Training
On
Seed Potato Production
(04th April – 13th April, 2018)

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Sponsored by: Govt. of West Bengal, WB
Organized by: ICAR–Central Potato Research Institute, Shimla & CPRS, Jalandhar

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### Training on “Seed Potato Production”

**Venue:** ICAR-Central Potato Research Institute, Shimla & CPRS, Jalandhar  
**Duration:** 04-13 April, 2018

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Potato is one of the non-cereal food crops that has the capacity to produce more food per unit time & area and can feed the ever increasing population of the developing countries. Seed constitutes a major and important input in potato (*Solanum tuberosum* L.) cultivation. Although several factors contributed towards its successful cultivation in the country, but the development of indigenous varieties and seed production system were the two main pillars that sustain this crop. During the last five decades, seed production has shown a spectacular progress in technology development and production strategies. India is the only leading country in Asia which has developed the scientific seed production technology for sub-tropics by taking the advantages of low aphid period and absence of soil borne insect pest and diseases. Ever since, development of “Seed Plot Technique” the seed production system has witnessed many technological breakthroughs; be it development of diagnostics based on ELISA/PCR protocol, tuber indexing, meristem tip culture, tissue culture based system of seed production or aeroponics. This compendium aims to deliver a broad range of seed production techniques in a form which is accessible to farmers, young entrepreneurs, students and research scientists of diverse backgrounds, including those with little or no previous experience. The themes aim to reflect those research areas which have been advanced by various diagnostic protocols, methodology and techniques that are required for production of quality potato seed.

I congratulate the contributors and editors of this manual for their efforts in compiling this highly informative and practical information with latest updates on “Seed potato production”. I am confident that this manual would be a great help for all stakeholders associated with seed potato production.

Shimla (SK Chakrabarti)
April, 2018
Director, ICAR-CPRI, Shimla
Potato scenario in India and World

SK Chakrabarti
Director, ICAR-Central Potato Research Institute, Shimla-171001 (HP), India

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 ad 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 initiating 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>
<thead>
<tr>
<th>Year</th>
<th>Area (million ha)</th>
<th>Production (million tones)</th>
<th>Yield (q/ha)</th>
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<tbody>
<tr>
<td>1949-50</td>
<td>0.239</td>
<td>1.54</td>
<td>65.9</td>
</tr>
<tr>
<td>1959-60</td>
<td>0.362</td>
<td>2.73</td>
<td>75.5</td>
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<tr>
<td>1969-70</td>
<td>0.496</td>
<td>3.91</td>
<td>78.9</td>
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<tr>
<td>1979-80</td>
<td>0.685</td>
<td>8.33</td>
<td>121.5</td>
</tr>
<tr>
<td>1989-90</td>
<td>0.940</td>
<td>14.77</td>
<td>157.1</td>
</tr>
<tr>
<td>1999-00</td>
<td>1.340</td>
<td>24.71</td>
<td>184.4</td>
</tr>
<tr>
<td>2003-04</td>
<td>1.270</td>
<td>23.12</td>
<td>182.0</td>
</tr>
<tr>
<td>2005-06</td>
<td>1.400</td>
<td>23.90</td>
<td>170.6</td>
</tr>
<tr>
<td>2006-07</td>
<td>1.482</td>
<td>22.09</td>
<td>149.0</td>
</tr>
<tr>
<td>2007-08</td>
<td>1.553</td>
<td>28.47</td>
<td>183.3</td>
</tr>
<tr>
<td>2008-09</td>
<td>1.810</td>
<td>28.58</td>
<td>157.8</td>
</tr>
<tr>
<td>2009-10</td>
<td>1.840</td>
<td>36.58</td>
<td>199.2</td>
</tr>
<tr>
<td>2010-11</td>
<td>1.860</td>
<td>42.34</td>
<td>227.2</td>
</tr>
<tr>
<td>2011-12</td>
<td>1.910</td>
<td>41.48</td>
<td>217.5</td>
</tr>
<tr>
<td>2012-13</td>
<td>1.992</td>
<td>45.34</td>
<td>227.8</td>
</tr>
<tr>
<td>2013-14</td>
<td>1.97</td>
<td>41.5</td>
<td>21.1</td>
</tr>
<tr>
<td>2014-15</td>
<td>2.07</td>
<td>48.0</td>
<td>23.2</td>
</tr>
<tr>
<td>2015-16</td>
<td>2.11</td>
<td>43.4</td>
<td>20.5</td>
</tr>
<tr>
<td>2016-17</td>
<td>2.17</td>
<td>46.6</td>
<td>21.5</td>
</tr>
</tbody>
</table>

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°C and day temperature is below 30°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 blights 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°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 Lauvka r, 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.

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 campaign 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.

**Table 2: State-wise Area, Production & Yield of Potato in India**

<table>
<thead>
<tr>
<th>States</th>
<th>Area (1000 Hectares)</th>
<th>Production (1000 Tonnes)</th>
<th>Yield (q/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uttar Pradesh</td>
<td>603.76</td>
<td>14430.28</td>
<td>239.0</td>
</tr>
<tr>
<td>West Bengal</td>
<td>386.61</td>
<td>11591.30</td>
<td>299.8</td>
</tr>
<tr>
<td>Bihar</td>
<td>322.50</td>
<td>6640.60</td>
<td>205.9</td>
</tr>
<tr>
<td>Assam</td>
<td>99.77</td>
<td>975.27</td>
<td>97.7</td>
</tr>
<tr>
<td>Madhya Pradesh</td>
<td>108.87</td>
<td>2299.00</td>
<td>211.2</td>
</tr>
<tr>
<td>Punjab</td>
<td>85.25</td>
<td>2132.31</td>
<td>250.1</td>
</tr>
<tr>
<td>Gujarat</td>
<td>81.27</td>
<td>2499.73</td>
<td>307.6</td>
</tr>
<tr>
<td>Jharkhand</td>
<td>47.21</td>
<td>659.61</td>
<td>139.7</td>
</tr>
<tr>
<td>Karnataka</td>
<td>44.40</td>
<td>698.30</td>
<td>157.3</td>
</tr>
<tr>
<td>Haryana</td>
<td>29.47</td>
<td>676.01</td>
<td>229.4</td>
</tr>
<tr>
<td>Others</td>
<td>183.2</td>
<td>2741.20</td>
<td>149.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1992.2</strong></td>
<td><strong>45343.60</strong></td>
<td><strong>227.6</strong></td>
</tr>
</tbody>
</table>

Source: Directorate of Economics & Statistics, Govt. of India.
The surplus potatoes in a season are stored in cold stores at 2-4°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°C. Only seed potatoes should be cold stored at 2-4°C. This would release at least 60 per cent of cold storage space that can be converted to store potatoes for processing and table purposes at 10-12°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.
Hi-tech seed production system
Tanuja Buckseth and RK Singh
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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 Pharmaceuticals Ltd., Ahmedabad; KF Bioplants, Pvt. Ltd., Pune/Banglore; Transgene Bioplants Pvt. Ltd., Chandigarh; Phulwari Bio-Tech Ltd., Chandigarh; Gufic Bioscience 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°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°C and 16 h photo period (120-200 µmol/m²/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 25°C and 16 h photo period (120-200 µmol/m²/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 healthy microplants are transferred to protays filled with sterilized peat moss. The microplants can be planted in portrait 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 portrait 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 portrait (4-5 days), these portrait are transferred to hardening chamber and kept at 27°C for 10-15 days. The hardened plantlets should be removed from portrait 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-1 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°C and 16 h photoperiod (120-200 µmol/m²/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 mg/l⁰ N⁶-benzyladenine (BAP) and 80 g/l⁻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°C 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

References:


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°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>
<thead>
<tr>
<th>Zone</th>
<th>Varietal requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>North-western hills</td>
<td>Long day adapted, highly resistant to late blight</td>
</tr>
<tr>
<td>Central hills</td>
<td>Long day adapted, highly resistant to late blight and bacterial wilt.</td>
</tr>
<tr>
<td>North-eastern hills</td>
<td>Long day adapted, highly resistant to late blight and bacterial wilt.</td>
</tr>
<tr>
<td>North-western plains</td>
<td>Short day adapted, early bulking, heat tolerance and moderate resistant to late blight, slow rate of degeneration. Tolerance to frost is an added advantage.</td>
</tr>
<tr>
<td>West-central plains</td>
<td>Short-day adapted, early bulking, moderate resistant to late blight and slow rate of degeneration. Tolerance to frost is an added advantage.</td>
</tr>
<tr>
<td>North-eastern plains</td>
<td>Short day adapted, early bulking, moderate resistant to late blight and slow rate of degeneration. Red skin tubers are preferred in some areas.</td>
</tr>
<tr>
<td>North Bengal hills and Sikkim</td>
<td>Medium maturity, resistance to late blight and immunity to wart. Red skin potatoes are preferred.</td>
</tr>
<tr>
<td>Plateau region</td>
<td>Early bulking, ability to tuberize under high temperatures, resistance to bacterial wilt, mites &amp; potato tuber moth and slow rate of degeneration.</td>
</tr>
<tr>
<td>Southern hills</td>
<td>Long day adapted, early bulking, resistant to late blight and cyst nematode.</td>
</tr>
</tbody>
</table>
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

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

*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 \((2n=4x=48)\) 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**

<table>
<thead>
<tr>
<th>Varieties/samples</th>
<th>Salient features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agra Red, Chamba Red, Coonoor White, Coonoor Red, Darjeeling Red Round, Desi,</td>
<td>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</td>
</tr>
<tr>
<td>Dhantauri, Gola Type A, Gola Type B, Gola Type C, Phulwa, Phulwa Purple Splashed,</td>
<td></td>
</tr>
<tr>
<td>Sathoo, Red Long Kidney, Shan and Silbilati</td>
<td></td>
</tr>
</tbody>
</table>


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

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Salient features</th>
</tr>
</thead>
<tbody>
<tr>
<td>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</td>
<td>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</td>
</tr>
</tbody>
</table>


**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 red-violet 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 cv 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**


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°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°C), spore production (18-22°C), spore germination (10-20°C) and for infection and disease development (7.2-26.6°C with optimum 18±1°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 23.9°C 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°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 (7.2-26.6°C) 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 (7.2-26.6°C) 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 7.2-26.6°C 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°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 (17-25°C) 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 wills 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 25-30°C and for the germination of sclerotia is 23°C.

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 28°C almost completely checks the disease. Therefore, in subtropics, 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 often 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°C, the optimum temperature for germination and infection is between 22 & 28°C. 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 up to 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°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 plantng, 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 rottting 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°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°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°C) if the moisture is favourable.

**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°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°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°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 subtropical plains where summer temperatures are very high and is practised 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 30
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:**

Potato is usually propagated by vegetative mode and the mode of propagation is overwhelmed with many problems; of which seed ‘degeneration’ is an important problem. It was reported that the degeneration of seed was due to viruses, mycoplasmas and viroids that multiply during successive clonal generations. Among the viruses reported globally, only eight are reported to occur in India i.e., PVY, PVA, PVX, PLRV, PVS, PVM, Potato Apical Leaf Curl Virus and Potato Stem Necrosis Virus.

Majority of the viruses are transmitted by insect vectors like aphids, white flies and thrips which are active under tropical and subtropical agroclimatic condition of India. Viral diseases cannot be directly managed by chemical means. Hence, exclusion of these viruses is a major step to manage, where detection is one of the effective pillars of effective pathogen exclusion strategy. Visual inspection of viral disease symptoms is a major step in detection causal virus. But the drawback was, similar symptoms could be produced by certain nutrient deficiencies or other abiotic or biotic factors. Therefore, there is a need to develop an accurate, sensitive and reliable detection technique for detection of viral diseases. Hence, diagnostic techniques like Electron microscopy, serological (ELISA), molecular (PCR & RT-PCR) based assays and on-site detection techniques like dipstick have been developed to screen pre-basic nucleus seed stocks and also for post-entry quarantine testing of imported material and mericlones during cleaning of virus infected stocks.

1. Electron Microscopy

**Introduction:** 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.

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

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 immuno 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.

