MICROPROPAGATION OF ORNAMENTAL PLANTS

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Abstract

An analysis has been made of commercial micropropagation in 15 West European countries. In 1988 Western Europe had 248 commercial tissue culture laboratories with a total production of 212.5 million plants. Most species micropropagated were ornamental plants (157 million). Special attention has been paid to the Netherlands which has 76 commercial laboratories and had a production of 80 million plants in 1989. A comparison is made of the various micropropagation methods used: single-node culture; axillary branching; adventitious organ formation and callus systems and the chances of obtaining genetic variation and mutations when using these methods is analyzed. The use of inflorescence explants for micropropagation is valuable for a number of ornamental crops, particularly bulbous species such as Hippeastrum, Nerine, and tulip. Ex vitro rooting of in vitro obtained shoots in so-called Rockwool plugs which reduces labour costs is described. One of the most complicated and complex factors influencing micropropagation is the solidifying agent agar, produced from seaweed. A survey is given of agar analysis together with the main effects of using various agars.

1. Commercial micropropagation in Western Europe

An analysis has recently been made of commercial micropropagation in 15 West European countries (Pierik, 1990b,c). In 1988 Western Europe had a total of 248 commercial tissue culture laboratories of which at least 37 each produced more than one million plants per year. The total production for 1988, in the various categories are summarized in Table 1, which shows that ornamental plant species (157 million or 74% of the total propagated) dominate micropropagation in Western Europe. The most frequently cloned ornamental plants are: Ficus, Synchonium, Spathiphyllum, Gerbera, Rosa, Philodendron, Saintpaulia, Nephrolepis, Cordyline, Anthurium, Calathea, Cymbidium, Dieffenbachia, and Rhododendron. The leading country in almost all categories of ornamental plants is the Netherlands, which in 1988 had 67 commercial tissue culture laboratories, followed by Italy (35), Spain (27), France (22), West Germany (21), United Kingdom (18), Belgium (16), Denmark (9), Ireland (8), Greece (6), Portugal (5), Switzerland (4), Finland (4), Sweden (4), and Norway (2). No data were collected from Luxembourg and Austria.

Table 1. Micropropagation in 15 countries of Western Europe in 1988. Numbers of plants are given in millions.

Categories		Categories	
1. Pot plants	92.34	8. Perennial garden plants	2.98
2. Cut flowers	37.84	9. Agricultural crops	2.42
3. Fruit trees	19.43	10. Miscellaneous ornamentals	1.94
4. Ornamental bulbs and corms	13.16	11. Vegetables	1.37
5. Small fruits	9.35	12. Trees (forestry)	1.29
6. Orchids	5.29	13. Herbs	0.03
7. Ornamental trees/shrubs	3.89	14. Not specified	21.13
		Total	212.46

Table 2. Micropropagation of ornamentals in the Netherlands in 1989 (Pierik, 1990a). Numbers of plants are given in millions.

Number of		The most important pot plants	
Plants produced per laboratory	Commercial laboratories	Nephrolepis	14.4
		Saintpaulia	5.1
		Spathiphyllum	4.7
Less than 0.1	39	Synchonium	3.3
0.1-1.0	21	Ficus	2.8
1.0-5.0	10	Anthurium scherz.	2.5
More than 5.0	6	Platycerium	0.7
		Cordyline	0.6
Total	76		
The most important cut flowers		Other important ornamentals	
Gerbera	17.1	Lilium	16.3
Rosa	1.1	Cymbidium	1.5
Anthurium andreanum	0.9	Other orchids	1.5

2. Commercial micropropagation in the Netherlands

Using detailed information available (Pierik, 1990a,c), special attention has been paid to this country, which accounted for 29% of micropropagation in Western Europe in 1988. A recent study (Table 2) in 1989 (Pierik, 1990a) showed that the Netherlands has a total of 76 commercial tissue culture laboratories with a production of 80 million plants. The majority of these are situated in the Western part of the country, where the ornamental industry is concentrated. The number has increased from 28 in 1980 to 76 in 1989. Table 2 shows the large number of relatively small-sized laboratories found in the Netherlands, many being micropropagation laboratories within plant breeding companies.

3. Micropropagation systems

Single-node culture

This method is the simplest, most natural and safe method (no problem with mutations) with plants which elongate (e.g. potato, tomato, lilac and grape), forming a stem with leaves with buds in their axils, but is difficult with rosette plants. The rate of propagation is strongly dependent on the number of nodes formed within a particular time interval. When cloning shrubs and trees, serious problems can arise with this method (dormancy of buds and failure to elongate the stem).

