Manipulation of Growth by Using Tissue Culture Techniques©

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INTRODUCTION

Looking at the whole production chain from breeder, selector, propagator, young plant producer, grower, retailer right through to the consumer, it is quite a long and time consuming process that these plants have to go through. Tissue culture is a tool or mechanism used by plant propagators to speed up the production of certain plants, as well as to create the desired type of growth. In the potted-plant industry, it is important for some plant types to be sold as a clump or cluster of plants rather than single plants. Tissue culture gives one the opportunity to determine the end result.

TISSUE CULTURE

What is tissue culture? Tissue culture is a technique by which any plant part can be cultured on a nutrient medium under sterile conditions with the purpose of obtaining growth. Tissue culture is also referred to as micropropagation, clonal propagation, in vitro propagation, or rapid propagation. The term "in vitro" refers to "in glass" or in an artificial environment compared to "in vivo" which means "in soil".

Normally plants can be propagated in two ways: vegetatively (asexual) and generatively (sexually, by seeds). In the sexual cycle new plants arise after the fusion of parental gametes. Seedlings will be variable and each one will represent a new combination of genes.

In the asexual cycle, unique characteristics of a plant are maintained, because during mitosis genes are copied exactly. Each plant produced in this way is part of the somatic cell line of an individual. Groups of plants produced in this way are called clones. Clonal propagation is defined as asexual reproduction of genetically uniform plants that originate from a single individual or explant. Micropropagation refers to the small size of explant required and the smaller amount of space required for maintaining and multiplying large numbers of small plants. Alternative terms are: rapid multiplication, because it is quick; clonal multiplication, because all the plants generated are clones and therefore genetically alike; and vegetative in vitro propagation, because plants are propagated asexually.

Advantages of Tissue Culture.

- A large number of clonal plants can be obtained. It is especially useful where plants are difficult to propagate vegetatively, are slow growing or where a single individual with good characteristics needs to be propagated.
- It is often much faster than conventional means. In 8 months 10,000 *Ornithogalum* plants can be produced from a single leaf in tissue culture. It would take 10 years to obtain the same number of plants with conventional means.
- With the use of meristem techniques it is possible to produce certified virus-free plants.

- New cultivars can be selected from cultures due to mutations of rapidly dividing cells.
- Micropropagation can be used for rejuvenation of plant material.
- Micropropagation can cause new temporary desirable characteristics, e.g., bushiness.
- Production is independent of season and can continue throughout the year.
- Vegetatively produced material can be stored for long periods.
- Plant material needs little attention between subcultures and no labour is required for weeding, spraying, watering, etc.

Disadvantages of Tissue Culture. There are also disadvantages to in vitro micropropagation:

- The main disadvantage of in vitro methods is that a specialized and expensive production facility is required, advanced skills are required for its operation, and specific methods need to be used to obtain optimal results. These may affect the cost of propagules.
- The plants obtained may be small initially and have undesirable characteristics.
- As in vitro plants are dependent on a carbon source they do not function autotrophically in culture and therefore have to undergo a transitional phase before they are capable of independent growth.
- The young plantlets are more susceptible to water loss in the external environment, since they are grown at a high relative humidity.
- The chances of producing genetically aberrant plants may be increased.

Stages of Micropropagation. There are various stages of micropropagation that should be considered before embarking on a micropropagation effort (Murashige, 1974; Debergh and Maene, 1981). These stages are as follows:

Stage 0: Mother plant selection and preparation. This includes the pre-treatment of starting material, e.g., rejuvenation, reducing contamination.
Stage I: Establishing an aseptic culture.
Stage II: The propagation phase.
Stage III: Preparation for growth in natural environment.
Stage IV: Transfer to natural environment.

Different methods of in vitro vegetative propagation are used in Stage 2 (George, 1993) and include:

a) Propagation from axillary buds/shoots.
   - Shoot (or shoot tip) culture.
   - Single, or multiple node culture.

b) Formation of adventitious shoots and/or embryos.
   - Directly, on pieces of tissue or organs (explants).
   - Indirectly, on callus tissues.

Propagation Form Axillary Buds. The production of plants from axillary buds has proved to be the most generally applicable and reliable method of in vitro propagation.

For example, single-node cultures are used for the rapid propagation of potato. Both apical and axillary buds are used. Both types of bud include a meristem of dividing cells that can give rise to a new shoot. This type of culture is routinely used for the propagation of crops such as potato, sweet potato, and tobacco.

