Embryo Culture of *Howea* Palms

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Abstract

This paper reports the findings of an experiment on embryo culture of *Howea belmoreana* and *Howea forsteriana*. It was demonstrated that growth regulators significantly affect the development of embryo culture. The medium containing 1 mg/-' of 2,4-D and 0.5 mg/-' of 2iP was found to give the best results for both of the *Howea* palms. Conventional germination of *Howea* palm seeds generally takes a year or more (Reynolds 1982). In our experiments, the embryo culture required only 12-20 weeks to develop fully rooted plantlets. This saving in time could be significant for commercial production.

Introduction

The *Howea* palms are ornamental plants which are arborescent monocotyledons with solitary vegetative shoots. They are relatively slow growing and consequently require much time and effort in production. Reynolds (1982) found that it normally took a year or more to germinate a *Howea* seed in a special germination bed.

Because of its lack of a tendency to form branches, *Howea* palms cannot be multiplied by conventional methods of vegetative propagation. In 1986, the Department of Botany, National University of Singapore, and the Parks and Recreation Department, Ministry of National Development, undertook a joint research project on the tissue culture propagation of tropical trees, palms and shrubs. The Tissue Culture Laboratory of the Botanic Gardens was assigned the task of studying the tissue culture propagation of *Howea* palms.

The study proceeded in two stages. In the first stage, embryo culture was produced from imported seeds of *Howea* palms. In the second stage, the plantlets and callus derived from the embryo culture were used for tissue culture experiments. This paper summarises our findings and observations of the first stage of the project: the development of *Howea* palm embryo culture under the influence of different combinations of growth regulators.

Materials and Methods

(a) *Plant Materials*

One thousand seeds each of *Howea belmoreana* and *Howea forsteriana* were imported from Australia. These seeds were excised for the embryos. Before excision, the seeds were soaked in tap water for 24 hours and then surface-sterilised by soaking for 15 minutes in 1% (w/v) sodium hypochlorite solution. The seeds were then sliced open with an hand cutter and the exposed embryos were carefully removed with sharp-tipped

forceps. The excised embryos were then sterilised in a 5% chlorox solution for a few minutes, rinsed with distilled water, and cultured on various culture media.

(b) *Nutrient Media*

For germination of embryos, the basal medium used was the Murashige and Skoog salts (1962) supplemented with 170 mg/-' NaH_2P02H_20, 100 mg/-^{1} meso-inositol, 0.4 mg/¯' thiamine-HCl, 3% (w/v) sucrose, 40 mg/~' adenine sulphate-$2H_20$, 50 mg/~' activated charcoal, and 0.8% agar. Three types of auxin; 2,4-dichlorophenoxyacetic acid (2,4-D), a-napthaleneacetic acid (NAA), and Indole-3-acetic acid (IAA), and two types of cytokinin; N^6-[A^2 isopentyl] adenine (2iP) and 6-benzylaminopurine (BAP), were used in the following combinations in our experiments:

(i) 1 mg 2,4-D and 0.5 mg 2iP;
(ii) 1 mg 2,4-D and 0.5 mg BAP;
(iii) 1 mg NAA and 0.5 mg 2iP;
(iv) 1 mg NAA and 0.5 mg BAP;
(v) 1 mg IAA and 0.5 mg 2iP;
(vi) 1 mg IAA and 0.5 mg BAP.

Three months after germination, the embryos were transferred onto an auxin/cytokinin-free medium which contained only the basal salts as described above, but at half the concentration.

The pH value of all the media was adjusted to 5.5 to 5.7. The media were dispensed into 25 mm x 150 mm culture tubes (10-20 ml of medium per tube) or 100 ml conical flasks (20-30 ml of medium per flask) and sterilised for 15 minutes at 120°C under 15 kg cm^{-2} pressure.

(c) *Cultural Conditions*

The cultures were kept under 2-3 klx lighting provided by true-lite tube on a 16-hour photoperiod throughout the experiment. The environmental temperature was kept at 26±1°C.

(d) *Experiments Conducted*

One thousand embryos each of *Howea belmoreana* and *Howea forsteriana* were excised. For each of the two palms, 150 embryos were cultured in each of the six media described in Section II (b). The remaining 100 embryos were cultured in the basal medium without growth regulators. These last 100 embryos were used as controls for the other experiments.

After three months, the germinated embryos were transferred to the auxin/cytokinin-free medium. Thereafter, transfers at 4-6 week intervals were necessary to maintain embryo/plantlet growth until they developed their first true leaf.

