The Development of a Program of Commercial Production of Staghorns from Plant Tissue Culture

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INTRODUCTION

Staghorns (*Platycerium superbum*) are naturally found in subtropical to tropical areas of Australia, Singapore, and the Philippines. They are a magnificent epiphyte and are sought after to such an extent that they are becoming rare due to the removal of their natural habitat through logging, clearing, and bush harvesting operations. This species has a single growing point and therefore cannot be propagated using off shoots as is the case with the elkhorn (*P. bifurcatum*). Spores are produced annually by mature specimens. Production from spores in a greenhouse requires a time frame of 2 to 4 years and is considered to be an unreliable method of propagation.

No references can be found for in vitro production of staghorns. Previous work with *P. stemaria* (Beauvois) Desu by Hennen and Sheehan (1978) produced 300 explants in a 16-month period using the shoot tip from a mature plant. By comparison there are numerous references to in vitro production of (*P. bifurcatum*) where 150 new shoots from one explant were obtained in 3 months using young excised leaves as the initial explant with no growth regulators in the culture media (Camloh and Gogala, 1991).

A program has been developed in our laboratory where commercial numbers have been produced, deflasked, and re-established which should ease the pressure of bush harvesting on this species.

MATERIALS AND METHODS

Spores were collected in autumn from a mature domesticated plant and were surface sterilised using the centrifuging techniques of Taji (1993). The spores are shaken in a centrifuge tube using sterile water with a drop of Tween 80 detergent and centrifuged at 2800 RPM for 5 min to settle the spores to the bottom of the tube. The supernatant is decanted and replaced with sterile water; this is then shaken and left at room temperature for 24 h. The tube is then re-centrifuged, the supernatant is again discarded, and 10 ml of 1% sodium hypochlorite solution is added and the tube allowed to stand for 5 min. The tube is centrifuged again, the supernatant discarded, and the spores planted aseptically onto a modified de Fossards (1976) medium level, broad-spectrum media (Table 1). The spores are planted without final washing with sterile water.

Spores were cultured in a 200-ml-capacity, glass screw-capped jar with an opaque polypropylene lid containing 25 ml of media, then sealed in a small plastic bag. This helped prevent desiccation and reduce microbial contamination from spores in the air or small insects such as mites in the incubation area.

Cultures were incubated using 36 W fluorescent tubes with an average light intensity of 1000 lux with a 16-h light 8-h dark photoperiod. A constant temperature of 25C±3C was maintained.

Table 1. Modified deFossard's medium (1976).

Macronutrients (mM)	NH_4NO_3	10
	KNO_3	10
	NaH_2PO_4	1
	$CaCl_2$	1.5
	$MgSO_4$	1.5
Micronutrients (µM)	H_3BO_3	75
	$MnSO_4$	50
	$ZnSO_4$	20
	$CuSO_4$	0.05
	Na_2MoO_4	0.5
	$CoCl_2$	0.05
	KI	2.5
	FeEDTA	50
Sucrose (mM)		90
Growth factors (µM)	Inositol	300
	Nicotinic Acid	20
	Pyridoxine HCl	3
	Thiamine HCl	20
Agar		8g per litre
Activated charcoal		150 mg per litre

Within a period of 3 months aseptic spores had germinated producing prothallus which could be subcultured at intervals of 2 to 3 months providing a source of gametophytic tissue. Large numbers of small sporophytic plants were spontaneously produced from these cultures without the need for flooding the surface with water to aid germination.

Shoot multiplication occurs either directly from the prothallus tissue or from adventitious buds formed in large numbers on the lower surface of larger leaves in close proximity to the media surface.

These small explants are subcultured to fresh media and incubated for a further 2 to 3 months where rapid growth occurs; typically these plants are 2 to 3 cm in diameter and are large enough to be deflasked.

Initially large numbers of plants were lost at this stage. The problems of re-establishing plantlets from in vitro conditions to greenhouse conditions are well known. The plantlets are very small and have been shown to have little crystalline wax on their leaves, they are not photosynthesising and usually have a poorly developed root system not adapted for handling the stresses of the outdoor environment.