2. Serological based diagnostic assays

Many serological techniques have been developed for identification and characterization of plant viruses and the advent of the enzyme-linked immunosorbent assay (ELISA) has facilitated the use of serology for virus identification in large scale. The serological methods can be subdivided into two broader categories involving liquid and solid phase methods. The liquid phase methods can be represented by the double immuno diffusion techniques in which the antigen and the antibodies react in agar media producing visible precipitates. In the solid phase methods, one of the reagents, usually the antibody, is trapped on a solid surface that could be nitrocellulose membrane, a microtitre plate, polystyrene or polyvinyl chloride plates. In this case the antigen-antibody reaction is detected by a labeled antibody as in the ELISA and its variations. In addition the virus particles are detected by direct visualization with serologically specific electron microscopy (SSEM).
i. **Enzyme-Linked Immunosorbent Assay (ELISA):** It is a very sensitive and specific serological technique introduced for identification of plant viruses in the 1970s. This technique is capable of detecting virus particles in very low concentrations and can be used with viruses of different particle morphology. Because of its adaptability, high sensitivity, and economy in the use of reagents, ELISA is used in a wide range of situations, especially for indexing a large number of samples in a relatively short period of time. The ELISA technique is based on the basic principle in which the virus antigens are recognized by their specific antibodies (IgG) in association with colorimetric properties. The ELISA method is commonly accomplished in a 96-well polystyrene plate by adding the antigens and antibodies into the wells in an established sequence, involving several stages. In the final stage, the positive reactions are detected when a colorless substrate, usually p-nitrophenyl phosphate, undergoes a chemical change resulting in a yellow colored product as the result of exposure to the enzyme alkaline phosphate linked to the antibody. The degree of color change indicates the degree of reactivity that is read by an ELISA plate reader apparatus. It is always recommended to include a homologous antigen for the specific virus antibody and extracts from healthy plants to compare the absorption readings and to obtain a correct interpretation of the results. The principle of ELISA techniques consists of detecting the antigen-antibody interactions by enzyme induced color reaction rather than by observing their precipitation. Although different variations of this serological technique have been developed i.e., direct and the indirect ELISA.

**Direct ELISA:** Direct ELISA is also called as double antibody sandwich (DAS-ELISA). The first step in this test is the adsorption of virus-specific antibodies to the wells of ELISA plates where unbound antibody is removed by washing, and the samples to be tested for virus antigen are added. Controls include extracts from known infected plants (positive control), and extracts from healthy plants (negative control) are to be used. After incubation and washing, the enzyme-antibody conjugate is added. If virus attached to the coating antibody is present, the enzyme-antibody conjugate will combine with the virus. Plates are washed, and the colorless substrate (p-nitrophenyl phosphate) is added. Positive wells will show a yellow reaction, due to the action of the conjugated enzyme (alkaline phosphatase) on the substrate and negative wells will remain colorless. The colorimetric changes are measured in an ELISA reader, using a filter for 405 nm wave length. The quality of the antiserum is critical in achieving certain objectives, but a good, broad spectrum polyclonal antiserum will give satisfactory results in most virus indexing programs. On the other hand, monoclonal antibodies could be useful for identification and characterization of specific plant virus strains.

**Indirect ELISA:** Indirect ELISA or plate-trapped antigen (PTA-ELISA) was developed to avoid the inconveniences and difficulties of conjugating the enzyme with the IgG specific for each virus species to be used in the second layer of antibodies in direct ELISA. For this reason, the indirect ELISA or PTA-ELISA requires antibodies produced in two different animal species and the virus particles are trapped in the wells of the ELISA plate. The indirect ELISA also requires the use of a universal IgG enzyme conjugate which can be used with the antibodies of all virus species. The so called universal conjugate is composed of an IgG produced against the IgGs from the animal in which virus antibodies are raised linked to the enzyme alkaline phosphate. If the virus antibodies are produced in rabbits (e.g.), an antirabbit IgGs are produced in a second animal species such as goats or mice. So, the detecting antibody conjugate binds specifically to the primary virus specific antibody. In this method, the wells of the ELISA plate are, initially, covered with extracts from infected plants and healthy plant samples.
Later the virus particles are covered with a layer of virus specific antibodies produced in a rabbit. The complex antigen-antibodies are covered with a universal conjugate that could be an anti-rabbit IgG produced in goats or mice linked to the enzyme alkaline phosphate. The linked anti-IgG-enzyme that react with the virus antibodies (IgG) which had reacted with the virus particles adsorbed to the bottom of the ELISA plate wells will be detected by colorimetric changes of a specific substrate that is added into the wells. Nevertheless, this method has certain disadvantages such as competition between plant sap and virus particles for sites on the plate wells and, consequently, high background reactions.

ii. Triple Antibody Sandwich (TAS-ELISA): Another widely used ELISA variation is the triple antibody sandwich i.e., TAS-ELISA, which is similar to the direct ELISA (DAS-ELISA), except for an additional antibody produced in another animal is used. First, the bottom of the ELISA plate wells are coated with a virus antibody produced in a species of animal (e.g., rabbit) and the virus antigen is linked in the trapped antibodies. The virus antigen is covered with a second layer of virus specific antibody produced in another animal species (e.g., mouse or goat) and the presence of this antibody is detected by adding an enzyme-conjugated specific antibody (e.g., rabbit anti-mouse IgG), that does not react with the plate well trapped antibody, followed by colorimetric changes of a specific substrate that is added into the wells.

iii. Dot Blot or Dot Immuno Binding Assay (DIBA): This technique is a simple and easier method to prepare and apply the samples on nitrocellulose or nylon membranes. The samples containing the virus antigens are prepared by grinding tissues in Tris-buffered saline and the extracts are applied directly on the membrane. The sample application on the membrane is usually accomplished through the use of a plastic mold with 96 wells which presses the membrane marking the places where the samples should be applied. Usually the spaces not occupied by the antigens on the membrane are blocked with neutral protein solution. The addition of virus IgG produced in rabbit and the anti-rabbit IgG produced in mouse follow protocols similar to indirect ELISA or PTA-ELISA, except that the positive reactions in DIBA are recorded as colored dots on the membrane. Considering that DIBA is a simple, less laborious and quick test, it can be used routinely for plant virus indexing and survey programs. One disadvantage of DIBA is the possibility of sap components interfering with the antigen-antibody reactions, resulting in subsequent problems with the diagnostic results.

iv. Lateral flow immuno assay: Mass express diagnosis of viral infections for virus-free plant culture industry or individual use requires inexpensive, rapid, and simple technologies that make possible analysis without special skill and equipment even under field conditions. One of the promising solutions for overcoming this challenge is lateral flow immuno assay (LFIA), based on the interaction between the target virus and immunoreagents (antibodies and their conjugates with colored colloidal particles) applied on the membrane carriers (lateral flow test strips). When the test strip is dipped into the sample being analyzed, the sample liquid flows through membranes and triggers immunochemical interactions resulting in visible coloration in test and reference lines. Test strips were developed for detection of five plant viruses varying in shape and size of virions: spherical carnation mottle virus, bean mild mosaic virus, rod shaped tobacco mosaic virus, and filamentous potato viruses X and Y by Byzova *et al.*, (2009). CPRI has developed LFIA kits for the detection of five viruses viz., PVX, PVA, PVS, PVM and PVY either individually or in combination of two viruses viz., PVA & PVX, PVA & PVS, and PVY & PVM using a single strip.
3. Molecular diagnostic techniques

i. Polymerase chain reaction (PCR): RT–PCR and PCR are popular techniques for detection and identification of RNA and DNA plant viruses respectively. The procedures are extremely sensitive, fairly inexpensive and require minimal skill to perform. In the case of RNA viruses, a cDNA strand complementary to the virus is made with reverse transcriptase (RT). Oligonucleotide primers, flanking part of the genome of the virus, are extended by a thermostable DNA polymerase in a series of denaturation and extension steps that exponentially increase the target DNA. For DNA viruses, the RT step is unnecessary. PCR-based methods can be adapted to high-throughput applications. In addition to detection of the virus, an additional advantage of the method is that the amplicon can be sequenced to provide further data about strain types. Possible drawbacks of the method are the need for a thermocycler, which can be expensive, and sequence information for design of primers. With databases containing ever-growing numbers of virus sequences, access to sequence information for many viruses is possible. Careful primer design is crucial, whether to detect only a single strain, or all the members of a genus. The sensitivity of the method is its major advantage. High sensitivity, however, can easily lead to false positive results from contamination; so adequate controls are essential.

RT-PCR protocols for detection of major potato viruses have been standardized that can detect very low level of virus inoculums. Several variations of RT-PCR like Immuno Capture PCR (IC-PCR), nested PCR and multiplex RT-PCR have been standardized for detection of potato viruses or to differentiate strain 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 from
the same sample. CPRI has designed primers and developed protocols for the uniplex and multiplex detection of PVX, PVY, PVA, PVS, PLRV, ToLCNDV-potato and Stem Necrosis and are routinely used to screen mother plants which meant for breeder seed production.

ii. Rolling circle amplification (RCA): RCA is an isothermal method which is reliable for diagnosis of geminiviruses and presumably all viruses with small single-stranded circular DNA genomes. The results showed the efficiency of this technique in characterizing viral DNA components of several geminiviruses from experimental and natural host plant sources. The advantages are: (a) that no expensive devices are necessary, (b) simple handling, (c) detecting of all infecting circular DNA components without any knowledge of sequence information in a single step, and (d) low costs per reaction. In addition, 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 increased the sensitivity and specificity of the assay. This technique is being used to detect ToLCNDV-potato, causing apical leaf curl disease in potato.

iii. Immuno-capture RT-PCR (IC–RT-PCR): This combines capture of virus particles by antibodies with amplification by PCR. In this method, the virus is adsorbed by the antibody bound to a surface, then removed by heating with a non-ionic surfactant such as Triton X-100. The nucleic acids are then amplified using RT–PCR. This method is especially useful in concentrating virus particles from plant species where virus titre is low, or where compounds that inhibit PCR are present; for example, plum tree sap containing Plum pox virus and sugarcane sap containing Sugarcane streak mosaic virus.

iv. Print capture RT-PCR (PC-RT-PCR): This technique involves spotting of samples onto nitrocellulose membrane (NCM), eluting viral RNA from the NCM in sterile distilled water (20 μl) at 95°C for 10 min, cDNA synthesis followed by PCR amplification and analysis of the PCR product.

v. Loop-Mediated Isothermal Amplification (LAMP): Recently, a technique called loop-mediated isothermal amplification (LAMP) of DNA has been developed. The technique uses four to six primers that recognise six to eight regions of the target DNA, respectively, in conjunction with the enzyme Bst polymerase, which has strand displacement activity. The simultaneous initiation of DNA synthesis by multiple primers makes the technique highly specific. The LAMP test is carried out under isothermal conditions (60–65°C) and produces large amount of DNA (amplified $10^9$–$10^{10}$ times) in 15–60 min. Alternatively, gene amplification can be visualised by the naked eye either as turbidity or in the form of
a colour change when SYBR Green, a fluorescent dsDNA intercalating dye, is employed. LAMP does not require a thermal cycler and can be performed simply with a heating block and/or water bath.

Gel electrophoresis showing LAMP based amplification and its visual detection.

vi. Next Generation Sequencing (Pyrosequencing): De novo sequencing of viruses using deep sequencing is a new technique that has successfully identified known and unknown viruses from long or short reads. Next Generation Sequencing (NGS) technology that is able to sequence viruses from samples without the need for laborious and costly purification, cloning and screening techniques. NGS technologies can be used as a diagnostic tool to identify a potato virus in an unbiased fashion when no prior knowledge of the aetiology of the virus is available.

Suggested Readings


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/900m² 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 methyl (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°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
Agrotis spinifera 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°C. Upper and lower threshold temperatures for PTM are 40°Cand 5°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 infestation and 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 by mass trapping PTM male adults. Chemicals-dusting of the tubers with 5% Malathion or 1.5% quino phos (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 circular 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/m² 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 mechanically. To the kill the pupae, expose them by ploughing 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.
Conventional seed production system in the country

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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°C and 10-16°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 dehauling 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:

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<tr>
<th>Zones</th>
<th>Crop Period</th>
<th>Varieties</th>
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<tbody>
<tr>
<td>North-Western hills</td>
<td>Summer (15th April –October)</td>
<td>Kufri Jyoti, K. Himalini, K. Girdhari, K. Kanchan, K. Chandramukhi,</td>
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<tr>
<td>North-Western plains</td>
<td>Autumn (1st week October –</td>
<td>Kufri Jyoti, K. Chandramukhi, K. Badshah, K. Pukhraj, K. Surya, K. Khyati, K. Satluj</td>
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**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 P₂O₅ and 100-120 kgK₂O/ha at planting, whereas, 100 Kg each of N, P₂O₅ and K₂O 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 rotting 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 flavescescence 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:**


Sharma AK, RS Chandel, V. Kumar and BP Singh. 2012. Potato Cultivation in North Western Himalaya. Technical Bulletin No. 95, Central Potato Research Institute, Shimla, India. 72p.


