

Foundation Potato Seed Program

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Propagation Procedures

- **Line selection:** Tubers and plants from superior clones are chosen.
- **Meristem Culture:** Excision of plant tissue and subsequent culturing *in vitro*.
- **Testing for diseases:** All mother plantlets are tested for major potato diseases.
- **DNA fingerprinting:** Potato plantlets in the clone bank are checked for genetic purity.

Line selection

Line selection combined with the eradication of bacterial and viral diseases provides a valuable tool for improving cultivars. The virus cleaning & subsequent multiplication program begins with selection of superior mother plants. Line selection is made for type, vigor, skin set, uniformity of tubers & foliage, and against diseases. *In vitro* plantlets are also selected for vigor, uniformity, and culturability. The selection of superior lines or clones of a particular variety requires knowledge's and expertise in potato production.

Meristem Culture:

The apical meristem together with one to three young leaf primordia, measuring 0.1-0.5mm, has been referred as meristem-tip. It is well known that the distribution of viruses in plants is uneven. In infected plants the apical meristems are generally either free or carry a very low concentration of the viruses. Meristem-tip culture although mainly used for virus elimination, it has also enabled plants to be freed from other pathogens, including mycoplasmas, bacteria, and fungi. Although the apical meristems are often free of viruses, there are evidences that suggest some viruses actually invade the meristematic region of the growing tips. Potato Virus S (PVS) and Potato virus X (PVX) are difficult to eliminate by thermotherapy or meristem-tip culture alone. In such cases it has been possible to obtain virus-free plants by combining meristem-tip culture with thermotherapy. Anti viral chemicals have also been used to eliminate viruses alone or in combination with meristem culture or heat treatment. In our laboratory we have combined meristem-tip culture with heat treatment and chemotherapy to achieve a high percentage of plantlet regeneration & eradication of viruses.

Testing for diseases

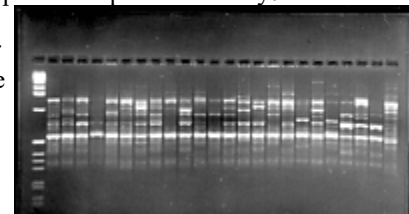
Meristem derived mother plantlets are cut into nodal sections for disease testing & further multiplication. The mother plantlets are tested as indicated in Oregon Potato Seed Certification Standards for the followings:

- Potato Virus Y (PVY), X, S, A, M (including the new strain of M), & Potato Leaf roll Virus (PLRV) by ELISA test.
- Electron microscopy test for the presence of viruses.
- Potato Spindle Tuber Viroid (PSTV) by cDNA, Dot hybridization or gel electrophoresis,
- Bacterial Ring Rot (BRR) by gram stain & immunofluorescent antibody stain (IFAS) and Richardson's media,
- Erwinia species by crystal violet pectate (CVP),
- All tests are done by independent laboratories.

DNA fingerprinting

Potato plants produced by meristem-tip culture, or micro propagation, are generally uniform in nature, and rare variants are usually attributed to spontaneous mutation. Chances that variations occur are much greater if the system is based on adventitious shoot formation, and still greater with embryogenesis, callus and cell systems. With any propagation system, no method can guarantee 100% true-to-type plants. However, there is a need to produce high quality & uniform plants to be competitive in potato industry.

Traditionally identification of potato cultivars depends on key morphological traits such as tuber type, leaf type, growth habit flower color etc. However, only a limited number of traits are stable over all environments, and several months maybe required to observe the distinguishing characteristics. Recently, a number of polymerase chain reaction (PCR) -based techniques have been developed for the analysis and isolation of genetic markers. Random amplified polymorphic DNA (RAPD) and more recently, the amplification of highly polymorphic



microsatellite or simple sequence repeat (SSR) sequences have become popular choice because  of their simplicity & ease of use for exploring genetic polymorphism's. To ensure genetic purity & uniformity of our stock plants in our laboratory we have combined RAPD and microsatellite markers to identify potato cultivars.

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