The following conditions need attention if successful acclimatisation is to be achieved:

Humidity. Plants take 6 to 8 weeks to become fully acclimatised. High relative humidity for the first few weeks is essential and can be provided by either mist or

a fogging system. Fog systems conserve plant moisture and cool the atmosphere with only small volumes of water being used.

Light. Plants are provided with up to 90% shade, especially during the hot summer months. Once roots have developed and leaves are fully functional the plants can be weaned through a series of structures designed to reduce the dependence on reduced light. A 70% shadecloth-covered structure offers excellent light and wind protection.

Temperature. Laboratory incubation rooms maintain a constant temperature of 25C year long. Plants transferred to a greenhouse are subjected to much greater temperature fluctuations ranging from 10 to 50C. Being sub tropical to tropical plants, a temperature range of 25 to 35C would be expected to be ideal.

Diseases and Pests. Sanitation and disease prevention is essential for transplanting success. Plantlets have come from the laboratory in an aseptic state and are therefore vulnerable to disease-causing microbes. Every effort is made to ensure that standard nursery hygiene procedures are adhered to. The two most commonly encountered groups of pests include root- and stem-rot fungi which can be controlled with Mancozeb™, mites which can be controlled with Rogor™, and fungus gnats which can be controlled with Mesurol™ or diazinon.

Bio-systems Environmental Growth Rooms. As an alternative to a conventional greenhouse with inherent problems associated with the management of

Figure 1. Proliferation of sporophytic plants from prothallus tissue ×2 magnification.

temperature, lighting, and humidity, we have deflasked large numbers of staghorns in the Biosystems Growth Rooms. The room is based on a shipping container with ten shelves and a central walkway down the entire length of the room. Each room has a shelf surface area of 50 m^2 and is lit with cool-white florescent tubes. Production is possible 12 months of the year since the controlled environment operates independently of ambient season and weather influences.

Minimal watering is required since the relative humidity is accurately controlled without the need for mist resulting in a lesser opportunity for either fungus or pest development. The computer-controlled cabinet offers precise control of temperature, lighting, humidity, and carbon dioxide enrichment of atmosphere. A 6- to 8-week turnaround of plants is possible throughout the year.

Potting Media. With loses of large batches of plants we realised that a plug was required that was sterile, free-draining, and would allow the staghorn to rotate from a horizontal position to a vertical position. It is thought that if the growing point is in this position it will shed excess water and help prevent rotting problems from occurring.

Using larger plants than initially tried, a high plant-out success rate using Oasis Wedge (Smithers - Oasis Aust. Pty. Ltd.) has been achieved. A small elastic band is used to secure the staghorn to the top of the wedge. Plants are place either under fog or in our environmental growth room (Gaincover-Biosystems) under a Maurix cover until the plants are sufficiently hardened off to be transferred to a 70% shadecloth-covered structure—a period of 6 to 8 weeks is normal.

Once plants have been hardened off they can be placed vertically on the side of a 50 mm-plastic tube with a 10 mm wedge cut from the top of the tube. A coarse free-draining potting mix of perlite and slashed pine is preferred. The next stage of production involves mounting the staghorn. Although none have yet reached full size it is believed that they will be of a marketable stage within a period of 2 years.

CONCLUSION

Platycerium superbum has displayed a high multiplication potential using a single hormone-free agar based media for all stages of production. Initiation from spores has two advantages, the mother plant remains intact, i.e., the single growing point is not destroyed, and surface sterilisation of spores does not present a major microbial contamination problem. Commercial numbers of plants have been produced and marketed through the College Nursery and tissue culture laboratory. This program should reduce the need to bush harvest this species.

LITERATURE CITED

Camloh, M. and **N. Gogala .**1991. *Platycerium bifurcatum*. Adventitious bud and root formation without growth factors*in vitro* . Acta Hort. Plant Biotechnology, 289:89.

de Fossard, R.A. 1976. Tissue culture for plant propagators. Department of Continuing Education. University of New England, NSW, Australia.

Hennen, G.R., and T. J. Sheehan. 1978. In vitro propagation of*Platycerium stemaria* (Beauvois) Desu. Hort Science 13(3):245.

Taji, A.M.,W.A. Dodd, and **R.R. Williams**. 1992. Plant tissue culture practice. University of New England Printery.