medium	3.700 g	9.250 g	18.500 g	37.000 g
$MnSO_4.H_2O$ 16.9 mg/L		169 mg	423 mg	845 mg	1690 mg
$ZnSO_4.7H_2O$ 8.6 mg/L		86 mg	215 mg	430 mg	860 mg
$CuSO_4.5H_2O$ 0.025 mg/L		0.25 mg (1.0 ml)	0.625 mg (2.5 ml)	1.25 mg (5.0 ml)	2.5 mg (10.0 ml)
Dissolve 25mg $CuSO_4.5H_2O$ in 100 ml dH_2O and then add required volume (within parenthesis) to MS 2 Stock					

3. MS Stock- 3

Chemical	**Strength (× 100)**	**100 ml**	**250 ml**	**500 ml**	**1000 ml**
$CaCl_2.2H_2O$ 440 mg/l	10 ml MS Stock 3 for 1L medium	4.400 g	11.000 g	22.000 g	44.000 g
KI 0.83 mg/L		8.3 mg	21.0 mg	41.5 mg	83.0 mg
$CoCl_2.6H_2O$ 0.025 mg/L		0.25 mg (1.0 ml)	0.625 mg (2.5 ml)	1.25 mg (5.0 ml)	2.5 mg (10.0 ml)
Dissolve 25mg $CoCl_2.6H_2O$ in 100 ml dH_2O and then add required volume (within parenthesis) to MS 3 Stock					

4. MS Stock- 4

Chemical	**Strength (× 100)**	**100 ml**	**250 ml**	**500 ml**	**1000 ml**
KH_2PO_4	10 ml MS	1.700 g	4.250 g	8.500 g	17.000 g

170 mg/L	Stock 4 for 1L medium				
H_3BO_3 6.2 mg/L		62.0 mg	155 mg	310 mg	620 mg
$NaMoO_4.2H_2O$ 0.25 mg/L		2.5 mg (1.0 ml)	6.25 mg (2.5 ml)	12.5 mg (5.0 ml)	25.0 mg (10.0 ml)
Dissolve 250mg $NaMoO_4.2H_2O$ in 100 ml dH_2O and then add required volume (within parenthesis) to MS 4 Stock					

5. MS Stock- 5

Chemical	**Strength (× 100)**	**100 ml**	**250 ml**	**500 ml**	**1000 ml**
$FeSO_4.7H_2O$ 27.8 mg/L	10 ml MS Stock 5 for 1L medium	278 mg	695 mg	1390 mg	2780 mg
$Na_2EDTA.2H_2O$ 37.3 mg/L		373 mg	933 mg	1865 mg	3730 mg
Store in amber colour bottle					

6. MS Stock- 6 (Vitamins)

Chemical	**Strength** (× **1000)**	**100 ml**
Thiamine-HCl (0.1 mg/L)	1 ml MS Stock 6 for 1L medium	10.0 mg
Pyridoxine-HCl (0.5 mg/L)		50.0 mg
Nicotinic acid (0.5 mg/L)		50.0 mg
Glycine (2.0 mg/L)		200.0 mg
Store at 0°C		

<u>Weigh and add directly</u>:

- Myo-Inositol: 100 mg/L
- Sucrose: 20.0 g/L
- pH: 5.8
- Gelrite: 2.0 g/L
- Autoclave-sterilize: 121 °C for 20 min