Culture of Adventitious Tissue. The term adventitious is defined as "to develop from unusual points of origin". Shoots usually develop from auxiliary buds, but callus, cotyledons, hypocotyls, leaves, petioles, stems, flower petals, roots, or other tissues can be stimulated to form shoots. These are then referred to as adventitious structures. Where the structure develops directly from the cultured explant, the process is called direct morphogenesis. If the structure develops from the undifferentiated mass of cells called a callus, the process is called indirect morphogenesis. Morphogenesis refers to the development of a differentiated structure.

- **Direct Organogenesis.** Adventitious shoots arise directly from the tissues of the explant and do not develop within previously formed callus. Direct shoot formation may be accompanied by some proliferation of unorganized cells. The chances of mutants arising with this method are far higher than with the single node or axillary shoot methods. Rates of propagation can, however, be very high. The induction of shoot regeneration depends on the plant organ from which the explant was excised and also depends on the plant species. This method is used very successfully for the propagation of African violets, *Saintpaulia*.
- **Indirect Organogenesis.** The indirect mode of regeneration consists of establishing an explant in culture, subsequent proliferation of callus, or a suspension culture, and then initiation of adventitious shoots or pre-embryos. Shoots are said to have regenerated indirectly when they are formed on previously unorganized callus or in suspension cultures. Separate root and shoot initials are formed in callus cultures. Callus cultures vary in their morphogenic potential or competence, but adjustment of the growth regulator in the culture medium can bring about shoot or root formation from callus from a large number of species. The chances of variability occurring are greatest in plants produced by indirect shoot formation.

Rooting and Acclimatisation. This is the part of tissue culture that must ensure that your product will survive and thrive outside the sterile container. The plant must be weaned from the protective, sterile, humid climate through a transitional phase to be able to survive the harsh, dry climate. This transfer is called hardening or acclimatisation.

At the beginning of Stage III, shoots or plantlets obtained from Stage II are often small and incapable of self-sufficient growth in soil. Therefore this material must be prepared so that individual plantlets are obtained which are capable of self-supporting photosynthesis and survival ex vitro. This includes the rooting of shoots. With some species, the rooting occurs on the shoots obtained from Stage II, e.g., potato, whereas others are rooted during the course of Stage III, e.g., rose rootstock. With other species, it is necessary that the shoots be elongated first in preparation for rooting that usually requires transference to another medium. Yet with

some species, like *Pistacia*, it is possible to remove shoots from in vitro conditions and root them directly ex vitro via conventional methods. Rooting ex vitro can be quicker and decreases costs in the laboratory. However, in vitro rooting is still commonly used, and it does offer more control over the rooting process.

Stage IV, where the plantlets are transferred from the in vitro to the ex vitro environment, is very important, as this stage can result in a significant loss of plants. The main reasons for this are that cultured plants have thin cuticles and are thus easily desiccated. Their stomata do not operate properly in vitro and in some cases are misformed or totally absent. In addition, they are not totally autotrophic in culture where they are grown with a carbohydrate source under low light, and are thus not self-supporting. Ex vitro plants are also more susceptible to fungal and bacterial diseases.

In vitro Rooting. Some plants easily form roots in vitro. This can be an advantage or disadvantage. In vitro roots are not always functional and they are also likely to be damaged during planting out. These roots usually die and new roots have to be formed once the plant is established. In such a case allowing the plants to root outside in the glasshouse would be better. However, some plants may need roots to survive the transplantation, although their roots are not fully functional. They usually recover faster than plants that still have to form roots.

Ex Vitro Rooting. As already mentioned, many tissue culture laboratories commonly use ex vitro rooting. This reduces the costs and time taken for production of rooted plantlets. Some crops root much easier ex vitro than in vitro, this is especially true for woody plants for example pistachio rootstock.

Shoots or clumps of shoots are removed from tissue culture and again it is necessary to ensure that the base of the shoot is cut cleanly with a sharp blade. Transfer of the shoots to the glasshouse must be as smooth as possible and the shoots must not dry out. In order to prevent this from happening, shoots can be transferred in a tub lined with damp paper towelling. Before planting out in a suitable porous growth medium, the medium must be watered and allowed to dry out a little. Shoots are often dipped in an auxin solution. Rooting powder (e.g., Rootone F1™, Seradix™) can also be used. Jiffy 7™ peat plugs are excellent for rooting unrooted cuttings.