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 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 necessitates 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/nomenclature. 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**

<table>
<thead>
<tr>
<th>Chromosome Number</th>
<th>Marker</th>
<th>Chromosome Number</th>
<th>Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome I</td>
<td>STM5127, STG0016</td>
<td>Chromosome VII</td>
<td>STM0031, STI0033</td>
</tr>
<tr>
<td>Chromosome II</td>
<td>STM1064, STM5114</td>
<td>Chromosome VIII</td>
<td>STM1104, STI0003</td>
</tr>
<tr>
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<td>STM1053, STG0010</td>
<td>Chromosome IX</td>
<td>STM1052, STI0014</td>
</tr>
<tr>
<td>Chromosome IV</td>
<td>STI0001, STI0012</td>
<td>Chromosome X</td>
<td>STG0025, STM1106</td>
</tr>
<tr>
<td>Chromosome V</td>
<td>STI0032, STPoAc58</td>
<td>Chromosome XI</td>
<td>STG0001, STM0037</td>
</tr>
<tr>
<td>Chromosome VI</td>
<td>STM0019, STI0004</td>
<td>Chromosome XII</td>
<td>STM5121, STI0030</td>
</tr>
</tbody>
</table>

**References**


Potato productivity is severely constrained by limited high quality seed tubers; this is precipitated by inefficiencies in various stages in the seed production system. In India, production of minitubers through aeroponics systems has been introduced. This paper discusses the production of potato minitubers using aeroponics. Important considerations that should be addressed before setting-up an aeroponics based potato minituber production system are also discussed.

Aeroponics refers to the process of growing plants in an air or mist environment without the use of soil or an aggregate medium. The word “aeroponic” is derived from the Greek word aero (air) and ponos (labour). Aeroponic culture differs from both conventional hydroponics and in vitro (plant tissue culture) multiproduction. Unlike hydroponics, which uses water as growing medium and essential minerals to sustain plant growth, aeroponics on the other hand uses a mixture of air and water.

In aeroponics, plants growth is facilitated by suspending them in air, in an enclosed environment, and providing them with necessary nutrients by spraying their roots with nutrient rich water solution. The nutrient solution is continuously re-circulated through the system and monitored and amended whenever necessary. The solution is sprayed in such a way that it creates a mist in the environment, thereby ensuring optimum availability of water & nutrient which ultimately results in vigorous plant growth and consequently better productivity.

Advantages

Availability of disease free planting material is a critical input in augmenting potato production in the country. The seed production system in India is based on tuber indexing for detection and elimination of all the prevalent potato viruses followed by clonal multiplication in four successive generations. Although this system is quite effective, it’s low multiplication rate and higher field exposure increases the risk of viral infection. 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 used substrate made of soil and mixture of various components. This method generally produces 8-10 minitubers per plant depending on cultivar. Aeroponics offers the potential to increase production in terms of number of minitubers per plant three to four times over in vitro multiplication and thus reduces the cost as compared to conventional method. Its other advantages are:

- It avoids exposure to unfavorable soil conditions and thus offers freedom from soil borne diseases
- Lot of vertical space available for aeration, growth and development of roots and progeny tubers
- Easy inspection of tuber zone and picking of minitubers of desired size by sequential harvesting
Aeroponic growing is considered to be safe and ecological friendly for producing natural, healthy plants and crops. The main ecological advantages of aeroponics are the conservation of water and energy.

Presence of oxygen in the rhizosphere (root zone) is essential for healthy plant growth. As aeroponics is conducted in air combined with micro-droplets of water, almost any plant can grow to maturity in air with a plentiful supply of oxygen, water and nutrients.

**Aeroponics: Technology Know How**

Aeroponic system mainly consist of an electrical unit, light proof growth chambers, nutrient solution chamber, high pressure pump, filters and spray nozzles. Interiors of growth chambers are covered with black lining to avoid any admittance of light to the root zone of plants. Aeroponic unit can be placed in insect proof net house under natural conditions or controlled environment conditions. For aeroponics, *in vitro* grown 15-21 days old microplants are required to be hardened before shifting to this system. For hardening, microplants are transplanted in peat moss, vermiculite or sand and hardened for 15 days at about 27°C in hardening chamber. These hardened plantlets of 10-15 cm height are planted in the 20 mm diameter holes made in the roof of the growth chamber of the aeroponic unit. If required, cuttings should be dipped in rooting hormone solution for five minutes before planting in the aeroponics to facilitate rooting. Very old and yellowish plants are not suitable for aeroponics and should be avoided. All the essential nutrient elements required for plant growth are dissolved the water in the solution chamber, and solution pH is maintained at the desired level throughout the crop period. The nutrient solution is compressed through nozzles by the high-pressure pump, forming a fine mist in the growth chambers. With the help of an automatically operated pump, the nutrient solution is sprayed inside the chamber for desired durations at desired intervals. In this way growth chambers are maintained at 100% relative humidity by misting nutrient solution round the clock. Nutrient solution is replenished form time to time and desired pH of the solution is maintained. The pH of nutrient solution should maintain at 5.6-6.2 throughout the crop duration. Roots, stolons and tubers develop inside the chamber and leaves are exposed to light. After a week, root systems start developing inside the growth chambers.

As in the soil system, stolon and tuber formation are initiated at different intervals depending upon the variety. After a month or so, lower leaves need to be removed with a dissecting blade following strict aseptic measures and the plant should be lowered. The process of lowering the stems is important and is equivalent to earthing up in the field. If stolons start to form in the upper part of the root system, lowering of plants lead to better stolon formation. Night temperatures lower than 4°C are too cold for aeroponic plants. Day temperatures higher than 30°C are too warm. When tuberization begins, it is desirable to have maximum night temperatures of 10-15°C and day temperatures around 20°C. Picking of the tubers started after 45-65 days when some of the tubers attain 15-17 mm diameter. Once the first flush is harvested, it triggers formations of more minitubers/plants. In this system, harvesting is done at regular interval when the tubers attain the desired size. On an average 35-60 minitubers can be harvested form a single plant, depending on varieties as against 8-10 minitubers under net house. Tubers are harvested sequentially as they attain the desired size. Minitubers should be allowed to cure in a dry and clean environment for ten to fifteen days before placing them into cold storage. These mini tubers are then stored at 2-4°C and used for planting in the next generation.
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. 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. The whitefly feeds on plants by inserting the stylets into the leaf and withdrawing sap from the phloem. Feeding by whiteflies causes direct damage to crops through excessive sap removal, excretion of honeydew that promotes growth of sooty mould fungi, and by inducing systemic disorders and 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, *Tomato Leaf Curl New Delhi Virus* (potato), ToLCNDV(potato)).

These categories are properties of the relationship between viruses and their vectors, but are often 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’ styles 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). 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. 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**

Bemisia tabaci 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.” 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). 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 behaviour 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).

Penetrating the leaf and locating a sieve element is a time-consuming and often unsuccessful process. 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).

**References**


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

- **APHID PERIOD**
- **52% VIRUS INCIDENCE**
- **AUTUMN CROP**
- **WINTER CROP**
- **SPRING CROP**

### Refinement of Seed Plot Technique

- **Hot weather cultivation, crop rotation**
- **Cold storage in plains and country store in hills**
- **Dehaul the crop before 20 aphids/100 compound leaves**
- **Regulate survey of vector species and inoculums**
- **Cull off type, diseased plant**
- **Remove off type, diseased plant**

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**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 while PALCV 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.gossypii* (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.
Aeroponics technology, a soil-less culture has capability to grow plants in a conditioned, protected and healthy environment. The term Aeroponics means to cultivate plants without using soil and water as medium; by maintaining all the parameters essential for growth of plants. The parameters are temperature, humidity, pH & electrical conductivity, light intensity, air circulation, nutrient solution etc. resulting in a healthy conditioned environment. Soil less cultivation (aeroponics/hydroponics) requires embedded electronic systems capable of precise, repeatable operations based on models of systems and processes. The efficiency and the performance of the mechanisms can be improved considerably through concurrent, integrated development of embedded systems. The electronic system comprises three parts- sensors, microcontroller (a single board computer) and actuators (Fig. 1). In this system clusters of sensor are employed to measure the above mentioned parameters, which are connected to the microcontroller/PLCs with appropriate amplifier/A to D convertor. The electronic signals (analog/digital) are being transmitted to the microcontroller for its processing, logging and decision making as per the predefined algorithms. After signal processing the actuators (motor, fans, solenoid valves, pumps, etc.) get actuated through a series of relay switches.

![Fig. 1 A block diagram of electronic system](image-url)
The user interface/control panel facilitates the operator to control or manipulate the system parameters or their threshold limits as per the requirement. A water pump with series of nozzles being used widely for intermittent spraying of nutrient solution in the root zone. Fan-pad system with temperature sensors is used in this system to maintain desired level of temperature and humidity. Apart from that a system can also be applied for continuous monitoring air quality, properties of nutrient solution, temperature of different zones through wireless system for effective operation of the unit.

Application of electronic systems in aeroponics: Automatic spraying of nutrient solution on crop roots is the vital operation of this system. A digital timer/PLCs/microcontroller can be employed with a suitable relay card for actuation of solenoid valves, which allows spraying fine droplet of nutrient solution in the grow box at an optimum pressure. The time, pressure and droplet size of spray can be adjusted as per the requirement of crop. Furthermore, a network of temperature, light intensity and RH sensor can also be employed with PLCs/microcontroller in order to maintain optimum range of temperature, humidity and light intensity in the aeroponics facility (Fig. 2).

Fig. 2 Structural diagram sensor network and control system in aeroponics system
In recent times, the potato seed production has emerged from the conventional seed production system to the scientifically more evolved Hi-tech potato breeder seed production system. The most prevalent among them is the tissue culture based and the aeroponic based Hi-tech system. However, the basic prerequisite for both these systems lies in the utilisation of quality planting materials namely microplants and microtubers which should meet the virus-free criterion as well as freedom from any other diseases. The basic technique used for production of these is the tissue culture. The chapter discusses in detail the production of these planting materials microplants and microtubers through the use of tissue culture.

The Hi-Tech based planting systems can be classified into microplant-based, minituber-based and aeroponics based seed production systems. The production of microplants in tissue culture channelizes all the three Hi-tech seed production systems (Fig 1).

**Fig 1: Interrelationships between the three hi tech seed production systems**

The basic or the starting planting material used in these techniques is the most crucial and critical step for determining the quality of breeder seed produced. The material has to be true to type.
and free from viruses and other diseases and has to be maintained in a similar manner during micropropagation and its subsequent seed production system. The microplants are channelized to respective seed production systems where they subsequently form minitubers which are then utilised for field multiplication and yield breeder seed. Irrespective of the type of the Hi-Tech based planting system the basic techniques of tissue culture is utilised in all the seed production systems for development of planting material at the earliest stage.

**Agro techniques for production of preparation of planting material**

The Agro techniques for preparation of planting material which in simple terms is tissue culture and related activities have been discussed in the following heads

**Lab organization**

The tissue culture facility requires the construction of a specialised laboratory where tissue culture facilities including culture room autoclave etc. are maintained. Completely sterile conditions are maintained inside the laboratory for multiplication of disease free quality planting material. It should have proper supply of water and electricity, away from traffic areas and contamination sources and have a proper drainage system. The laboratory consists of different areas:

**Washing area**: It is the area where the containers and vessels involved in tissue culture are washed.

**Medium Preparation room**: Here the medium is prepared and should have the storage of chemicals used in medium preparation and other small-related equipment like stirrer, pH meter, refrigerator, medium dispenser, microwave etc. It should lead directly to sterilization area.

**Sterilization room**: It is the area where the nutrient medium is autoclaved in an autoclave.

**Culturing room**: It is the room where the plants are transferred to the nutrient medium under proper culturing conditions using a laminar flow.

**Culture room**: It is a specialised room for incubating the cultures at ambient temperature humidity and photoperiod for optimum growth of the cultures. Air conditioning, systems heaters temperature controllers’ photo period controllers humidifiers etc. may be installed along with automatic digital controllers for proper monitoring of the culture room conditions.