Results and Discussions

During the first three months of the experiments, the embryos exhibited 10 growth patterns: no growth/contaminated; developed into swollen heads only (Fig. 1); developed into cotylendonary sheaths without roots (Fig. 2); developed into callus (Fig. 3); developed into cotyledonary sheaths with roots (Fig. 4); developed into callus with roots only (Fig. 5); developed into callus with shoots only (Fig. 6); developed into shoots only (Fig. 7); developed into callus with shoots and roots (Fig. 8); and developed directly into shoots and roots (Fig. 9). Some of these growth patterns had also been

Fig. 1 Embryos develop inlo swollen heads only.

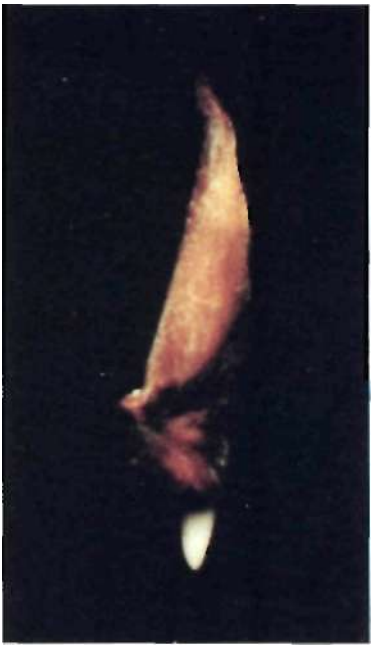

Fig. 2 Embryo develops into cotyledonary sheaths without roots.

Fig. 3 Embryo develops into callus.

Fig. 4 Embryo develops into colyledonary sheaths with root.

Fig. 5 Embryo develops into callus with roots only.

Fig. 6 Embryo develops into callus with shoot only.

Fig. 7 Embryo develops into shoot only.

Fig. 8 Embryo develops into callus with shoot and roots.

Fig. 9. Embryo develops into shoot and roots directly.

observed in date palm culture (Tisserat 1979, Tisserat 1982, Gabr and Tisserat 1985) and oil palm culture (Hodel 1977, Nwankwo and Krikorian 1986).

Table la lists the percentages of *Howea belmoreana* embryos that took on each of the growth patterns when cultured in the various media. Table lb lists the corresponding figures for *Howea forsteriana.* Both of the tables show that growth regulators are important as the percentages of no-growth embryos for the control experiments are very much larger than those for the other experiments. For the various media except the control, the percentages of *Howea belmoreana {forsteriana)* embryos that showed no growth are close to each other, ranging from 46.00-49.33% (13.33-30.00%). There is a slight hint that the Media with 2,4-D/2iP, 2,4-D/BAP, and NAA/BAP favour direct development into shoots and roots. However, at this stage, it is still not clear whether the differences in the growth regulators significantly affect the success of the embryo culture.

After the first three months, all the germinated embryos were transferred on the auxin/cytokinin-free medium. After repeated transfers, some of the embryos developed

Table la
Initial developments of the embryos of *Howea belmoreana* in percentages

Embryos develop into	Control	2,4-D/2iP	2,4-D/BAP	NAA/2iP	NAA/RAP	IAA/2iP	IAA/BAP
swollen heads only	1.00	6.67	4.67	16.67	14.67	6.67	8.67
cotyledonary sheaths without roots	2.00	3.33	2.00	4.67	6.67	8.67	6.67
callus	0.00	3.33	3.33	5.33	5.33	4.67	5.33
cotyledonary sheaths with roots	2.00	7.33	2.00	2.00	1.33	3.33	3.33
callus with roots only	0.00	1.33	2.67	4.00	0.67	2.00	4.00
callus with shoots only	1.00	8.00	4.67	2.67	1.33	1.33	2.00
shoots only	2.00	1.33	10.00	2.00	0.67	9.33	8.00
callus with shoots and roots	0.00	2.00	4.67	1.33	0.67	6.67	6.67
shoots and roots directly	2.00	20.67	20.00	13.33	19.33	10.00	6.67
no growth/contamiinated	90.00	46.00	46.00	48.00	49.33	47.33	48.67

