ingily propagated by conventional methods, tissue culture may be used as an alternative. The demand for tropical ornamental plants, the feasibility of the use of asexual propagation is not rapid enough to meet the sharp increase in stocks in a much shorter time. The objective of this paper is to summarize our work on the tissue culture of tropical ornamental plants.

Because clonal multiplication through conventional methods of asexual propagation is not rapid enough to meet the sharp increase in demand for tropical ornamental plants, the feasibility of the use of tissue culture for rapid clonal propagation has been increasingly investigated. Through this method, it has become possible to asexually propagate rapidly some plants which are difficult or slow to increase through conventional means. Even in cases where plants can be readily propagated by conventional methods, tissue culture may be used advantageously for rapid clonal increase of newly selected cultivars or cultivars available in limited numbers to establish propagation stocks in a much shorter time. The objective of this paper is to summarize our work on the tissue culture of tropical ornamental plants.

Orchidaceae

Since 1960, when Morel reported his success in rapidly propagating Cymbidium axially through meristem culture (16), other orchid genera have been successfully propagated in similar manner. However, instead of meristems, shoot tips have been used as explants in most cases because the sole objective was rapid clonal increase. The disadvantages of using meristems are: 1) the necessity of using a microscope for dissection, 2) the size of these explants, 3) high mortality, and 4) slow growth.

Culturing shoot tips, we have clonally propagated cattleyas (20), dendrobiums (9, 11), vandas (13, 22), and phalaenopsis (6, 8). The source of explants from sympodial orchids is the young shoot arising from the rhizomes and from monopodial orchids, the stem terminal.

From these sources, axillary and apical buds are removed and cultured in a liquid nutrient medium. The liquid culture is constantly agitated at 160 rpm on a New Brunswick Model V rotary shaker under constant illumination of about 2.2 klx and at temperatures ranging from 25°C to 30°C. Proliferations will eventually emerge on these explants and are continuously increased by subculturing in fresh liquid medium. The proliferating bodies are then plated on agar medium for differentiation of shoots and roots. Besides axillary and apical buds, explants of leaves from asexually grown plantlets (6), young inflorescences (7), and roots (10) have also been used to initiate clonal increase.

Our basic nutrient formulation for rapid clonal increase of orchids is that of Vacin and Went (24). The medium is always supplemented with coconut water (liquid endosperm) because it contains the necessary plant growth regulating factors (14, 21, 25) for inducing proliferations and for maintaining vigorous growth. It has not been necessary for us to use additional growth regulators such as auxins and cytokinins. Coconut water is collected from immature fruits, filtered, and stored frozen until use.

In the course of our work on orchids, we have found that sucrose at the recommended amounts (20–30 g/liter) in tissue culture and orchid seed germination media has a deleterious effect on the proliferating bodies of some monopodial orchids (7, 13, 22). The proliferations rapidly become chlorotic and, in severe cases, die on sucrose medium. Proliferating bodies of sympodial orchids can tolerate these amounts of sucrose. Sensitivity to sucrose is only

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restricted to the proliferating bodies and therefore sucrose is not added to the medium at this stage of propagation. Prior to this stage when initial explants are induced to proliferate and after this stage when shoots and roots have developed on the proliferation, addition of 20–30 g/liter sucrose will have no deleterious effect. Sugars are always present in our medium because of the addition of coconut water which may contain more than 9 mg of fructose, glucose, and sucrose in 1 ml (23). Based on this, our medium may have almost 1.5 g of sugars in a 15% by volume coconut-water formulation. Similar deleterious effect has been obtained with glucose and fructose but not with mannitol, indicating that the increased osmotic concentration of the medium with the addition of sugars was not the responsible factor for this sensitivity.

This finding may also have application in the aseptic germination of orchid seeds, in cases of poor germination although seeds appear normal. Germination may be improved by lowering the sugar content in the medium or, if coconut water is used, additional sugar need not be added to the medium.

**Araceae**

Pierik et al. have asexually propagated *Anthurium andreanum* through tissue culture by first promoting callus formation on explants and then producing plantlets on the callus (19). In our work on this species, instead of promoting callus formation, we want to minimize its formation because of reports on other plants of genetic variants arising through callus culture (1, 4, 15, 17). For clonal increase, node explants of asexepically grown plantlets are placed on agar medium of Murashige and Skoog (MS) basic nutrients (18) supplemented with 0.2 mg/liter of 6-benzylaminopurine (BA). At this BA concentration, callus formation is minimal, but adventitious shoots are produced and rooting is not inhibited. Our method of clonal increase is not as rapid as through the callus route but maintaining of genetic uniformity of cultivars developed through our breeding program is of prime importance in addition to rapid clonal increase. As yet, no genetic aberrants have been detected when plantlets selected at random from each subculture were transferred to natural conditions.

The major difficulty encountered with *A. andreanum* is in the obtaining of sterile cultures of initial explants. The high contamination necessitates the use of minute explants of vegetative buds but this results in greater mortality. Pre-culture treatments such as heat and soaking of plant material for different periods in surface-sterilizing agents have not increased the yield of sterile cultures. Hartman and Zettler in Florida also encountered this problem of high contamination with another aroid genus, *Dieffenbachia*, and indicated that the contaminating agents may reside within the plant (3). This may be the case because when we cultured only internal tissue of the stem contamination was still very high.

Cultures of not all ornamental aroids are difficult to initiate aseptically. Hartman was very successful in culturing *Scindapsus aureus, Philodendron oxycardium, P. lacerum, Alocasia cucullata,* and *Spathiphyllum sp.* through the use of node explants (5). With *S. aureus,* although plantlets can be obtained more rapidly with node explants, leaves from asexepically grown plantlets can also serve as explants. Medium of basic nutrients of Murashige and Skoog (MS) must be supplemented with an auxin and a cytokinin when petioles and portions of the leaf blade are used, and only a cytokinin is required when entire leaves and nodes are cultured.

**Iridaceae**

Clonal multiplication of *Neomarica coerulea* through tissue culture can be accomplished by first promoting adventitious shoot formation on node explants on MS medium supplemented with BA and then promoting root development on plantlets on an auxin-containing medium (5).

**Lilaceae**

Numerous adventitious shoots have been produced on node explants of *Asparagus myriocladus* on MS medium of low BA concentrations. However, attempts to promote rooting on these plantlets have not been successful even though different auxins at various concentrations, alone or in combination with a cytokinin, were used (5).

**Agavaceae**

In Hawaii, plants of *Cordyline terminalis* with very attractive, exotic foliage may have economic importance. This species is easily propagated through cuttings and, therefore, tissue culture may be used in the efficient establishment of propagation stock plots. This has been accomplished, further clonal increase may be more easily and economically done through cuttings.

Because the objective of the initial step is to obtain contamination-free cultures, stem explants are placed on MS agar medium without any growth regulator. Plantlets from the initial culture will then be the source of explants for the next step, clonal increase. Stem sections are placed on a medium of 0.5 ppm BA, which promotes multiple shoot formation with minimal callus formation. In the final step of aseptic culture, the many plantlets produced on an explant are separated and rooted on MS agar medium without growth regulators. After roots have developed, plants can be transferred to natural conditions (12).

The use of tissue culture for rapid clonal increase has developed very rapidly for herbaceous ornamental plants, and for some crops like orchid, it has already given the industry a great boost. As procedures are developed to encompass a wider array of plants, like woody and shrubby species, tissue culture will have an important role in agriculture for the production of uniform, disease-free plants.

**Literature Cited**

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