Axillary branching

Axillary buds have their dormancy broken by breaking apical dominance with cytokinin. This method has become the most important propagation method being simple and quite safe. Another advantage is that the propagation rate is relatively fast and the genetic stability is usually preserved. However, mutations can occur when adventitious buds are formed as a result of high cytokinin levels.

Regeneration of adventitious buds/shoots

This method includes the formation of adventitious buds/shoots on explants from leaves, petioles, stems, scales and floral stems. However, the percentage of plant species that can regenerate adventitious buds is relatively small and is often restricted to herbaceous plants. The chances of obtaining mutations is much higher with this method than with the first two methods described, particularly with so-called chimaeric plants. However, it is successfully applied for Saintpaulia ionantha, lily, hyacinth, Achimenes, and Streptocarpus (Pierik, 1988).

Regeneration of plants from callus, cells, and protoplasts

Despite claims to the contrary, cloning of higher plants through callus, cells, and protoplasts has been found to have many disadvantages. The greatest difficulty with callus cultures is their genetic instability. Only a few plant species such as Anthurium andreanum (Pierik, 1988), are cloned (partially) through a callus phase in the Netherlands.

4. Use of floral stem and inflorescence explants for micropropagation

The first reports on regeneration of shoots from floral stem and inflorescence explants were recorded for tobacco by Aghion-Prat (1965) and for Lunaria annua (Pierik, 1967). It was later concluded that isolated floral stem explants or parts of inflorescences can be an extremely valuable starting point for adventitious bud/bulblet/shoot formation, particularly in bulbous and cormous plants: tulip (Wright and Alderson, 1980); Hippeastrum hybrids (Pierik et al., 1985; Koopman et al., 1987; Smits et al., 1989); Nerine bowdenii (Pierik and Steegmans, 1986); Eucharis grandiflora (Pierik et al., 1985); Freesia hybrids (Pierik and Steegmans, 1976) and Begonia venosa (Pierik and Tetteroo, 1987).

Since 1985 (Pierik et al., 1985) the regeneration of a large number of bulblets on floral stem explants of various Hippeastrum hybrids has been extensively studied (Pierik et al., 1991). An efficient micropropagation method

has now been developed for this expensive bulbous crop. Floral stems (3-6 cm etiolated stems, forced in darkness) are cut into 1.5 mm discs and placed on a regeneration medium (Table 3, medium A), with their basal ends positioned downwards on the medium. On this medium most Hippeastrum hybrids readily regenerate bulblets within 3 months of isolation. Optimal physical conditions for bulb regeneration are 25°C and continuous darkness. Although most hybrids only require auxin for bulb regeneration, it is advisable to use medium A with both auxin and cytokinin. Under the conditions described above between 80-90% of the floral stem explants regularly produce shoots. The bulblets produced should first be subcultured on a new medium (Table 3, medium B) in the light at 25°C to regenerate roots, form leaves, until a minimum bulb size of 0.6 cm diameter is reached. They are then split longitudinally into 4 segments and subcultured again on medium B in the light at 25°C. After 12 weeks each quartered segment regenerates on average 1.3 bulblets. The in vitro produced plants flower after 2.5 years and appear to be true-to-type. Since the bulblets regenerating on floral stem explants are multicellular in origin (Smits et al., 1989), the chance that mutations come to expression is very small.

Table 3. Media for the micropropagation of Hippeastrum hybrids. All media contained Murashige and Skoog's (1962) macro- and microsalts (except iron), NaFeEDTA 25 mg/l, Daishin agar 0.7% (w/v) and were adjusted to pH 6.0 before autoclaving.

	Medium A	Medium B
Saccharose (%)	6-8 *	4.5
Meso-inositol (mg/l)	100	0
Vitamin B_1 (mg/l)	0.4	0
BA* or 2iP* (mg/l)	2.0	0
NAA (mg/l)	0.2-0.4 *	0

*The concentration or compound to be used is dependant on the cultivar.

5. Ex vitro rooting in Rockwool microplugs

In recent years inert synthetic supports for micropropagated shoots have been developed e.g. by Baumgartner Papier in Lausanne (Sorbarods) and by Milcap France (substrates 'Milcap', lumps and plugs). These supports are useful (Roberts and Smith, 1990), but the cost price is relatively high. However, the cost price of Rockwool supports is much lower (U.S. $ 0.03).