The culture room and culturing room should be kept most sterile and separate from the other areas.

**Hardening Chambers** these are required for transferring of the cultured micro plants to the external environment for its acclimatization the cultured plants and hardened under...

**Lab equipment/ instrumentation/ glassware**

The major equipments required to be installed in the various areas of the Tissue Culture Lab have been summarised in table 1.
Table 1: Basic equipment for Plant tissue culture laboratory

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoclave</td>
<td>For sterilization of nutrient medium and other glassware and equipment used in tissue culture</td>
</tr>
<tr>
<td>Laminar air flow hood</td>
<td>For carrying out culturing under sterilized conditions</td>
</tr>
<tr>
<td>Balances preferably electronic</td>
<td>For weighing of chemicals for nutrient medium preparation</td>
</tr>
<tr>
<td>Magnetic stirrer</td>
<td>For proper mixing of components of nutrient medium</td>
</tr>
<tr>
<td>Oven</td>
<td>For heat sterilization of glassware and metallic equipments</td>
</tr>
<tr>
<td>PH metre</td>
<td>To adjust the pH of the nutrient medium</td>
</tr>
<tr>
<td>Refrigerator</td>
<td>To store growth regulators and other heat labile chemicals at optimum temperature</td>
</tr>
<tr>
<td>Water distillation assembly</td>
<td>For production of distilled water for use in medium preparation</td>
</tr>
<tr>
<td>Culturing shelves preferably with lights and photo period controls</td>
<td>For proper arrangement of culture tubes or bottles inside the culture room and providing proper photo period</td>
</tr>
<tr>
<td>Heating and cooling equipment</td>
<td>Air conditioners and heaters with temperature controllers are used for maintaining ambient temperature inside the culture room</td>
</tr>
<tr>
<td>Humidifier/ dehumidifier with humidity controller</td>
<td>For maintaining proper humidity inside the culture room</td>
</tr>
<tr>
<td>Media dispenser</td>
<td>For pouring exact volume of nutrient medium into culture vessels</td>
</tr>
<tr>
<td>Microwave oven</td>
<td>For heating the nutrient medium during its preparation</td>
</tr>
<tr>
<td>Bead sterilizer</td>
<td>For sterilization of small metallic equipments like forceps scissors scalpel IT sector during culturing</td>
</tr>
</tbody>
</table>

The glassware of borosilicate glass of good quality resistant to breakage, scratch and autoclaving may be used. Container type may vary according to culture.

**Nutrient Medium preparation**

The nutrient medium utilised for tissue culture consists of different macro and micronutrients along with a carbon source and growth regulators which promote the growth of cultures full stop the most commonly used medium in tissue culture is the Murashige and Skoog medium which is suitable modified with growth regulators and vitamins to promote the growth of cultures. The major component of culture medium are as follows:

**Inorganic nutrients**: Major salts: K, N, CA, Mg, P and S; Minor salts: Fe, Mn, B, Cu, Zn, I, Mo and Co

**Carbon and Energy source**: 2-5% sucrose
**Vitamins**: B1 thiamine, B Nicotinic acid, B6 Pyridoxine, pantothenic acid, biotin folic acid, p-amino benzoic acid, choline chloride, riboflavin, ascorbic acid etc.

**Gelling agents**: Agar, alginate, Gelrite, clarigel etc.

**pH of medium**: It is very important and adjusted to 5.8 in potato.

**Culturing process**

The culuring is carried out in specialized chamber referred to as the Laminar air flow system which is fitted with the HEPA (High Efficiency Particulate Air) filters, which do allow microbes to settle on culture medium during the culturing process, which may otherwise cause contamination. The cultures need to be handled carefully and aseptically during the transfer with proper precautions for surface sterilization of hands, surfaces and plant parts. There should not be any obstacles between the culture medium and the Laminar air flow. The cultured plants shold be transferred to the culture room under ambient conditions.

**Culturing conditions**

- **Culture medium for Microplant micropropagation**: MS + B5 Vitamins + Calcium Pantothenate @ 2mg/l kept at 50-60 µmol/ m2/ s light intensity at 22 ± 1 °C and a photoperiod: 16 h. The culturing of microplants is done upto 12-14 cycles and after 3-4 weeks of culture, in culture tubes or bottles. Perforated caps may be used or cotton plugs to improve culture ventilation and growth.

- **Culture medium for Microtuber production**: Culture 2-3 nodes cuttings in erlenmeyer flask for 3-4 weeks in liquid Microplant micropropagation medium (without agar) at 100-120 µmol/ m²/ s light intensity at 25 ± 1 °C and a photo period: 16 h. After 4 weeks of culture drain out the medium and add microtuber induction medium (MS + 10 mg/l N-benzyladenine (BAP) and 80 g/l sucrose without agar) and keep in dark at 15°C for 60 -90 days. The microtubers are form epigeally at the apical as well as axillary buds of the shoots approximately 15-20 in numbers and weighing 50-300 mg each in a single culture flask with 2-4 nodal cuttings. The 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. These are then manually harvested carefully and treated with 0.1 % Carbenazim for 10 min and allowed to dry in dark for 2 days. These are subsequently stored in a perforated polythene covers in a refrigerator at 4°C.

**Sterilization techniques:**

1. Media sterilization using Steam (wet) sterilization in an autoclave at 121 °C temp, 15 psi pressure and duration of autoclave at 20 min for standard 20 to 500 ml media. Upto 35 min for 500 to 5000 ml media flasks

2. Sterilization of glassware and metallic instruments: thorough washing followed by dry heat sterilization in an oven for 3 h at 160 -180°C

3. Sterilization of thermolabile compounds: eg. Growth regulators, amino acids, vitamins which are heat labile are filter sterilized using filter membranes of 0.45 to 0.22 µm.
4. Surface sterilization of laminar hood and workers hands with 70% alcohol/spirit

5. Flame sterilization of instruments used in culturing

6. Use of UV light for disinfection prior to working in laminar flow (caution with UV light).

7. No blockage between culture and airflow.

8. Sterilization of explant: using chemical sterilants like 1% sodium hypochlorite, Calcium hypochlorite, 1% bromine water, 0.1% mercuric chloride, 1% silver nitrate etc.

**Hardening process**

The hardening regime is most crucial for planting in hot weather conditions and may be surpassed if planting in cooler weather conditions of 15-20°C day temperature. However, it results in more vigorous plants with greater survival rates. Hardening is carried out in specialized hardening chamber, where 3-4 week old microplants are transferred in sterilized peat moss filled in portray. The adhering medium needs to be thoroughly washed with luke warm water as it may cause contamination to microplants. These may be either dipped in Mancozeb 2% for half hour before transplanting or may be drenched into peat moss after planting. The plants are kept in dark for two days and subsequently transferred to hardening chamber at 27°C for 10-15 days. Alternatively, peat moss, vermiculite and perlite may be used in 1:1:1 ratio.

**Transplantation**

The hardened plants are planted in net house at 20-30 X 10-15 cm plant geometry under insect free net house conditions. Proper care after transplantation ensures higher survival, plants should be irrigated regularly, preferably with a sprinkler system. Proper quality check by way of virus testing for 5% of the net house population must be adhered.

It is also important to maintain soil health under net house condition for which practices of deep ploughing, soil solarization and green manuring are recommended.

The microtubers may be kept in a well ventilated room under diffused light for sprouting and planted in a similar geometry as microplants and similar cultural conditions.

**Post-harvest handling**

The microplant crop is allowed to mature when haulms are cut and mintubers are harvested after 15-20 days of haulm cutting. The seed tubers are thereafter graded into less than and more than 3 gm minitubers and stored accordingly. The spray of 3 per cent boric acid is recommended to prevent tuber borne diseases. These are then cold stored in cold stores at at 3-4°C. The less than 3 gm minitubers may be recycled once more in G0 generation. The minitubers so formed are planted in G1 and G2 generations to produce Breeder seed.
The semi-perishable nature of potatoes makes their handling and storage difficult. Under tropical and sub-tropical conditions, losses due to poor handling and storage may increase up to 40-50%. Therefore, it is of greatest importance to minimize losses during handling and storage. Good post-harvest management begins at the production stage itself. The good quality of the tuber is greatly influenced by the cultural practices such as improperly prepared fields, mechanical harvesting etc. Sometimes conditions for high yield and for good storage characteristics may be contradictory. For better storage quality and life, potatoes should be harvested in dry weather; irrigation should be stopped two weeks before dehauling. Potatoes harvested under wet soil conditions must be dried before storage to avoid infection and rotting during storage. Only mature tubers should be stored. Immature tubers have poor keeping quality due to lower dry matter content and a weak skin. Harvesting should be done 10-15 days after haulm cutting to facilitate proper skin curing. In spite of best efforts, cutting and bruising cannot be completely eliminated while harvesting and therefore, curing is essential to heal the wounds. Suberization is the process by which wounds are healed in potatoes and the optimum conditions for suberization are storage at 25°C and relative humidity of 95%. Further, adequate pest and disease management is also essential for producing potatoes with good storage quality. Therefore, proper management of pre-storage factors that affect the keeping quality of potatoes is the first step in good storage management. Whatever may be the storage method, its success depends on the quality of the tubers entering storage. If the tubers entering storage are not sound, the tubers that come out of storage will also be of poor quality.

**Post-harvest losses**

At present a higher proportion of potatoes in the country is wasted as post-harvest losses (PHLs) than that used as seed or processing. Post-harvest losses lead to reduction in the quantity as well as quality of potatoes. 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. Quantitative losses are apparent and attempts are made to reduce these losses whereas qualitative losses are not apparent but their importance cannot be ignored. Since qualitative losses can greatly reduce the value of potatoes therefore, adequate attention should be paid to prevent loss in quality. Various factors which play key role in post-harvest loss of potatoes are being discussed in subsequent heads.

**Losses at harvesting and handling**

The mechanical damage during harvest is responsible for loss initiation in potatoes and it depends on several factors like, if the soil is very dry, especially for hard land, cuts and bruises will take place, while if it is very humid, the potato peel will be very delicate. Hence, the soil should be at the right tillage at harvesting and the 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 transport**

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 have handled the absolute minimum number of times possible. Clearly, damage is often proportional to the number of times that potatoes are transferred. All labour involved with potato handling should be supervised carefully to guarantee an appropriate operation.

**Losses during storage**

Various factors like physiological tuber condition, mechanical damage suffered during harvest and handling, as well as by storage temperature and humidity conditions affects the losses during storage. Mechanical damage such as cuts and bruises, facilitates invasion and multiplication of microorganisms that cause spoilage and rotting. Weight loss in potatoes is linearly related to nature or magnitude of physical damage. Whereas, losses by rottage increase exponentially with regard to magnitude of physical damage. Therefore, it is necessary to reduce tubers physical damage to minimize losses during storage.

**Physiological losses**

Physiological losses occur through natural respiration and evaporative loss of water through skin. The magnitude of these losses depends largely on the environmental conditions. Two important storage environmental factors that affect the storage behaviour of potatoes are temperature and relative humidity. Optimal holding temperatures for potatoes in storage depend on the potato variety and the intended end use of the product. Physiological damage can occur from exposure to high or low temperatures both before and during storage. Physiological weight loss is the most relevant quality reducing process. Exposure to high or low temperatures both before and during storage leads physiological damage. Black heart symptoms may develop due to overheating of tubers either by direct exposure to sun light or during high temperature and non-refrigerated storage. At high temperature, the high rate of respiration leads to increased oxygen requirement, which results in asphyxiation leading to discolouration and breakdown of inner tissues of tubers. Tubers exposed to freezing temperatures also suffer internal damage. It should be taken into consideration that temperature change in storage should be made in gradual way. This gradual temperature reduction results in little changes in the sugar content of tubers and also avoids deterioration of quality of processed product.

Respiration is a natural biochemical process of the tuber tissue. Respiration rate varies with variety and response to storage temperature. It can be controlled by an appropriate temperature control.
to reach and keep low physiological activity of the tubers. Respiration consumes oxygen and as a result converts starches to sugar. The tuber cell, oxidize glucose into nutrient that is required by tuber to stay alive, produce water, carbon dioxide, heat and by-products. Respiration also increases when tubers are mishandled, transported over rough tracks or subject to bruises or cuts. For most varieties, temperatures above 15°C may cause dramatic increases in respiration. In general, tuber respiration is relatively low at low storage temperatures and increases as storage temperatures are elevated. During the early part of the storage season higher tuber respiration rates, wound healing, and high transpiration results in rapid weight loss.