It is extremely important that these microcuttings must be prevented from drying out at all times. The transfer from the lab to the greenhouse must be accompanied with as little stress, drying out, and in the shortest time frame as possible. To prevent the cultures from drying out after planting, they can be kept in high humidity tents. A thick layer of wet sand in the tent will keep the humidity high. It was also found that a fogging system works better than a mist system.

In order to keep fungal diseases under control, a fungicide solution (e.g., Benlate™ or Folpet™) can be sprayed on the shoots. However, these fungicides can suppress root formation, and so the fungicides must be used sparingly. Some commercial rooting powders contain both fungicides and auxins.

Many growth media suitable for ex vitro rooting are available, and it is important to select a medium with the correct characteristics, as there can be a marked difference between root production by the same species on different media. Mixtures of peat, sand, vermiculite, perlite, and bark are popular choices. Alternatively, a compressed peat plug (e.g., Jiffy™ plugs) can be used. Rockwool plugs are another good alternative. Seedling trays filled with different substrates can also

be used for unrooted cuttings. The polystiren trays are dipped in a copper containing solution (Styroseal™) to prevent the roots from growing into the trays.

Because ex vitro plantlets are so susceptible to pests and pathogens, it is necessary to sterilise the growth medium used, usually by steam. After planting, nutrients must be supplied to the plantlets, and this can either be in the form of a weak solution (e.g., ½ MS) or by applying fertilizers (e.g., Multifeed™). Slow-release fertilisers can also be added to the substrate depending on the type of plant. Be careful not to burn roots that were already formed in vitro. Concentration of the nutrients should be gradually increased over time. Again, fungicides can be supplied if necessary. Sanitation is very important, and it may be necessary to use sterilised water if the contamination rate of the plants is high.

Transfer to the Environment. Transfer to the environment (usually the glasshouse) and establishment of the rooted plantlets is also called acclimatization or "hardening off". In the case of ex vitro rooting of shoots, rooting and hardening off can occur simultaneously.

Facilities. The type of facility needed depends on the budget and the scale of the project. If only a small experiment has to be transplanted a big expensive glasshouse is not necessary. On the other hand if you are producing plants on a large scale and your income depends on the production of the plants it might be worth it to invest more money in a well-equipped glasshouse. Such a glasshouse would include a mistbed or plastic tunnels to keep the humidity high and a very good temperature control system. A method to control the light intensity would also be needed, as plantlets should be gradually exposed to an increase in light intensity during hardening off.

If only a few plants need to be hardened off they can be planted in a small tray and can be covered by a sheet of plastic or glass or a plastic beaker can be inverted over the plantlets to maintain a high humidity.

Ex Vitro Plantlets. Pots or trays in which ex vitro plantlets have been placed can either be covered with plastic or placed in a mist bed. The humidity must be kept very high for the first 3 weeks. After that the humidity can be decreased slowly. Start by opening the tunnels slightly for half an hour per day and then gradually increase the time. As the shoots produce roots, they gradually become more exposed to drier air and increased light intensity. After 6 weeks most plants could be transferred to a climate where the humidity is not controlled any more. It is however, still important that the temperature must be controlled.

In vitro Plantlets. When in vitro shoots are first rooted in tissue culture, the plantlets must be healthy and well proportioned. No callus must be present between the root and the shoot. If sugar was included in the rooting medium, it is necessary to wash away the agar. Sucrose found on the surface of the roots can cause the plantlets to be infected with disease causing micro-organisms. Otherwise, plantlets must be treated with fungicide and nutrient solution, and rooted in growth medium as previously described for ex vitro rooting.

It is essential to work as quickly as possible with the plantlets as soon as they have been removed from the tissue culture vessels. They may desiccate in a couple of minutes under hot, dry conditions. Often, it is easier to work with the plantlets when the roots are still quite short. The longer the roots are, the more likely they

are to be damaged. When using a seedling tray or container where a lot of plants are planted in the same container, the plants must be sprayed with water regularly. As soon as the leaves start to curl up, the plant is under stress. A spray bottle with distilled water works very well.

Transplantation Time. The best time of the year to harden plants off is the time when the temperature and humidity can best be controlled. Greenhouses in which the temperature and humidity can be controlled can be used year round, although light intensity will have to be adjusted. The best time to plant is the natural growing season of the specific crop, e.g., *Lachenalia* should be planted in March, as it is a winter crop. The duration of the hardening off process depends on the type of plant that is transplanted. Plants that have to form roots ex vitro also need a longer period and should never dehydrate. The more gradual the transformation process can be made, the better the results that can be expected.