In 1988 Grodan-Rockwool, in the Netherlands, introduced Rockwool microplugs which are especially developed for rooting of micropropagated shoots in vivo. Rockwool, already well known as a growing medium in artificial substrates without soil, for many horticultural crops (3 000 ha in the Netherlands), is at present mainly used for Gerbera and Anthurium andreanum microcuttings. The microplug of mineral wool has now also been introduced in the Netherlands, especially to reduce labour and material costs and to increase the quality of the micropropagated plants.

The following are some of the unique properties of Rockwool:
1. It replaces soil so that export of plants becomes possible to those countries who refuse soil as part of their plant protection regulations.
2. It has the capacity to achieve a homogeneous water/air balance, including an optimal distribution of water, nutrients and air in the root environment.
3. Roots can penetrate the well drained substrate very easily and shoots can easily be transferred to other Rockwool blocks of larger size or to soil to continue further growth. Rockwool enables transport without damaging the root systems and quick regrowth after transplantation.
4. It is sterile, eliminating the need for soil steaming or chemical sterilization.
5. It allows the regeneration of normal roots in comparison to abnormal root formation in agar media. The plugs have a special fibre structure to promote rooting.
6. It is almost chemically inert, containing no plant nutrients, or contaminants of organic origin normally present in agar.
7. Tray systems for microplugs facilitate transport.

However, there are a number of special requirements to be kept in mind:
1. Plugs should be fully saturated with a weak nutrient solution (Electrical conductivity 0.5-1.0 μS/cm) before shoot cuttings are inserted. Plugs should be allowed to leach out before inserting the cuttings.
2. Auxin requirement for cuttings is strongly dependant on the plant species and cultivar, but also on the hormonal composition of the culture medium just before shoot cuttings are taken.
3. Shoots should be washed to remove sugar from the culture medium, otherwise infections easily occur.
4. Since Rockwool has initially a slight basic reaction, the nutrient solution (dependant on the plant species) should be used at a pH of 5.2.
5. Relative humidity (90-95%) should be carefully controlled , especially during the first 1-2 weeks after inserting the shoot cuttings. Hardening off should take place by gradually decreasing the relative humidity.
6. Regular application of water and nutrients is required, preferentially by hand, depending on water loss and location in the greenhouse.
7. The quality and size of the minishoots is more critical in Rockwool plugs than in agar media.
8. The greenhouse climate (temperature and relative humidity) needs to be carefully controlled during rooting and acclimatization in Rockwool. This has been shown particularly for gerberas.
9. When transplanting rooted plants in plugs to soil, care must be taken that plants do not dry out in the first week, since soil easily removes water from the relatively small plugs.

6. Agar quality

Agar is a hydrophilic colloid extracted from seaweeds, belonging to the Rhodophyceae. It consists of 2 fractions (agarose and agaropectin) and is the classical gelling agent used in plant tissue culture (Armisen and Galatas, 1987). Although a natural product, it is in principle purified by the manufacturers and should contain no toxic compounds. The ash content (inorganic salts) of agar varies from 2.5-5.0% (w/w). The ash content of agarose is much lower than that of agar due to the absence of ionic groups.

Analyses of agars have shown that they contain many organic and inorganic impurities (Kohlenbach and Wernicke, 1978; Debergh, 1983; Pierik, 1987; Scherer et al., 1988; Kordan, 1988). It is not known which undesired contaminants should be removed to obtain a qualified agar or even which ash content is acceptable and which ranges of the various elements in the agar can be tolerated. Toxicity is certainly dependant on the element under consideration. Debergh (1983) demonstrated that inorganic impurities introduced with the agar are responsible for significant differences in the concentration of several ions in addition to the normal ions added with the mineral nutrition. Nothing about the nature of organic contaminants can be found in literature.

Remarkable effects of various agar brands and agarose have been described in the literature. The following processes were strongly influenced by the agar brand: pollen germination and pollen tube growth (Kordan, 1988), differentiation of tracheary elements (Roberts et al., 1984), anther regeneration (Kohlenbach and Wernicke, 1978), regeneration of sugar cane (Anders et al., 1988) and Kalanchoë (Hauser et al., 1988), and shoot proliferation of apple and pear (Singha, 1984).