Evaporation on the other hand is a physical process which can be managed by controlling the storage environment in terms of temperatures, air humidity and air flow rate. The relative humidity of the storage also affects transpiration weight loss and hence, it is recommended that relative humidity should be kept as 95% or above. Most of the tuber shrinkage that occurs during the first month of storage results from water lost before the completion of the wound healing process. Maintaining high relative humidity in potato storage prevents some of the early season tuber dehydration and helps controlling the total shrinkage during the season. Shrinkage in storage is directly proportional to the length of the storage season and inversely proportional to the relative humidity conditions maintained within the store.

Pathogenic losses
Pathogenic losses in potatoes are greater than due to physiological losses. Physical damage during harvesting and handling intensifies the attack of bacteria and fungi resulting into sever quantitative loss. Late blight, dry rot and pink rot are the more common storage diseases caused by fungi. Soft rot is the most severe bacterial disease that causes rotting. 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. Qualitative losses are caused by diseases such as common scab, powdery scab, black scurf and wart which affect the appearance of the tuber and thus reduce quality of potatoes. 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. The larval damage results in weight loss and tuber moth infection greatly reduces the market value of tubers.

Storage of seed potato
In India about 90% of the potatoes produced in the Indo-Gangetic plains are harvested in February-March. Seed potatoes harvested in February have to be stored until October and refrigeration is essential for such a long-term storage. For storage of seed potato cold storages are maintained at 2-4°C and 90-95% relative humidity which is ideal for storing seed potatoes at the right physiological age for 6-8 months. The proper storage environment for potatoes may be defined as the environment, which will reduce physiological and pathological losses to the minimum. Therefore, temperature and relative humidity inside the cold chamber have very significant effect of seed quality. A fairly important storage loss due to high temperature is rotting of the potatoes. The centre-most product in a stack is more frequently subject to rotting, where high temperature is developed due to long cool-down time and high respiration heat of potatoes. Large stack dimension and inadequate circulation of air throughout the cold store also leads to long cool down time. If this cool-down time is more than the time required for initiation of deterioration, the rotting phenomenon starts. Loading of potato bags in haphazard manner, leaving very less space for air circulation around the stacks would increase the cool-down time, thus increasing losses during storage. Low cool-down time can be achieved by lowering the storage air
temperature, increasing the storage air velocity and limiting the size of stack as small as economically feasible.

Similarly, too low storage temperature also leads to deterioration in seed quality. In stacks, potatoes near the surface of the bag achieve the temperature close to the storage air temperature relatively in a short time and remain at this temperature for rest of the storage period. If temperature of potatoes is lower than the threshold temperature, it causes cold injury (often below 2°C). The chilled potatoes become weakened because they are unable to carry on metabolic processes. Often these potatoes look sound when removed from cold store. However, symptoms of chilling become evident in a few days at warmer temperature. Tubers exposed to freezing temperatures (about minus 2°C) are injured because of internal ice formation. Even slightly frozen tubers exhibit discoloration in the vascular ring. More prolonged exposure leads to a blue-black necrotic discoloration of the pith in addition to necrosis of the vascular tissue. Tubers frozen for 4 to 5 hours seldom show internal discoloration symptoms, but death of tissue is so widespread that the thawed tuber becomes wet and soft and oozes liquid. Both the time and temperature are involved in chilling injury. Damage may occur in a short time if the temperatures are considerably below the danger line. Inside the cold chambers high temperature also leads to the condensation of moisture on the surface of tubers which may serve as a potential source for the development and growth of the pathogens. Under favorable storage conditions such type of infection may cause rapid rotting. The affected tubers also produce extra heat, temperature rises very rapidly and within a few days, the entire potato stock may rot. To counteract the spread of diseases in the cold store, care must be taken for proper sorting out at the beginning of storage. Also the potatoes must be maintained at desired low temperatures and kept dry throughout the storage. For long term storage potatoes are generally packed in gunny bags and then arranged in stacks within the cold store on wooden platform having slots on it.

**Importance of refrigerated storage for seed potatoes**

Seed potatoes are very delicate material and their storage requires special attention to provide acceptable quality and physiological condition at planting. Seed storage methods must provide the desired number and size of sprouts prior to planting. The number of sprouts per tuber, which determines the number of main stems per plant, is influenced by degree of apical dominance which is influenced by storage temperature. If a potato tuber is stored at a temperature that promotes a short dormant period, the young bud at the apex start growing while growth of older buds is suppressed. This is known as apical dominance. A tuber with apical dominance have few main stems. As a result of apical dominance a smaller number of tubers may be formed and they may grow too large for proper market size. If storage of seed potatoes is controlled to suppress apical dominance then the proper number of main stems usually 3-5 will develop. This permits the proper amount of seed to be planted to yield the maximum amount of tubers of suitable market size. Storing of seed at 2-4°C beyond the end of natural dormancy and until a few weeks before planting result in 3-5 main stems per planted tuber. After removal from cold storage seed tubers should be stored in light (natural or artificial) at about 15°C for a week before planting to provide multiple green sprouting. Storage of seed potatoes in cold storage is thus ideal to facilitate the availability of potato seed tubers in right physiological age for planting.

**Recommended conditions for long-term storage of potatoes**

**Storage temperatures**

- Seed potatoes: 2-4°C
- Potatoes for fresh consumption: 4-5°C
Potatoes for chipping: 7-10°C
Potatoes for French frying: 5-8°C
Potatoes for granulation (mashed): 5-7°C
*Ware potatoes must be treated with sprout inhibitor at storage temperature above 4°C
RH of storage atmosphere should be 95% or more
CO₂ concentration should not exceed 1% and O₂ level should be 20-21%

Pre-cold storage treatment to reduce losses
Before placing seed potatoes for cold storage a short period of pre-cold storage treatment at somewhat higher temperature, about 15°C is a desirable practice to prevent the physiological breakdown of tubers. This pre-cold storage treatments enable the potatoes to acclimatize with the low temperature that exists in the cold storage chamber itself. Before the transfer of seed material to low temperature storage chamber tubers should be treated with 3% aqueous boric acid solution. It helps to treat surface borne diseases like Black scurf (Rhizoctonia), common scab (Streptomyces), dry rot (Fusarium), and soft rot (Erwinia) etc. 1.5 Liter of aqueous boric acid solution is sufficient for 1 quintal of tubers. Cost of treatment is 5-6 Rs/quintal.

Management of cold storage
Successful management of storage system requires monitoring and control of storage environment. Temperature and relative humidity must be constantly controlled and monitored
For measuring air temperature surrounding the potatoes direct reading instruments like glass thermometers or remote station indicators may be used. Always use some ordinary glass thermometers as a back-up and check on proper calibration. Humidity monitoring can be conveniently done with the use of dry-wet thermometers or humidity probes. After completion of storage remaining potatoes lying in the stores serve as a potential source, host or reservoir for diseases and pests. They should be carefully disposed off before filling the stores. For the sanitation of cold stores disinfectants (Formalin 1:20) may also be used.
Introduction: The potato (*Solanum tuberosum* L.) introduced in India by Portuguese in seventeenth century, is the most important non-cereal food crop and is next only to rice, wheat and maize in terms of potato production. Agricultural Mechanization embraces the use of tools, implements and machines for agricultural land development, crop production, harvesting, preparation for storage and on-farm processing. It includes three main power sources: human, animal, and mechanical. Mechanization play a pivotal role in timely seedbed preparation, planting and harvesting of the crop as well as in the precision placement and efficient utilization of the various crop inputs. With the emergence of new crop rotations and in a bid to further enhance the cropping intensity and productivity, many farmers experience scarcity of time and labour. As such, there appears to be a need for developing additional potato equipment especially automatic potato planters, haulm cutters and improved potato diggers etc. Potato being a relatively short duration crop in the plains of India, its mechanization assumes a special importance in order to accomplish the various crop operations in the available time. Though potato production practices in India vary a great deal from region to region, yet basic operations like the seed bed preparation, fertilizer application, planting inter-cultivation, plant protection, haulm separation, harvesting, handling and grading are common to all the regions.

Seed bed preparation: Potato requires soils with good tilth to ensure covering of stolons and tubers and also to provide adequate aeration for respiration of roots and tubers. Potatoes can be grown in all types of soils except saline and alkaline soils. To get the best yield, the soil should be loose and friable with good drainage and aeration. Traditionally fine tilth is maintained for potato cultivation. The main objects of the tillage are;

1) The production of a suitable soil tilth or soil structure.
2) The control of soil moisture.
3) The destruction of weeds.
4) The burying and cleaning of rubbish and the incorporation of fertilizer in to the soil.
5) The destruction or control of pests.

In places, where the green manuring crop proceeds the potato crop, it has to be incorporated in the soil well in advance. For burying of the green crop, two or three bottom tractor drawn mould board plough is a suitable implement. The animal or tractor drawn disc harrow is used for seedbed preparation. In light soils, three tines animal drawn or nine tine tractor operated tiller are used for seedbed preparation. A fine seed bed can be achieved by cross harrowing or cross tilling the field followed by one or two plankings.

Fertilizer application and line marking: An essential feature of all the permanent systems of farming is the return to the soil of the nourishment taken from it by the crops that are removed. This is commonly done by the application of farm yard manure and artificial or organic fertilizers. Fertilizer drill
cum line marker is a machine which ensures placement of fertilizer in furrows and marks the impressions at 600 or 650 mm fixed row spacings for tuber placement for uniformity in row to row spacing. With this machine fertilizer rate can be adjusted between 200-1200 kg/ha and this machine has field capacity of 4.0 ha/day.

**Planting:** Results achieved in potato planting by machines depend upon complex relationships between the technical quality of the machine performance and ability to work with a minimum demand for labour. After preplanting tillage operations, potato is planted by different methods in various parts of the country. However, ridge and furrow method is the most popular method which has received wide acceptance in India. Planting of potatoes can be grouped into two types as under.

**Manual planting:** Potato is planted at a row spacing of 55 to 60 cms and seed spacing of 15-20 cms. The traditional method of potato planting comprises the making of ridges with the help of spade and placing of the whole or cut tubers with a khurpa. This practice is slow and tedious and requires about 400 man-hours/ha. Some improvements in manual planting of potato have been made in the recent years. These include marking of rows with a manually operated marker and placing of the potato tubers on the marked rows through visual judgment. The ridges are later formed by gathering the soil from either side with a spade. This requires on an average 250-300 man-hours/ha. Another improvement in the manual planting introduced on some farms in the mid sixties involved the marking and spacing of tubers manually in the rows and forming of the ridges, to cover the tubers, by means of an animal drawn or tractor drawn ridger. The labour requirement for planting by this method is reduced to about 150-175 man-hours/ha.

**Mechanical planting:**

A potato planter is essentially required to perform three functions viz;

i) Opening of a tiny /shallow furrow.

ii) Metering of the seed with a manually assisted or fully automatic seed dispensing device and

iii) Forming of ridge to cover the seed.

Application of fertilizer and other chemicals may or may not form an integral function of a potato planter. The uniqueness of a potato planter lies in efficient planting and ridge making accomplished by a substantial reduction in labour and cost of planting. Potato planters can be broadly categorized as “semiautomatic” and “automatic” potato planters. Tractor drawn two or four row marker cum fertilizer drill is used for marking furrows and placing of fertilizer in the furrows followed by planting with 2 or four row potato planter cum ridger. Ridge planting is the most common potato planting practice followed in India. Results achieved in potato planting by machines depend upon complex relationships between the technical quality of the machine performance and ability to work with a minimum demand for labour. With planters of semiautomatic type, the potatoes have to be placed in cups by the operators sitting on the machine.
Weeding, fertilizer top dressing & earthing up

In potato, inter row cultivation for weeding, applying second dose of nitrogen and then earthing up the loosened soil to make good ridges again are the important operations for good yield. For these operations tractor operated three row and five row machines in different combinations (inter row cultivator alone, cultivator-cum-fertilizer applicator, cultivator-cum-fertilizer-cum-thimet applicator etc.) are used by potato farmers. For earthing up operation three/five row ridge makers are available. Single bottom desi plough and ridgers are used where animals are the source of power.

