Tissue Culture of Perennials

Paul Nihot
Paul Nihot, Tissue Culture, Burg. Engelbertsstraat 86, 2042 KP Zandvoort, Netherlands
Email: Paul@Nihot.com

INTRODUCTION

Tissue culture refers to the culture of complete plants or, more often, excised plant organs under sterile conditions on a nutrient medium. The techniques for successful tissue culture were developed in the previous century: growth under sterile conditions, satisfactory inorganic and organic nutrients, and plant growth regulators. Tissue culture has been utilised in horticulture for the removal of pathogens (by meristem culture), various biotechnological breeding techniques (among others, embryo rescue, genetic engineering), and vegetative propagation. In this paper I will focus on vegetative propagation in tissue culture, also referred to as micropropagation. This technology was established in the 1950s and 1960s, with the first commercialization immediately following. Rapid growth of micropropagation occurred through to the 1990s, and ever since, growth has been steady, both with respect to the total numbers of plants produced, and the number of micropropagated crops.

THE ADVANTAGES OF MICROPROPAGATION OVER CONVENTIONAL VEGETATIVE PROPAGATION

Micropropagation is significantly more complex, and can appear more expensive than propagation by conventional means. Thus, it requires a very specialised scientific knowledge base, sophisticated and expensive laboratory facilities, and is also highly labour-intensive. As a result, micropropagation can be more expensive than conventional vegetative propagation, unit-for-unit. Other drawbacks include enhanced variations in various genotypes, and the special skills required when micropropagated plants are acclimatized to ex vitro conditions (or weaned). Nevertheless, the advantages far outweigh the disadvantages.

- With conventional propagation it can take 10 years to build up numbers, while micropropagation may reduce this period to 2 to 3 years. In this way, the actual costs of getting a plant into production can, in fact, be far less with micropropagation than with conventional propagation. A plant can be on sale and returning investment far quicker, and, very importantly, ahead of the competition.
- Conventional methods of propagation are prone to many plant pathogens (with regulations on pesticides becoming ever more strict). Micropropagated plants are grown in a totally sterile and clean environment and, provided the culture is started from disease-free material, the plants produced, are disease-free, and of superior quality. Many major growers are now specifying disease-free material, produced from elite stock; only plants produced by micropropagation can comply!

- Micropropagated plants rejuvenate during the production process. As a consequence, they are more vigorous and produce more branches than plants propagated by conventional means.
- Conventional propagation methods often yield non-uniform batches of plants. Micropropagated plants are far more uniform, not only in their genetics but also in size and vigour (genotype and phenotype). This is particularly relevant when nursery practices are being mechanised, and drastically reduces the need for size and quality grading on the beds.

MICROPROPAGATION STAGES

In micropropagation, five main stages can be identified: Stage 0, Preparation; Stage 1, Initiation; Stage 2, Multiplication; Stage 3, Rooting; Stage 4, Weaning.

Stage 0: The Preparative Stage. Micropropagation requires the choice of appropriate starting material. Elite plant material needs to be selected for initiation. The elite material will either be selected for phenotypic characteristics, or the plant will be the bearer of elite seed. Tests for virus and bacteria contaminants can be an important part of the preparative procedure. Further preparation includes measures to reduce contamination problems in the initiation stages, for example, avoiding overhead irrigation, pre-treatment with plant growth regulators, warm water treatment. The growing status of the plant also determines the success of the next stage; in general, at the time of initiation, the plants need to be vigorously and actively growing, rather than entering dormancy.

Stage 1: Initiation of Culture. Having prepared and selected this "true-to-type" elite plant, tissue is selected, excised, and initiated in tissue culture. This can be an apical or axillary bud, or a rhizome. In all three cases, existing meristems resume/continue growth in tissue culture. However, tissues without pre-existing meristems can be used; e.g., leaves or stem segments. Cells in these tissues may de-differentiate and then re-differentiate into new meristems. This process is referred to as adventitious regeneration. It should be noted that, depending on the genotype, adventitious regeneration may yield unwanted variation, especially when the process involves an intermediate period of callus growth.

The main goal of Stage 1 is to produce sterile plant material, growing on the selected sterile, nutrient medium. Only a very small part of one plant is needed as starting material for the rapid production of thousands of genetically identical progeny.