EUROPEAN PLANT TISSUE CULTURE

According to a survey done in 1996/1997 of plant tissue culture laboratories in Europe, there are approximately 179 million plants produced per year. A total of 505 laboratories were located of which 193 were commercial. The rest were made up of official labs at universities and research institutes. As far as I know, no such survey was done in South Africa before.

Averages of 0.54 million plants were produced per lab per year with a mean number of 7.5 people per lab. *Prunus* is the species most propagated by tissue culture. This species replaced *Solanum* as number one. The highest number of plants produced from a single lab in Europe then, was 15 million.

Plant genera listed in order of most commonly propagated by the commercial labs in Europe are as follows: *Prunus,* orchids*, Ficus, Rosa, Solanum, Spathiphyllum, Calathea, Fragaria, Rhododendron, Begonia, Pelargonium, Rubus, Geranium, Syngonium, Brassica, Drosera, Betula, Hosta, Actinidia, Anthurium, Chrysanthemum, Hydrangea, Malus, Nephrolepis, Syringa, Cordyline, Pyrus, Dianthus, Lobelia, Philodendron, Saintpaulia, Sorbus,* and *Vitis.*

CONCLUSION

A lot of plants are already propagated by means of tissue culture techniques in South Africa: *Solanum, Musa, Syngonium, Spathiphyllum, Fragaria,* and *Rosa*, to name but a few. In the laboratory at ARC-Roodeplaat, Vegetable and Ornamental Plant Institute, we propagate potatoes (*Solanum tuberosum*) for the Seed Certification Scheme. Rose rootstock as well as strawberries, *Haworthia*, indigenous bulbs, sweet potato (*Ipomoea batatas*), garlic (*Allium sativum*), cassava (*Manihot*), *Plectranthus*, *Chrysanthemum*, *Aster*, etc. are propagated on request.

Tissue culture as a tool for propagation of plants is on the increase in South Africa. Several commercial tissue culture laboratories have European linkages and export on a regular basis ex-agar plants.

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Getting the Most From Your Lettuce Seedlings: International Nutrition Research©

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INTRODUCTION

Lettuce (*Lactuca sativa*) transplants grown with a floatation irrigation system often show limited root growth, which results in root systems not pulling out completely from the transplant flat and poor establishment in the field. In the present investigation, 'South Bay' lettuce transplants were grown in a peat and vermiculite medium in the greenhouse. They were fertilized with varying concentrations of N, P, and K via floatation irrigation at selected fertigation frequencies to determine optimum nutrient and water management requirements for production of high quality lettuce transplants, with sufficient roots to fill a 10.9 cm^3 tray cell and that ultimately establish in the field rapidly. To avoid inconsistency in the duration of the light period, natural photoperiod was extended to 16 h in all experiments.

FERTILIZER MANAGEMENT

To determine the optimum P concentration necessary for production of high quality transplants, plants were propagated by floating flats in nutrient solution containing either 0, 15, 30, 45, or 60 $mg \cdot L^{-1}$ P in summer and autumn experiments, and either 0, 15, 30, 60, or 90 $mg \cdot L^{-1}$ P in factorial combination with 60 or 100 $mg \cdot L^{-1}$ N in a winter experiment. When the concentration of P in the medium (saturated paste extract) was more than 12 $mg \cdot L^{-1}$ (summer experiment), P at 0, 15, 30, 45, or 60 $mg \cdot L^{-1}$ subirrigated every 2 to 4 days, did not influence fresh or dry root mass. However, when the concentration of P in the medium was about 0.5 $mg \cdot L^{-1}$ (autumn experiment), fresh and dry root mass increased with each level of P fertigated every 2 to 4 days. When the fertigation frequency was every 2 days (winter), fresh and dry root mass increased in response to 15 $mg \cdot L^{-1}$ P, with no further increases in root mass at higher P concentrations up to 90 $mg \cdot L^{-1}$ even though the medium P concentration was only 0.4 $mg \cdot kg^{-1}$.

The major transplant growth responses to applied P occurred between 0 and 15 $mg \cdot L^{-1}$ P, regardless of fertigation frequency and medium P concentration. Added P increased fresh and dry shoot mass, root length and area, leaf area, pulling success, leaf tissue P, relative growth rate (RGR), specific leaf area (SLA), leaf area ratio (LAR), leaf mass ratio (LMR), but reduced root : shoot ratio (RSR), net assimilation rate (NAR), and root mass ratio (RMR). Only about 30% of plants grown with 0 P could be pulled from the transplant flat, compared to approximately 90% pulling suc-