Plant protection equipment: As the application of science to farming progresses, spraying is employed for an ever widening variety of purposes. The chief crop protection operations are the application of herbicides to reduce the competition from weeds, protective fungicides to minimize the effect of fungus diseases, insecticides to control various kinds of insects/pests and hormones to regulate the crop growth. There are large numbers of manually operated back pack type spray machines which are used by Indian potato farmers. In some designs source of power is small engine or a battery. High capacity tractor operated sprayers have also been successfully adopted. Main components of these machines include frame, tank, hose pipe, spray gun and triplex pump. These are PTO (power take off) operated machines with single/double jet type spray guns. Due to high pressure spraying, even the lower leaves of the potato plants get completely drenched with chemical. In some designs instead of spray guns a long boom fitted with nozzles is also used.

Haulm cutting: Seed potato producers will often kill vines 10-12 days prior to harvesting which helps in hardening of the skin, controlling of the tuber size or to reduce transmission of the disease organisms from plant to the tuber in addition to reduce the vine volume before harvest. Farmers in India using mechanical diggers employ manual labour to cut the vines with the help of hand sickles. This operation requires 100-125 man-hours/hectare. The use of haulms cutter can reduce labour requirement to the extent of 75%. Following types of the potato haulm cutters have been developed at CPRI.

Cutter bar type of potato haulm cutter: This two row machine mainly consists of three mechanisms namely vine lifting mechanism, cutting mechanism and power transmission system. Three numbers of V-shaped vine collectors and two numbers of rollers with MS rod catch fingers constitute the vine lifting mechanism of the machine. Cutting mechanism of the machine is provided with cutter bar having ten numbers of 75 mm wide serrated triangular cutting knives and six numbers of two tined guards. Power to both the cutter bar and rollers is given from tractor PTO through a gear box. During actual field testing of the machine with different potato varieties, it was observed that the average field capacity of the machine was 1.4 ha/day. The optimum speed of operation was found as 1.75 to 2.5 kmph. The
uncut vines were found to be less than 3%. Haulms are usually cut in one or two pieces and most of them are dropped on the ridges at the rear of the machine.

**Flail type of the potato haulm cutter:** A two row flail type of potato haulm cutter, has been designed and developed at CPRI. It consists mainly of three mechanisms namely vine lifting mechanism, cutting mechanism and power transmission system. Three numbers of spring loaded V-shaped vine lifting attachments, constitute the vine lifting mechanism of the machine. Cutting mechanism of the machine is provided with cutting blades of varying lengths, sharpened on either side to cut the haulms all along the ridge profile. These blades which rotate in a vertical plane, have been contoured to match the ridge profile. A gearbox having reduction ratio as 5:1 has been provided in the power transmission system to give power to the cutting mechanism of the machine. During actual field testing of the machine with different potato varieties, it was observed that the average field capacity of the machine was 1.2 hectares in a day of 8 hours. The optimum speed of operation was found as 1.75 to 2.5 kmph. The uncut vines were found to be less than 4%. The haulms are usually chopped off into pieces and are dropped on the ridges at the rear of the machine. To get the best results out of both the types of haulm cutters and also to minimize the potato damage, it is essential to plant the crop with a tractor operated planter so as to have uniform size of ridges and furrows.

Harvesting: In spite of all the mechanization efforts intended to increase the production of the potato crop, the harvesting has received the scant attention in the past. Traditionally the harvesting of potato is carried out manually by digging the ridges with hand tools such as spade, forked hoe or khurpa and simultaneously picking the potatoes which employs a huge labour force of about 900 man-hours/ha. Besides this, the manual method of digging has other shortcomings such as 5-7% cut tubers and also the left over tubers in the field. Efforts have been made during the last two decades to develop potato harvesting equipment of different types.

**Animal drawn potato diggers:** The animal drawn equipment used for potato harvesting, are the soil raising plough and double mould board type potato diggers. It is a bullock drawn, single row, open throat plough type potato digger provided with high carbon steel blade. The equipment weighing approximately 18 kgs has upwardly bent rods at the rear for better exposure of the dug potato tubers. This equipment with digging efficiency 98% exposes potatoes to the tune of 80%. Tuber damage is less
than 2%. Overall dimensions of the digger are 800X 470 X880 mm. The effective field capacity of this implement is 0.125 ha/hr.

Open throat type potato digger-animal drawn  Potato digging with animals

**Tractor drawn potato digger:** A mechanical potato digger performs the following operations, in sequence a) digging b) separation of loose soil and small clods. Potatoes are then picked up by hand. The use of an animal drawn potato plough reduces the labour requirement to 300 man-hours/hectare, whereas with a tractor operated digger, it further reduces to 80-90 man-hours/hectare depending upon whether it is a single or two row digger. At present the mechanization of potato cultivation in India is partial and selective.

The tractor drawn implements for potato harvesting are the raising plough diggers, spinner diggers and digger elevators. After digging manually picking of potatoes from the fields is never complete (100 %), irrespective of the method or machine used for digging. Estimated left over percentage is 5-15 % depending upon method of digging, field conditions and efficiency of pickers.

Two row potato elevator digger  Passive blade potato digger  Oscillating type potato digger

**Potato Combine Harvester :** One and two row potato combine harvesters are commonly used in the western countries. Two type of the prototype potato combine harvesters (1) to dig and collect tubers in a trolley (Prototype-I) and (2) to dig and collect tubers in the bunker hopper of the prototype.(Prototype-II), have been developed at CPRI.
POTATO GRADING: Grading of potatoes is essential in order to get the remunerative returns and ensure uniform quality of seed and table potatoes to the buyers. Mechanical planting of potatoes, which is gradually picking up in the country, has further necessitated the grading of seed potatoes accurately. Grading is also one of the essential requirements for production of the certified seed. Manual grading is labour and time consuming and delays the cold storage of potato resulting in deterioration of quality. It requires 20 man-hrs. per tonne of produce. Mechanization of grading operation is desirable, both for reducing the cost and time required for operation and ensuring uniformity in grades. Grading of potatoes in India is generally done manually either by hand picking or through sieves. The main drawback with manual sorting is that uniformity within the different grades is not maintained as the sizing mainly depends upon the visual judgment of the persons engaged in the grading process. Thus mechanizing the grading operation is desirable for both reducing the cost and time required for the operation and ensuring uniformity in the grades. Various crops are graded by size, in sieves, rollers and belt conveying surfaces and cylindrical graders. The geometric size of the particulate material is governed by three dimensions i.e. thickness, length and width. However most of the potato grading machines separate potato tubers on the basis of their size generally maximum width or maximum thickness. Damaged, diseased, insect infested, off type (mixture), green or otherwise unwanted potatoes are picked manually from the grader or before feeding into it. Potato-grading machines designed to work on small-scale farms in the developing world must satisfy both technical and economic requirements, as well as considering social factors for successful technology adoption. Machines must not only be suitable for use in harsh physical conditions, but they must also be profitable to the farmer. Such machines must be able to grade a wide range of tuber sizes and shapes efficiently and cost-effectively in the short and long term.
Potato considered as a future food crop because it produces maximum edible energy and edible portion per unit area per unit time among major food crops.

**Potato Statistics in India**

<table>
<thead>
<tr>
<th>Year</th>
<th>Area(M ha)</th>
<th>Production (M ton)</th>
<th>Yield(tons/ha)</th>
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<td>1980-89</td>
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</tr>
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<td>1990-99</td>
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<td>2000-09</td>
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<tr>
<td>2010-11</td>
<td>1.83</td>
<td>36.5</td>
<td>19.9</td>
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<tr>
<td>2011-12</td>
<td>1.86</td>
<td>42.3</td>
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<td>2012-13</td>
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<tr>
<td>2013-14</td>
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<tr>
<td>2016-17'</td>
<td>2.17</td>
<td>46.6</td>
<td>21.5</td>
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</table>

(Source: FAO, 2016-17 * 2nd Advance estimates)

**Current Status of Potato and Seed Potato**

During the last six decades, there is 8.4 times expansion of area and 29 times more in production. NE states and SW areas are fully dependent on northern plains for the quality planting material. To cultivate this area our country requires 4.53 million tonnes of seed material. Best management practices by quality seed material and nutrient management can only be a way out for productivity enhancement to fulfill the requirement of potato in the scenario of increasing population pressure of the country, export quality production for doubling farmer’s income and processing sector demand.

Soil is a living medium which is natural nutrient source for growth of plants. The capacity to produce usable potato plant biomass depends upon the adequacy and balance of macro and micro-nutrients in the plant. Potato is one of the most sensitive crops to low nutrients supply of soils, i.e. potato is a plant of lower nutrient efficiency than cereals like wheat, rice etc. Potato being a shallow rooted crop the fertilizer use efficiency for N is 40-50% and for K it ranges between 50-60% while it is only 10-15% for P. The basic philosophy of nutrient management should be to apply fertilizers at rates to ensure high fertilizer use efficiency so that the cost of cultivation of potato is reduced as well as the amount of unutilized fertilizers is also reduced to environmentally acceptable levels. Following information on role of nutrients/deficiency symptoms; and on suitable source, dose, time and method of fertilizer/manure application can be of help to efficiently manage nutrient requirement of potato crop.
Role of nutrients in potato

Fertilizer nutrients NPK and micronutrients promote root and shoot growth and tuber yield. Nitrogen increases plant height, number and size of leaves, leaf area duration, thickness of stem, tuber initiation, number and size of tubers. Phosphorus promotes early growth, plant height, number of shoots, leaf area index (LAI), leaf area duration, size and number of tubers particularly small size tubers. Potassium increases leaf expansion particularly at early stages of growth, extends leaf area duration by delaying leaf shedding near maturity. It aids in translocation of photosynthates from leaf to tuber. It increases both the rate and duration of tuber bulking. Potassium increases the size of tubers and not the number. It provides resistance against frost and diseases. Micro-nutrients play a specific role in the growth and development of a plant. Even though these elements are needed in only minute quantities, many soils do not supply them in sufficient quantity for healthy growth and optimum yield of potato. Potato plants produce specific deficiency symptoms under nutrient stress conditions.

Vital aspects of nutrient management in potato

Optimum rate of fertilization

General recommendations:

<table>
<thead>
<tr>
<th>General Recommendation</th>
<th>Fertilizers Source</th>
<th>Fertilizers Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urea, DAP, MOP</td>
<td>Urea, SSP, MOP</td>
</tr>
<tr>
<td></td>
<td>Split -I</td>
<td>Split -II</td>
</tr>
<tr>
<td>N = 175 kg/ha</td>
<td>100kg/ha</td>
<td>200kg/ha</td>
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<td></td>
<td>190kg/ha</td>
<td>190kg/ha</td>
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<tr>
<td>P&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt; = 100 kg/ha</td>
<td>220kg/ha</td>
<td>625kg/ha</td>
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<tr>
<td>K&lt;sub&gt;2&lt;/sub&gt;O = 150 kg/ha</td>
<td>250kg/ha</td>
<td>250kg/ha</td>
</tr>
</tbody>
</table>

Soil test for nutrient management: Fertilizer management of potato crop is usually based on averages on regional basis known as package of practices. Such an approach results in suboptimal or excessive application of inputs resulting in inefficient utilization of costly fertilizers on one hand and risks environmental degradation on the other. Moderation in rate of fertilization is possible on the basis of soil test value (STV). Soil test based fertilizer recommendation results in fertilizer economy through balanced application and enhances tuber yield. Therefore, to save on fertilizer input, apply fertilizer based on soil test values after getting soil tested from soil testing laboratories. Some of the potato growing soils have already accumulated medium to high available phosphorus and do not need full dose of phosphorus application each year. Use/Grow nutrient efficient potato cultivars like Kufri Pukhraj and Kufri Gaurav (Trehan SP, 2005).

Application of QUEFTs model for site specific nutrient management for potato:

Farmers are often resorting to apply more than the recommended dose of fertilizers. As a result of heavy applications of fertilizers over the year, there has been a decline in soil health and also environmental degradation and yield of many crops including potato has stagnated. Moreover, higher than the recommended application of fertilizers not only results in increased cost of cultivation but also
Maximizing input use efficiency is the only alternative left to enhance the productivity in a sustainable manner from the limited natural resources without any adverse consequences on soil health and environment (Kumar et al., 2016). Models can help in making precise fertilizer recommendations. QUEFTS (Quantitative Evaluation of the Fertility of Tropical Soils) is one such model. It is a site specific nutrient management approach which describes the quantitative evaluation of the native fertility of tropical soils, using yields and NPK uptake trials (Dobermann et al. 2004). QUEFTS has been calibrated and validated for best fertilizers management of NPK in rice, wheat, maize, cassava, elephant foot yam and sweet potato. We have calibrated this model for potato. Uttar Pradesh is a leading state in potato production accounting about 34 per cent share in national production. This decision support tool is developed to give recommendations on NPK application in potato in different districts of Uttar Pradesh. The decision support tool is based on the output derived for QUEFTS model. The tool requires information on date of planting, district and available NPK status of the soil. The output is for recommended NPK doses for different target yields based on the available nutrients and planting date of the particular district. In case the information is not available for any or all the nutrients, the tool takes the default value for the particular district on the basis of information on available NPK available in literature for making the recommendations.

