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The Canon of Potato Science:

22. *In Vitro* Multiplication through Nodal Cuttings

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What is it?

The potato crop can be propagated in two ways, vegetatively (clonally) and sexually (true seeds). Currently, commercial potato production throughout the world is almost completely based on vegetative propagation. True seeds are predominantly used only in breeding work for introduction of new cultivars. Although the potato plant is multiplied using a number of different techniques, *in vitro* nodal cuttings are probably the most common propagules used in early stages of commercial seed potato production. Since the genetic stability and the production of uniform, true-to-type plant material are very important for the industry, the multiplication methods which utilize nodal or apical cuttings and microtubers are preferred in seed potato production. New plants are produced from existing buds assuring identical genetic make-up of the parent plants.

In *in vitro* nodal cutting systems, plantlets are regenerated and multiplied under artificial, aseptic conditions in the laboratory. Depending on the genotype or potato cultivar, 5 to 10 fold multiplication rates per 4 weeks are reported under lab conditions. If virus elimination is needed, cultures are initiated from meristems (dissected portions of the meristematic region of a shoot tip) from plants exposed to a heat treatment or to chemotherapy (i.e. Virozole) prior to culturing. Normally, up to 40% regenerants from meristems are virus-free. Meristems of the treated plants are all disease-free as the pathogens have not been able to enter the less differentiated cells of the meristem. The meristems are then placed on the liquid nutrient media (usually on paper bridges in test tubes) for plant regeneration. They grow into plantlets in approximately 6–8 weeks and then the disease tests are performed on them. Once the disease/virus-free status is determined, the meristem-derived plantlets are used for further rapid multiplication by the *in vitro* nodal cutting system. The healthy plant material is cut into individual stem portions (single-node

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cuttings) with one axial bud and an attached leaf. These individual nodes are placed on agar media (usually in jars) and grown for about 4 weeks. Due to loss of apical dominance, the axial buds grow into stems with numerous nodes. These, in turn, are cut again into nodal pieces containing one axial bud and leaf and are placed on a growth medium again. This process can be repeated many times, until the required number of plantlets is produced. Although the plantlets could be then directly planted to the screenhouse, greenhouse or even in the field, a normalization phase and/or a hardening period is recommended prior to transplanting into soil. During the normalization phase, the single-node cuttings develop into fully rooted *in vitro* plantlets on media containing auxins, and might be subjected to some other growth regulators (e.g., Alar application) that will produce sturdy plants with larger leaves that will better survive transfer to the greenhouse. The quality of plantlets coming from the *in vitro* nodal cutting system is of extreme importance and cannot be overlooked. Multiplication via nodal cuttings offers possibilities of producing large quantities of high quality, genetically identical, disease-free plantlets in a short period.

Why is it Important in Potato Science?

The development of *in vitro* single-node multiplication systems was a breaking point in the commercial production of high quality potato seed. Although the method has been around for about three decades, there is still plenty of scientific research conducted around the globe studying various aspects of regeneration, physiological responses and production of plantlets. The method helped to reduce the number of field generations and consequently the problems (mostly pathogens) associated with the conventional seed production methods. The basic requirement for *in vitro* plantlets is the disease/virus-free status. This minimizes disease incidence (fungal, bacterial and/or viral) and in later generations assures high quality of the seed. Numerous tests have been developed to tests the plantlets against the diseases to assure the pathogen-free status.

The production of plantlets via nodal cuttings has opened a new area in research on nutrient requirements and nutrient uptake of plantlets during the multiplication stage as well as effects of light and temperature on culturing. Since the quality of plantlets produced from nodal cuttings has been a priority, over the years, the effects of liquid and solid media on the growth of micropropagated potato shoots have been studied extensively in relation to the availability of nutrients and water in the culture medium. Nitrogen and sugar assimilation in the new grown shoots and their water content have been evaluated for various cultivars in various regeneration systems.

Multiplication through nodal cuttings has also shed new light on physiological responses of single-node microcuttings to applied growth regulator treatments, rate of growth in culture, lateral branching, shoot/root ratio, and planting density in a culture vessel. Studies on the removal of the leaves from single-node potato cuttings *in vitro* that would allow propagules to be placed in culture vessels at increased densities (with the intent of saving resources and space) proved the subtending leaf to be essential for quality of regenerating shoots. Nodal microcuttings with no leaf produce shoots with fewer nodes, shorter plantlets, smaller leaf area, and lower fresh and dry weights which in turn results in inefficient multiplication protocols.

Why is it Important for the Potato Industry?

At present, the base for seed potato production in most parts of the world is the pre-elite potato tuber. Pre-elite tubers are predominantly produced from greenhouse-grown minitubers (nuclear seed tubers) derived from tissue cultured, *in vitro* plantlets. *In vitro* multiplication through nodal cuttings is the basic method for the production of these plantlets. The plantlets are produced using various protocols in different laboratories around the world. Optimisation of conditions and techniques of the multiplication phase has ultimately led to maximum numbers of nodal microcuttings entering the normalisation step. Since the *in vitro* derived plantlets have high vigour and possess a very high health status (they are tested and they are pathogen-free), they are invaluable in the production of top quality, true-to-type, disease-free seed material. The *in vitro* plantlets are sold to selected specialist seed potato growers who use them to produce minitubers and multiply them further producing later generations of seed tubers. The above is extremely important for the highly competitive potato industry.

The *in vitro* systems for production of plantlets based on single node multiplication have become very efficient and inexpensive allowing large quantities of plantlets be produced in short time at relatively low cost. Recently, to reduce the production cost even further, a number of efforts have been made to mechanize or to use a vision based robotic system for the production of nodal cuttings. A robot capable of removing morphologically regularized plantlets from a vessel, cutting them into internodes and transplanting cut pieces to new vessels has successfully been tested in the production of potato plantlets. The efficiency of such robots could reach 4,000,000 plantlets per year. The single-node microcuttings are also excellent initial material for the multiplication systems via bud clusters and in the production of microtubers. The system uses axillary buds of *in vitro* produced plantlets where these buds are multiplied in liquid media and produce bud clusters that could be multiplied again and again or be induced to develop microtubers (bioreactor). The above may reduce the production cost even further.

Scientific Developments

The *in vitro* multiplication through nodal cuttings technology is very well established and used extensively around the world. The technology is essential for mass production of top quality, true-to-type, disease-free material for the potato industry. Although the subject has been researched extensively for over three decades, there is always some room for additional improvement especially in optimisation of regeneration from nodal cuttings, increasing the number of nodes and leaf size, multiplication by clusters produced from axillary buds, mechanization of the system (robots), growth-room environmental conditions. Since the nodal microcuttings can be used in production of a number of different propagules including plantlets, bud clusters and microtubers, adequate nutrient media additives and growth regulators (e.g., jasmonates for production of microtubers) can be determined for the proper pre-treatment of the nodal cuttings according to the final product. Also, a multiplication system of multiple shoots from stem cuttings of

in vitro plantlets where the stem sections with 3-4 nodes are multiplied in flasks can be further explored in order to find additional mass multiplication systems for quick delivery of large quantities of plantlets.

Further Reading

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