Table Ib

Initial developments of the embryos of *Howea forsteriana* in percentages

Embryos develop into	Control	2,4-D/2iP	2,4-D/BAP	NAA/2iP	NAA/BAP	IAA/2iP	IAA/BAP
swollen heads only	13.00	8.67	8.67	12.00	11.33	5.33	6.00
cotyledonary sheaths without roots	0.00	13.33	13.33	12.67	5.33	20.00	13.33
callus	2.00	8.00	8.00	8.67	9.33	19.33	24.00
cotyledonary sheaths with roots	0.00	2.00	2.67	8.00	6.67	6.67	8.00
callus with roots only	1.00	1.33	2.67	6.00	3.33	2.00	1.33
callus with shoots only	1.00	2.67	2.67	10.00	4.00	8.67	4.00
shoots only	2.00	6.67	8.67	11.33	5.33	6.67	6.67
callus with shoots and roots	1.00	2.00	2.67	3.33	4.67	3.33	3.33
shoots and roots directly	2.00	42.00	33.33	8.00	20.00	6.67	16.67
no growth/contaminated	78.00	13.33	17.33	20.00	30.00	21.33	16.67

into normal plantlets, while others remained as roots, shoots, or callus only, or even browned off and died. There was however a strong dependence of this later development on the initial growth pattern of the embryo as shown in Table 2a, b. In general, only those embryos which initially developed both shoots and roots stood a good chance of developing into normal plantlets, and nearly all that initially developed into swollen heads, cotyledonary sheaths, or callus with shoots or roots only, had browned off and died.

Table 2a
Further developments of *Howea belmoreana* embryos in percentages

Embryos develop into	Browned off and died	Callus only	Roots only	Shoots only	Normal plantlets
swollen heads only	100				
cotyledonary sheaths without roots	85			5	10
callus		60	20	10	10
cotyledonary sheaths with roots	98		2		
callus with roots only	100				
callus with shoots only	98				2
shoots only				70	30
callus with shoots and roots					100
shoots and roots directly					100

Table 2b
Further developments of *Howea forsteriana* embryos in percentages

Embryos develop into	Browned off and died	Callus only	Roots only	Shoots only	Normal plantlets
swollen heads only	100				
cotyledonary sheaths without roots	92			3	5
callus		75	10	10	5
cotyledonary sheaths with roots	95		5		
callus with roots only	100				
callus with shoots only	99				1
shoots only				80	20
callus with shoots and roots	5.				95
shoots and roots directly	*5* V				95

Considering only development into normal plantlets as success, we may work out the success rate figures for each culture medium by summing the products of the figures of the last column of Table 2a(b) and the corresponding figures of the columns of Table la(b). For instance, the success rate figure for *Howea belmoreana* in the medium IAA/BAP is given by

(0 X 8.67 + 0.1 x 6.67 + 0.1 X 5.33 + 0 x 3.33 + 0 X 4.00
+ 0.02 x 2.00 + 0.3 X 8.00 + 1 X 6.67 + 1 x 6.67)% = 16.98%.

The success rate figures for the various media are tabulated in Table 3 and the corresponding bar-chart is shown in Fig. 10. It is evident that the media 2,4-D/2iP and 2,4-D/BAP yielded the best results, especially for *Howea forsteriana*. *Howea belmoreana* appears to be not as sensitive to the media as *Howea forsteriana*. Our results agree with Tisserat (1981) and Gabr and Tisserat (1985) who observed increased germination rate and accelerated growth of date palms in 2,4-D/2iP medium, and also Nwankwo and Krikorian (1986) who made similar observations for oil palms. However, both of these previous studies used very much higher concentrations of 2,4-D (100 mg/-') and 2iP (3 mg/-')- Fig. U shows the various stages of normal growth of a healthy embryo of *Howea forsteriana* over a period of eight weeks.

Table 3
Success rates of the media for the two *Howea* palms in percentages

	Control	2,4-D/2iP	2,4-D/BAP	NAA/2iP	NAA/BAP	1AA/2iP	IAA/BAP
Howea belmoreana	2.82	23.90	28.30	16.31	21.43	20.83	16.98
Howea forsteriana	3.36	44.23	37.03	14.20	25.28	12.89	22.24

Our observations suggest that the size of the embryo could be a factor affecting its growth. Most of the embryos that died or showed no growth at all were very small in size. Those smaller embryos that survived generally took a long time to show any sign of growth, and they often developed into swollen heads, callus, or shoots or roots only. On the other hand, the large embryos in suitable media tended to grow within three months into normal plantlets. It is possible that the size of the embryos is an indication of the age of the embryos. The age of the embryo and the condition of the explant have been shown to have an effect on the growth pattern of date palm and oil palm culture (Jones 1974, Hodel 1977, Zaid and Tisserat 1983). Unfortunately, since the seeds were imported, we did not have information on the age and quality of the embryos used in our experiments. We therefore cannot draw a definite conclusion on this aspect about *Howea* palms.

Conclusions

This study demonstrates that growth regulators are important for embryo culture of *Howea belmoreana* and *Howea forsteriana*. Of the combinations tested, the medium containing 1 mg/-' of 2,4-D and 0.5 mg/$^{-1}$ of 2iP gave the best results for both *Howea* palms.