Stage 2: Multiplication. In a classical multiplication stage, the culture generates shoots for subsequent propagation, as well as material required to maintain stock.

When the sterile explants have resumed growth they are transferred regularly to fresh medium. A well-used multiplication method is the production of shoot clusters (clusters consisting of multiple shoots) by the outgrowth of axillary buds. At the end of a cycle, the shoot clusters are divided into individual shoots and transferred subsequently onto fresh medium to develop new clusters until the desired number of shoots has been produced. This transfer of the shoots is labour intensive and is done under sterile laboratory conditions. During this production process, great care is taken to maintain the axenic (contaminant-free) status of the culture, utilising a range of strict laboratory procedures and quality assurance methods.

Stage 2 is the core of micropropagation. In comparison to conventional propagation, more new shoots may be formed by forcing dormant axillary buds to resume growth

by applying a plant growth regulator of the cytokinin type. Most importantly, many propagation cycles can be achieved per year (ca. 10), compared to the single cycle in conventional propagation.

Stage 3: Rooting. After the desired quantity of plants is obtained in the multiplication stage, the shoots are transferred to an auxin-based media for additional shoot development, shoot elongation and, of course, the formation of roots. The main goal at this stage, besides root formation, is to harden the plants to ensure the weaning stage can take place with a minimum of loss.

Stage 4: Weaning. The skills and the facilities required in the weaning stage, do not, in themselves, guarantee minimal losses. Much depends also on the quality of the plant material produced in the previous stage. The main goal during the stage of weaning or acclimatization (as it is also known), is the adaptation of the plants from a sterile environment to greenhouse conditions.

During the multiplication and the rooting stage, plants have been growing in a sterile environment with high humidity, in a controlled atmosphere and on a sugar-based medium. Once transferred to soil, plants need to adapt to greenhouse conditions with lower humidity, varying temperature, and the presence of microorganisms. At this stage, the plants become self-sustaining, taking up water and minerals via their roots, and photosynthesising in their leafs, to produce their own supply of nutrients, in the usual way of green plants. A major factor is that the water-retention-capacity of micropropagated plants is compromised because of limited stomatal control.

PLANT HORMONES

The two important plant hormones used in micropropagation are auxin and cytokinin. At present we are using discoveries made ca 50 years ago: cytokinins to break apical dominance and auxin to achieve rooting.

NEW DEVELOPMENTS

- Technological: TIB (temporary immersion bioreactor).
- Biological: In the new plant biology many discoveries are being made about plant development and the effects of the environment on plants. This may yield very significant breakthroughs.
- Many small discoveries about the propagation of specific crops.

Table 1. Example with *Geranium* (cranesbill) schedule in time and quantity.

Before micropropagation:

- Evaluation of a particular plant to determine if it is superior in comparison to existing selections.
- Evaluation to ensure tissue culture propagation is the right means to start production.
- Selection of an elite plant, suitable for initiation in tissue culture.
- Testing for virus and other plant pathogens.
- Virus elimination, where applicable.
- Preparation of the plant for initiation.

	Week	Year	Plants used (no.)	Plants not used (no.)	Remarks
Initiation	1	1	25		
Growth of meristems	10	1	25		
Visual selection	15	1	18	7	Loss of 28% of clean material because of contamination
Growth of new shoots (multiplication)	20	1	36		
Selection of clean cultures by test	25	1	30	6	Additional loss of 17% because of contamination
Multiplication factor 2 per 5 weeks	30	1	60		Multiplication
Multiplication	35	1	120		
First rooting	40	1	60	60	If the rooted plants "fail" to flower properly, the procedure should be modified
Conservation of cultures for ca 9 months					To wait for the outcome of the growing trials
Start multiplication	25	2	50		
Multiplication	30	2	100		
Multiplication	35	2	200		
Multiplication	40	2	400		
Multiplication	45	2	800		
Multiplication	50	2	1600		
Multiplication	3	3	3200		
Rooting	13	3	6400		
To greenhouse for weaning	18	3	6000		Loss of 6% because of non-rooting
Weaned and delivered in plugs	25	3	5600		Loss of 7% during weaning