To find a correlation between agar quality (physical and chemical properties) and growth response of 3 plant species on 20 different agar brands the following analyses and biotests were carried out:

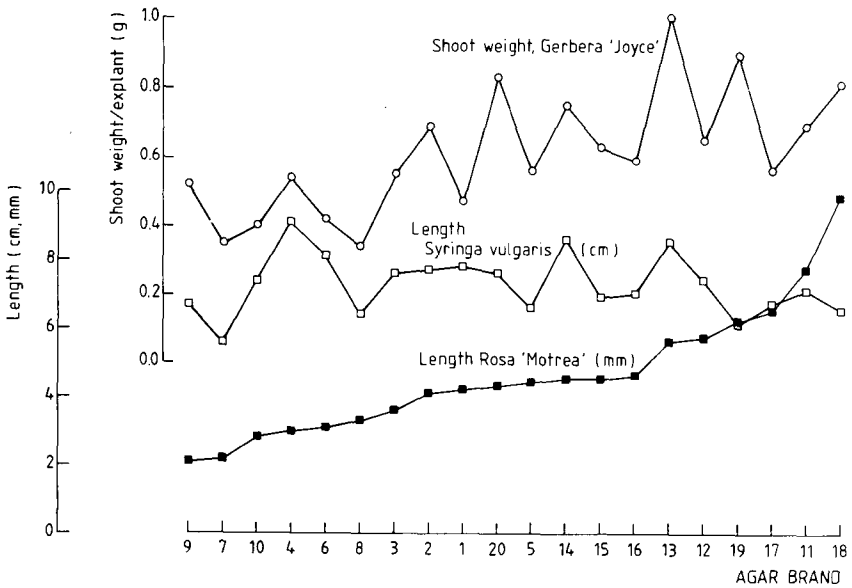
1. A number of elements in the agars (N, P, K, Ca, Mg, Na, Cl, Br, I, Fe, Mn, Zn, Co, Cr, Th, Sr, V and several others) were determined spectrophotometrically or by means of atomic absorption spectroscopy.
2. Electrical conductivity, moisture content, colour, and pH before and after autoclaving of the agars were determined.
3. Shoot elongation of the rose cv. 'Motrea' was examined on a basic culture medium with 4.5% (w/v) sucrose as suggested by Marcelis-van Acker (1990).
4. Shoot elongation of the lilac (Syringa vulgaris) rootstocks A2 and A3 were followed under the conditions previously described by Pierik et al. (1988).
5. Shoot fresh weight and shoot multiplication of the Gerbera cv. 'Joyce' were determined using the shoot multiplication system described by Pierik et al. (1982) with 2.5 mg/l kinetin.

From this work the following conclusions can be drawn:

1. The brand of agar strongly influences the shoot length of rose and the fresh weight of Gerbera (Figure 1). However, lilac is very irregularly affected, indicating that impurities in the agar are probably of little importance for the growth and elongation of shoots of this species. This can be explained by its toleration of a medium with a very high inorganic salt content (Pierik et al., 1988).
2. There was a large difference in shoot elongation in rose between the worst (No. 9) and the best agar (No. 18). Fresh weight of Gerbera shoots also showed large differences on the worst agar (No. 8) and the best agar (No. 13).
3. When the growth of rose and gerbera are compared (Figure 1), a similar pattern of growth is seen, although there are exceptions to this rule, e.g. agar No. 17.
4. A strict correlation between the growth response of rose or Gerbera and moisture content, pH before or after autoclaving, colour, or EC was not found. A high pH before autoclaving generally coincided with a high EC.

5. Agar No. 9, on which growth of rose as well as Gerbera was poor, can be characterized by a very high EC (415 μS/cm), a relatively high pH before autoclaving (7.0), a 'bad' colour (grey-brown), and a high concentration of N, Ca, Na, Mn, Cl, Br, I, V, Cr and Sr.
6. Agar No. 13 the best agar for Gerbera and a good agar for rose, has a 'good' colour (white), a low EC (122 μS/cm), a low pH before autoclaving (6.2), and a low concentration of N, Na, Br, Cl, I and V, but a relative high concentration of Ca, Mn, Mg, Co, and Fe.
7. Agars bad for both rose and Gerbera can be characterized by high levels of several elements (Ca, Na, Mg, I, Br, V and Cr), a relative high EC, a higher pH before autoclaving, and a grey-brown colour.

Figure 1. Effect of the agar brand on shoot length (mm) of the rose cv. 'Motrea', shoot length (cm) of Syringa vulgaris and shoot weight per explant of the Gerbera cv. 'Joyce'.



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