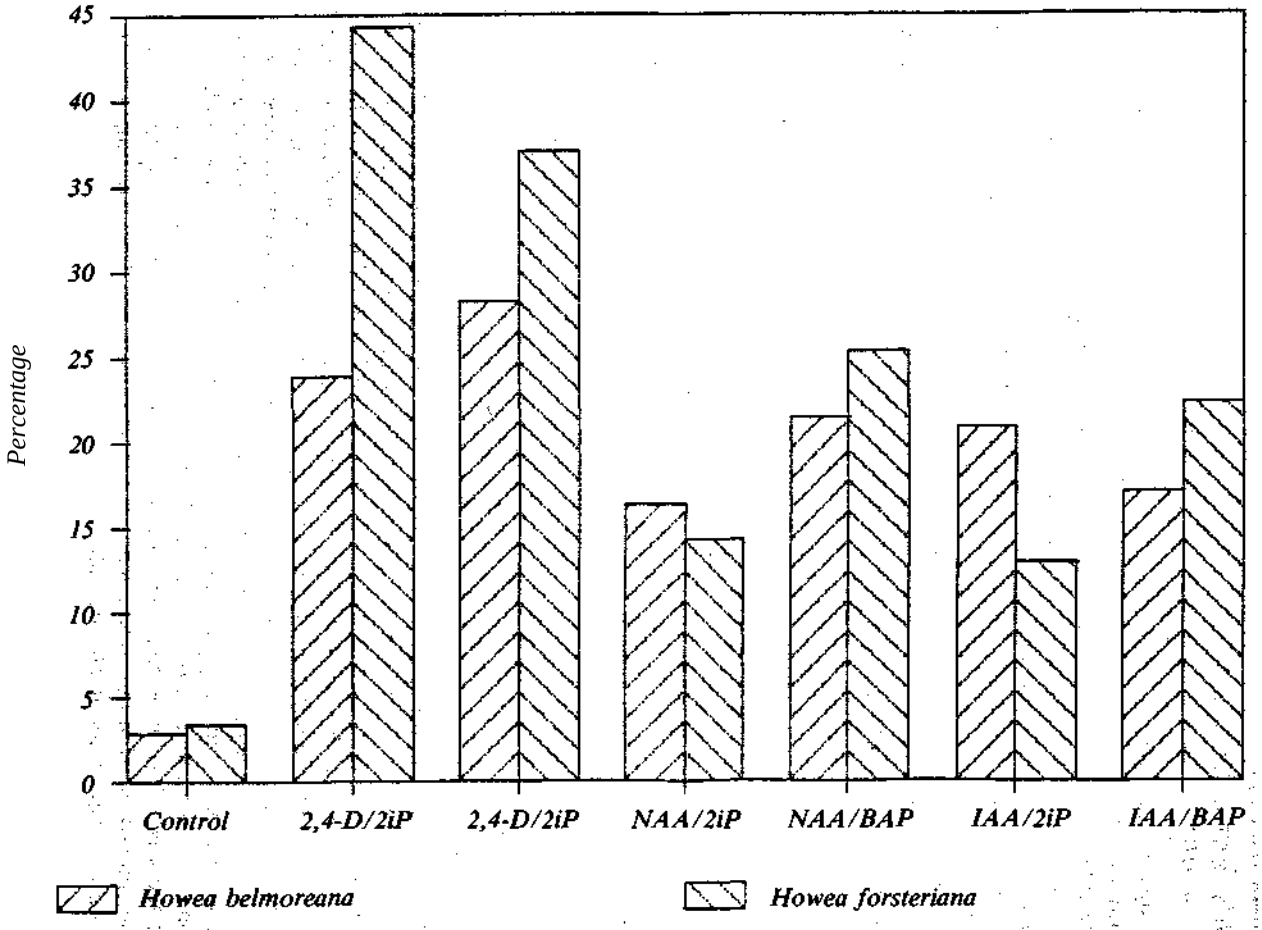

Fig. 10 Success rate of the media for the embryo culture of the two palms.

Fig. II. The development of an excised embryo of *Howea forsteriana* in the presence of growth regulator over a 40-day period.

Germination of *Howea* palm seeds normally takes a year or more (Reynolds 1982). In our experiments, the embryo culture required only 12-20 weeks to develop fully rooted plantlets. This saving in time could be significant for commercial production. Similar results were obtained by Hodel (1977) for oil palm, which took 20-24 weeks to grow from seed to seedling, but only 10-12 weeks to achieve the same stage of development in embryo culture.

The age of the embryos may also affect the growth of the *Howea* embryos. This possibility is worth a further study. A better knowledge of the physiology and the biochemistry of the embryo will also be helpful in understanding the requirements of embryo growth in vitro.

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