(Source: http://14.139.61.86/Nutrient_index/homepage.aspx).

Sources of nutrients

Nitrogen: Urea the cheapest and most available (80%) source of N in India is 10-15% less efficient than ammonium sulphate (AS) and calcium ammonium nitrate (CAN). Urea is equally efficient, when broadcast and ploughed under during land preparation at least 48 hrs before planting potato.
dressing of urea between rows at earthing up is equally efficient to other sources of N. Urea alone at planting in excess of 60 kg N/ha is harmful for emergence of potato.

**Phosphorus:** Readily soluble sources of P viz, single super phosphate (SSP), triple super phosphate (TSP) and DAP are more suitable for potato.

**Potassium:** Although potassium sulphate has been found to be 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 in potato crop. However, in sulphur deficient soils, it can be more effective because of S present in it. The potassium chloride (MOP) is commonly used in potato crop and constitutes 97% of K fertilizers consumption in potato.

**Micronutrients:** If soil is deficient in any of the micronutrients, it can be applied through soil, foliar spray or seed tuber treatment, but for iron and boron, foliar spray method should be preferred. The foliage spray should be avoided between 11 am to 3 pm. The micronutrients could be sprayed mixed with fungicides for spray purpose. In case of deficiency, micronutrients may be applied at 25 kg Zinc sulphate, 50 kg Ferrous sulphate, 25 kg Manganese sulphate, 20 kg Copper sulphate, 2 kg Ammonium molybdate, 2 kg Sodium borate per hectare through soil or 0.2% Zinc sulphate, 0.3% Ferrous sulphate, 0.3% Manganese sulphate, 0.2% Copper sulphate, 0.1% Ammonium molybdate, 0.1% Sodium borate through foliar spray.

**Method and time of fertilizer application:** Split application of N (1/2 at planting + 1/2 at earthing) is essential for maximizing efficiency. Spraying 2% urea solution 40-50 days after planting corrects mild visual deficiency symptoms of N, if any. Split application of K is advantageous only in light textured loamy sand soils. No benefit from split dose of P is reported, because it is required mostly for early root and shoots growth. Only small benefits of foliar application of P and K are reported in case of visual deficiency of these nutrients in crop. Band placement of P fertilizers invariably is better than broadcast, because of fixation of P in most soils. However, methods of placement of K fertilizers in band at sides or above or below seed tubers or broadcast were equally efficient.

**Sustainable Integrated nutrient management**

It recommends conjoint application of chemical fertilizer, organic manures and bio-fertilizer, in addition to inclusion of legumes in cropping systems and incorporation of on and off-farm generated crop residues to constitute an efficient integrated nutrient management strategy. Integrated nutrient management through green manuring before potato once in 2-3 years, manuring with farmyard manure and incorporation of crop residue into the soil maintains soil fertility and help sustain productivity. Application of 15 t/ha of FYM takes care of the half of fertilizer phosphorus and potassium requirement of the crop, whereas 30 t/ha of FYM can take care of whole of fertilizer P and K needs of the crop. Therefore, adjust the dose of P and K fertilizers according to the dose of FYM applied. Role of green manuring in INM is to supplement nutrients in combination with chemical fertilizers and to improve physical condition of the soil. Green manuring usually does not help save or reduce N fertilizer needs of potato, yet for fixed yield targets, some saving in nitrogen is possible, because tuber yield level is raised by green manuring. Green manuring helps achieve 20-30% higher produce of tubers of uniform shape and size, and superior quality. It is concluded that monitoring nutrient imbalances in soil through soil test is essential for moderation in rate of fertilization of potato crop in the long term for sustained
productivity and economy. Incorporation of crop residues/green manure into the soil should be an integral part of nutrient management to maintain soil fertility and sustain productivity.

<table>
<thead>
<tr>
<th>Organic manures</th>
<th>Biofertilizers</th>
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<tbody>
<tr>
<td>(FYM, vermicompost and neem manure)</td>
<td>(Azotobacter, Azospirillium and PSB)</td>
</tr>
<tr>
<td>IPNM</td>
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</tr>
<tr>
<td>Green manuring (Dhaincha)</td>
<td>Chemical Fertilizers</td>
</tr>
<tr>
<td>(Neem coated urea)</td>
<td></td>
</tr>
</tbody>
</table>

References:


DNA fingerprinting/Genetic fidelity
Jagesh Tiwari and CM Bisht
Division of Crop Improvement, ICAR-CPRI, Shimla-171001 (HP) India

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. MgCl₂ (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 µl and for 5 reactions.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>20.0 µl</td>
</tr>
<tr>
<td>Taq pol. buffer (10X)</td>
<td>10.0 µl</td>
</tr>
<tr>
<td>Mgcl₂ (25 mM)</td>
<td>08.0 µl</td>
</tr>
<tr>
<td>dNTPs (2.5 mM)</td>
<td>10.0 µl</td>
</tr>
<tr>
<td>Primer (F)</td>
<td>05.0 µl</td>
</tr>
<tr>
<td>Primer (R)</td>
<td>05.0 µl</td>
</tr>
<tr>
<td>Taq DNA poly. (5U)</td>
<td>02.0 µl</td>
</tr>
</tbody>
</table>

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
<table>
<thead>
<tr>
<th>Cycle</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95°C</td>
<td>12 min</td>
</tr>
<tr>
<td></td>
<td>94°C</td>
<td>15 sec</td>
</tr>
<tr>
<td>10</td>
<td>50°C</td>
<td>15 sec</td>
</tr>
<tr>
<td></td>
<td>72°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>20</td>
<td>89°C</td>
<td>15 sec</td>
</tr>
<tr>
<td></td>
<td>72°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>1</td>
<td>72°C</td>
<td>30 min</td>
</tr>
</tbody>
</table>

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°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’.
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

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the chemical</th>
<th>Molecular formula</th>
<th>Quantity (g)</th>
<th>(10X)</th>
<th>(40X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Potassium nitrate</td>
<td>KNO₃</td>
<td>19.0</td>
<td>76.0</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Ammonium nitrate</td>
<td>NH₄NO₃</td>
<td>16.5</td>
<td>66.0</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Potassium di-hydrogen phosphate</td>
<td>KH₂PO₄</td>
<td>1.7</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Magnesium sulphate hepta-hydrate</td>
<td>MgSO₄.7H₂O</td>
<td>3.7</td>
<td>14.8</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the chemical</th>
<th>Molecular formula</th>
<th>Quantity (g)</th>
<th>(10X)</th>
<th>(40X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Calcium chloride dehydrate</td>
<td>CaCl₂.2H₂O</td>
<td>4.4</td>
<td>17.6</td>
<td></td>
</tr>
</tbody>
</table>

Take 500 ml double distilled water in a 2.0 liter beaker, weigh and add CaCl₂.2H₂O, 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

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the chemical</th>
<th>Molecular formula</th>
<th>Quantity (mg)</th>
<th>(10X)</th>
<th>(40X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Boric acid</td>
<td>H₃BO₃</td>
<td>62.0</td>
<td>248.0</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Manganese sulphate mono-hydrate</td>
<td>MnSO₄.H₂O</td>
<td>169.0</td>
<td>676.0</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Zinc sulphate hepta-hydrate</td>
<td>ZnSO₄.7H₂O</td>
<td>86.0</td>
<td>344.0</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Potassium iodide</td>
<td>KI</td>
<td>8.3</td>
<td>33.2</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>*Sodium molybdate di-hydrate</td>
<td>Na₂MoO₄.2H₂O</td>
<td>2.5</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>**Copper sulphate penta-hydrate</td>
<td>CuSO₄.5H₂O</td>
<td>0.25</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>**Cobalt chloride hexa-hydrate</td>
<td>CoCl₂.6H₂O</td>
<td>0.25</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

* 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:** $\text{Na}_2\text{MoO}_4\cdot2\text{H}_2\text{O}$ should be dissolved separately in approximately 20 ml of distilled water and added to the stock and $\text{MnSO}_4\cdot\text{H}_2\text{O}$ takes time to dissolve.

**D. Preparation of iron stock Murashige and Skoog (MS-IV)**

Iron-EDTA stock (MS-IV) in 1000 ml (10X)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the chemical</th>
<th>Molecular formula</th>
<th>Quantity (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(10X)</td>
</tr>
<tr>
<td>1.</td>
<td>Sodium EDTA di-hydarte</td>
<td>$\text{Na}_2\text{EDTA}.2\text{H}_2\text{O}$</td>
<td>373.0</td>
</tr>
<tr>
<td>2.</td>
<td>Ferrous sulphate hepta-hydrate</td>
<td>$\text{FeSO}_4.7\text{H}_2\text{O}$</td>
<td>278.0</td>
</tr>
</tbody>
</table>

Take 1000 ml double distilled water in a 2.0 liter amber colored bottle, and warm the water near boiling. Now weigh and add $\text{Na}_2\text{EDTA}.2\text{H}_2\text{O}$ while stirring under a magnetic stirrer; after $\text{Na}_2\text{EDTA}.2\text{H}_2\text{O}$ has been dissolved, add gradually $\text{FeSO}_4.7\text{H}_2\text{O}$ while still under mild magnetic stirring. This will yield a clear yellow solution. Immediately after adding $\text{FeSO}_4.7\text{H}_2\text{O}$ 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

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of the chemical</th>
<th>Quantity (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(10X)</td>
</tr>
<tr>
<td>1.</td>
<td>myo-Inositol</td>
<td>1000.0</td>
</tr>
<tr>
<td>2.</td>
<td>Glycine</td>
<td>20.0</td>
</tr>
<tr>
<td>3.</td>
<td>*Thiamine HCl</td>
<td>1.0</td>
</tr>
<tr>
<td>4.</td>
<td>*Nicotinic acid</td>
<td>5.0</td>
</tr>
<tr>
<td>5.</td>
<td>*Pyridoxine HCl</td>
<td>5.0</td>
</tr>
</tbody>
</table>

* 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**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Stock solution</th>
<th>Quantity (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10X</td>
</tr>
<tr>
<td>1.</td>
<td>MS-I</td>
<td>100.0</td>
</tr>
<tr>
<td>2.</td>
<td>MS-II</td>
<td>100.0</td>
</tr>
<tr>
<td>3.</td>
<td>MS-III</td>
<td>100.0</td>
</tr>
<tr>
<td>4.</td>
<td>MS-IV</td>
<td>100.0</td>
</tr>
<tr>
<td>5.</td>
<td>MS-V</td>
<td>100.0</td>
</tr>
</tbody>
</table>
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 g l\(^{-1}\) or gelrite at the rate of 2.0 g l\(^{-1}\).
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 lb 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**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the Disinfectants</th>
<th>Concentration (%)</th>
<th>Exposure time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Calcium hypochlorite</td>
<td>9-10</td>
<td>5-30</td>
</tr>
<tr>
<td>2.</td>
<td>*Sodium hypochlorite</td>
<td>0.5-5.0</td>
<td>5-30</td>
</tr>
<tr>
<td>3.</td>
<td>Hydrogen peroxide</td>
<td>3-12</td>
<td>5-15</td>
</tr>
<tr>
<td>4.</td>
<td>Ethyl alcohol</td>
<td>70-95</td>
<td>1-5.0</td>
</tr>
<tr>
<td>5.</td>
<td>Silver nitrate</td>
<td>1.0</td>
<td>5-30</td>
</tr>
<tr>
<td>6.</td>
<td>Mercuric chloride</td>
<td>0.1-1.0</td>
<td>1-5</td>
</tr>
</tbody>
</table>

*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.*
Viruses are systemic pathogens, and therefore, perpetuate through seed tubers. Thus, the losses caused by viral diseases are not only confined to the year when infection occurs, but continue as long as the diseased tubers are used as seed. While plants infected with bacteria and fungi respond to treatments with bactericidal and fungicidal compounds, there is no commercially available treatment to protect virus-infected plants. Being dependent on host for DNA replication and protein synthesis, selective interference of viral multiplication by chemical means without adversely affecting the plant nucleic acid and protein synthesis is almost impossible.

The term 'meristem culture' denotes *in vitro* culture of meristematic dome of actively dividing cells located at the extreme growing tip of the shoot is called as “apical meristem” and if it is located at axillary bud means it is called as “axillary meristem”, along with a portion of the subjacent tissue containing one or two leaf primordia. This piece of tissue is called apical shoot tip about 0.1-0.3 mm in size. The production of pathogen-free mother plants is achieved by tissue culture. Elimination of viruses by meristem excision and culture is a rather old practice; it is based on the fact that not all cells from an infected plant carry the virus and in rapidly growing meristematic tips viruses are either absent or their concentration is very low. Meristematic tissue from roots and sprouts may be virus-free. Despite the phenomenal success of meristem culture in elimination of plant viruses, it remains still unclear as to why the apical/axillary meristems contain a little or no virus? There are several hypotheses. Some of these are given below:

- Lack of true vascular tissue in this part of the plant. Most viruses translocate efficiently inside a plant through the phloem or by passage from cell to cell through plasmodesmata. Since cell-to-cell movement is slow, the concentration front of a virus is slowed in rapidly dividing tissue.
- High metabolic activity in the meristematic tissue. Because viruses replicate using the metabolic process of the host, they find it difficult to compete with this high metabolic activity.
- Chromosome replication during mitosis and high auxin content in the meristem may inhibit virus multiplication through interference with viral nucleic acid metabolism.
- Existence of virus-inactivating systems with greater activity in the apical region than elsewhere.

**Protocol: Potato meristem culture**

The protocol for potato meristem culture consists of:

(i) Selection and testing of apparently healthy plants from the field or harvested tubers.

(ii) Establishment of *in vitro* cultures.

(iii) Virus elimination through meristem culture.

(i) **Selection and testing of plants/tubers**

Select apparently healthy plants from the field or sample tubers

Test these plants/tubers for freedom from viruses using enzyme-linked immunosorbent assay
(ELISA) or PCR

If no plant/tuber is found free from all viruses then one has to resort to meristem culture

Select a plant/tuber that is infected with minimum viruses for use in meristem culture

(ii) Establishment of *in vitro* cultures

*From infected plant:* Excise nodal stem segments from the third and fourth nodes from the stem apex. Each nodal cutting should be 1-2 cm long, and the leaves should be detached. Such single node cuttings (SNCs) are used to initiate *in vitro* cultures.

*OR*

*From infected tuber:* Treat the freshly harvested tubers with a fungicide (Mencozeb 75 WP @ 0.2%) for 15 min and dry them. If not required for immediate use, the tubers can be stored at 4°C till dormancy breaking. For immediate use, give dormancy breaking treatment (GA3 @ 1 ppm and thiourea @ 1%) and allow the tubers to sprout in dark at 24°C. Harvest sprouts measuring about 2-3 cm long.

In the laminar flow, clean air work station, surface sterilize the SNCs/sprouts for 8-10 min in 20% of commercial sodium hypochlorite solution (4% w/v available chlorine), rinse in sterile distilled water three times, trim both ends of the explants by a scalpel and place the explants inside culture tubes (25 x 150 mm) each containing ~13 ml of semisolid propagation medium. The propagation medium is based on MS (Murashige and Skoog, 1962) basal nutrients supplemented with D-calcium pantothenate (2 mg/l), gibberellic acid (0.1 mg/l) and 30 g/l sucrose. The medium is solidified with 7.0 g/l agar

Incubate the cultures under a 16 h photoperiod using cool white fluorescent lights (50-60 μE/m²/s light intensity) at 24 °C

Allow the explants to grow up to 6-8 nodes stage, and then subculture through SNCs on fresh medium under the cultural conditions described above. Shoot cultures can be maintained and multiplied *in vitro* by sub-culturing on fresh medium after every 3 weeks
(iii) Virus elimination

**Thermotherapy:** Thermotherapy is given to *in vitro* cultures or tubers prior to meristem culture. This is done as under:

Place 7-days-old cultures in a thermotherapy chamber or BOD incubator at 37 °C under a 16 h photoperiod at 20-30 µE/m²/s light intensity, and incubate for 3 weeks.

Treat infected tubers with GA (2mg/l) and allow them to sprout at 37 °C under dark till 2-3 cm long sprouts are formed.

Dissect meristem from the *in vitro* plantlets/sprouts by the method described below.

**Chemotherapy:** Chemotherapy involves the use of chemicals like antibiotics, plant growth regulators, amino acids, purine and pyrimidine analogues to inactivate viruses or inhibit replication/movement of viruses in tissues. These chemicals can either be sprayed on growing plants prior to excision of meristems or incorporated into tissue culture media. Of all the chemicals tested for plant virus elimination, synthetic nucleotide analogues like ribavirin @ 20 mg/l (Virazole: I-D-ribofuranosyl- 1,2,4-triazole-3-carboxamide) and DHT (5-dihydroazauracil) have been particularly effective in inhibiting different plant viruses. *In vitro* chemotherapy of meristematic explants with antiviral chemical ribavirin has been found to be most promising for elimination of major potato viruses.

The concentrations of many antiviral chemicals required during chemotherapy to inhibit virus multiplication are very close to the toxic concentration for the host plant. In addition, there is always a
possibility of mutations when the plants are exposed to antiviral chemicals (synthetic nucleotide analogues). Therefore, *in vitro* ribavirin therapy at low concentrations combined with thermotherapy has been used to eradicate viruses from infected potato cultivars.

**Meristem excision and culture**

Excise meristems (terminal as well as axillary) from thermo-treated *in vitro* plantlets/sprouts under laminar flow cabinet using a stereoscopic zoom microscope, scalpel and needle. Protective leaves on the buds should be removed carefully using a needle. Use a drop of sterile distilled water to avoid meristem desiccation during excision.

Trim the meristematic dome plus one set of leaf primordia with a scalpel to 0.2-0.3 mm.

In case of sprouts, surface sterilization using 20% of commercial sodium hypochlorite solution is essential before dissecting meristems.

Place the excised meristems on semi-solid meristem culture medium in a culture tube (1 meristem/culture tube), and incubate at 24 °C under a 16 h photoperiod with 50-60 µE/m²/s light intensity.

Test meristem-derived plantlets for freedom from viruses by ELISA.

Multiply and maintain virus-negative counterparts of meristem-derived clones by single node culture *in vitro* as described above.

Several meristem culture media have been reported in the literature. The meristem culture medium used at the ICAR-CPRI is based on MS basal nutrients supplemented with 2mg/l D-calcium pantothenate, 0.1 mg/l GA3 and 30 g/l sucrose, and solidified with 7.0 g/l agar. For chemotherapy, meristem culture medium is also supplemented with suitable concentration of anti-viral compounds.

It takes about 5-6 months for meristems to grow into full plantlets (mericlones). At this stage sub-culture the plantlets individually for maintaining their clonal identity.
Double Antibody Sandwich ELISA (DAS-ELISA) Technique

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Division of seed technology, ICAR-CPRI Shimla-171001 (HP) India

Principle of the test: Double Antibody Sandwich-Enzyme Linked Immuno Sorbent Assay (DAS-ELISA) uses antibodies which are bound to the surface of a microplate to capture the antigens. The presence of the antigen is detected using specific antibodies coupled with alkaline phosphatase. Finally, the addition of the substrate of the enzyme (pNPP) induces a yellow product, detectable at 405 nm, when the antigen is present.

Buffers and Reagents:
Bicarbonate/carbonate coating buffer for 1000ml pH 9.6
Antigen or antibody should be diluted in coating buffer to immobilize them to the wells:
Na$_2$CO$_3$ 1.59g
NaHCO$_3$ 2.93g

PBS (Phosphate buffered saline-PBS): WASH BUFFER for 10X, pH 7.4
NaCl 80.00g
KH$_2$PO$_4$ 2.00g
Na$_2$HPO$_4$.12H$_2$O 11.50g
KCl 2.00g
Tween-20 5.00g
NaN$_3$ 2.00g

CONJUGATE BUFFER for 1000ml, pH 7.4
TRIS 2.40g
NaCl 8.00g
PVP 20.00g
Bovine serum albumin (BSA) 2.00g
MgCl$_2$ 2.00g
KCl 0.20g
Tween-20 5.00g
NaN$_3$ 2.00g

SUBSTRATE BUFFER for 1000ml, pH 9.8
Di-ethanolamine 97.00ml
NaN$_3$ 0.20g

SAMPLE BUFFER for 1000ml, pH 7.4
TRIS 2.40g
NaCl 8.00g
PVP 20.00g
KCl 0.20g
Tween-20 5.00g
NaN$_3$ 2.00g
Egg albumin (add just before use) 10.00g
The test is performed in micro-ELISA plates as follows:

1. Dilute specific antibody (IgG) in coating buffer i.e. 20µl in 20 ml buffer at a recommended dilution of 1:1000 or 40µl in 20 ml buffer at a recommended dilution of 1:500. Dispense 100-200µl to each well of the microtiter plate.

2. Cover the plates and incubate at 37°C for 2-4 h.

3. Decant and wash the plate with PBS-Tween using wash bottle, soak for a few minutes and repeat washing three to five times. Blot plates by tapping upside down on tissue paper.

4. Extract samples 1:20 (w/v) in extraction buffer. Add 100-200 µl aliquots of the test sample to duplicate wells.

5. Cover the plates and incubate 2-3 hrs at 37°C or overnight at 4°C. Wash the plate three to five times as in step 3.

6. Add 100-200 µl enzyme lablled IgG conjugate (in extraction buffer).

7. Cover the plates and incubate at 37 °C for 2-4 hours.

9. Wash three to five times as in step 3.

10. Add 100-200 µl aliquots of freshly prepared substrate (0.6-1mg/ml para-nitrophenyl- phosphate in substrate buffer) to each well.

11. Cover the plate and incubate at room temperature for 30-60 min, or as long as necessary to obtain clear reactions.

12. Stop reaction by adding 20 µl 3M NaOH to each well.

13. Assess results by:
   a) Visual observation
   b) Spectrophotometric measurement of absorbance at 405 nm

Advantages:

• High specificity, since two antibodies are used the antigen is specifically captured and detected.
• Suitable for complex samples, since the antigen does not require purification prior to measurement.
• Flexibility and sensitivity, since both direct and indirect detection methods can be used.
Precautions:

1. Before opening the tubes containing coating antibody (IgG) and IgG-AP Conjugate, do spin down all the liquid by a short centrifugation (approx. 3000rpm for a few seconds).
2. Always prepare IgG enzyme conjugate within 10 minutes before use.
3. Include negative (healthy) and positive control to validate the assay. Use tissue from a non-infected plant (tested negative for the test virus) of the same species as the healthy control.
4. Do not wash or rinse microtitre plates prior to use. Plates once used, should not be reused.
5. Use separate beakers for preparing, antiserum, conjugate and substrate dilutions.
6. Dilute antiserum (IgG) according to the titre value and commercial conjugates as per the manufacturer’s directions.
7. Always use freshly prepared substrate buffer.
8. PNPP is photo-degradable. Hence, cover with aluminium foil to avoid direct contact with light. Use PNPP solution immediately after preparation.
9. Samples preparation: When possible select samples showing symptoms. Leaf tissue is often used in ELISA testing. Stem, seed potato and other tissue can also be tested. To prepare the samples, you can use a mortar and a pestle, or other grinding devices to grind samples. If you are using a mortar and a pestle, wash and rinse them thoroughly between each sample. After extraction, the material can be kept at +4°C during a maximum of 12 hours. The sensibility of the test can be altered because of a bad conservation, so it is better to deposit samples directly after their grinding.
10. Use of positive and negative controls is deeply recommended during the tests to be able to validate the results.
11. A sample is positive if the ratio (OD405 sample/OD405 healthy plant extract) is at least 2 or more than 2.
12. The use of other dilutions for the reagents will cause differences in reactivity, specificity, selectivity and detection limits.
13. Lower reaction volumes will cause higher detection levels and lower ratio’s.
14. Different incubation times and temperatures will cause differences in sensitivity and background reactions.
Agrisearch with a human touch