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Micropropagation note

In vitro multiplication of *Vriesea fosteriana*

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Abbreviations: BA – 6-benzyladenine, FAA – 50-formalin: glacial acetic: ethanol: water, IBA – indole-3-butyric acid, KC – Knudson C, MS – Murashige & Skoog, 1/2 MS – half strength Murashige & Skoog, NAA – α-naphthaleneacetic acid

Vriesea fosteriana L.B. Smith, a bromeliad with highly ornamental qualities, occurs in a restricted area of south-eastern Brazil. This species has especially attractive and characteristic leaf pigmentation patterns. Harvesting of the plant for sale has been destructive, and widespread and continued use will hasten extinction (Leme 1984).

Bromeliads are frequently propagated by seeds or by division of lateral shoots, although both methods present certain disadvantages (Jones & Murashige 1974). In subfamily Tillandsioideae, which includes the genus *Vriesea*, only one or two offshoots are formed in the post-flowering stage (Mekers 1977). However, application of tissue culture to clonal propagation of *Tillandsia cyanea* was recently reported (Pierik & Sprenkels 1991). The present paper describes conditions for multiplication and growth of *Vriesea fosteriana*.

Mature seeds of *Vriesea fosteriana* were disinfested by washing with 70% ethanol for 3 min, then in 20% commercial bleach (5% of NaOCl) for 40 min, and were rinsed 3 times in sterile distilled water. Two seeds were inoculated into each 25 × 150 mm metal-capped glass tube containing 20 ml of basal medium. Knudson (1946) (KC) and Murashige & Skoog (1962) full strength (MS) and half strength (1/2 MS) were used as basal media, with KC modified by the addition of MS micro-nutrients. Comparative effects of agar-gelled (0.8% W/V Difco Bacto-agar), and stationary liquid media were studied. pH was adjusted to 5.8 prior to autoclaving.

The 30-day-old seedlings were transferred (1 per tube) to gelled KC media supplemented with NAA (0.54, 1.1, 2.2, 3.2, 4.3 or 5.4 μM) alone or NAA (0.54, 2.7 or 5.4 μM) in combination with BA (8.9, 22.5 or 44.4 μM). Subsequent transfers of seedlings to stationary liquid KC medium with (2.7 μM) NAA and (8.9 μM) BA were tried.

For experiments on bud regeneration from leaf explants, entire leaves with a minimum length of 2 cm were removed from aseptically-grown seedlings and re-cultured (1 per tube) on gelled KC medium with added NAA, in combination with BA at the same concentrations as described for seedlings. Subsequent transfer of leaf explants to stationary liquid KC medium supplemented with 2.7 μM NAA and 8.9 μM BA was tried.

The clusters of shoots obtained from seedling and leaf cultures were cultivated for 3 months on gelled KC medium supplemented with 0.54 μM NAA for re-establishment of apical growth. For rooting, shoots ca. 2 cm tall were transferred (10 per flask) to 90 ml wheaton flasks containing 30 ml of gelled KC medium with 1.1 μM NAA. All cultures were maintained at 26 ± 1°C in a

16-h photoperiod with a total irradiance of 10 Wm^{-2} at culture level. Each experiment consisted of 20 replicates.

Rooted shoots (3-4 cm) were transplanted into pots containing a mixture of peat and vermiculite (1:1) and were grown in a greenhouse under natural day length.

For histological observations, seedlings incubated in gelled KC medium containing 2.7 μM NAA and 8.9 μM BA were fixed in FAA-50, dehydrated in an ethanol series and then embedded in paraffin. Embedded tissue was sectioned at 11 μm thickness and stained with safranin and fast-green.

Seeds of *V. fosteriana* germinated 10 days after sowing onto either liquid or gelled basal media (KC, MS and 1/2 MS). Gelled KC medium was the best, since seedling growth was prolonged. In contrast, in liquid medium, seedlings showed a progressive decrease in vigor until death, which took place after nearly a month of culture. However, Murashige (1974) observed that most bromeliads studied in his laboratory could be started only when liquid media were used.

Seedlings showed better growth on gelled KC medium than on MS and 1/2 MS (Table 1). Mathews et al. (1976) found that high phosphate levels favored both growth and differentiation of *Ananas sativus* plantlets cultured *in vitro*. The fact that KC medium is 1.5 and 3 times more concentrated in phosphate than MS and 1/2 MS respectively could be related to the differences in growth rate found in *V. fosteriana*.

After 3 months of culture, promotion of root and shoot growth was evident at the lower NAA levels, particularly 1.1 μM. At this concentration the seedlings showed an increase of 60% in average length and the number of roots increased nearly threefold, by comparison with seedlings incubated on gelled KC medium without NAA. Concentrations higher than 2.2 μM induced toxic effects.

Seedlings of *V. fosteriana* gave rise to greenish protuberances from which buds formed in all

Table 1. The effect of various gelled basal media on *in vitro* growth of *Vriesea fosteriana* seedlings after 6 months of culture.

Medium	Number of leaves	Seedling length (cm)
KC	10.0 ± 1.3	3.6 ± 0.3
MS	8.1 ± 1.0	2.4 ± 0.3
1/2 MS	6.2 ± 1.0	1.5 ± 0.3

Data given a means ±SD.
N = 20.

Table 2. Effect of combinations of BA and NAA on the frequency of regeneration and the number of buds formed per explant of leaf (*) and seedling (+) cultured on gelled KC medium, after 1 and 3 months, respectively.

NAA (μM)	BA (μM)		
	8.9	22.2	44.4
	Regeneration (%)		
0.54	50*– 85^{+}	30*– 85^{+}	10*– 50^{+}
2.7	50 –100	40 – 90	27 – 70
5.4	10 – 70	10 – 75	10 – 55
	Buds per leaf		
0.54	6.6 ± 2.3	10.9 ± 2.1	1.3 ± 0.6
2.7	15.2 ± 2.4	2.1 ± 0.9	2.8 ± 1.4
5.4	3.5 ± 1.4	4.9 ± 1.2	4.8 ± 1.3
	Buds per seedling		
0.54	9.9 ± 1.3	5.6 ± 1.6	1.6 ± 1.0
2.7	22.5 ± 2.0	18.2 ± 1.2	1.4 ± 1.0
5.4	21.4 ± 1.9	4.1 ± 1.3	1.3 ± 0.6

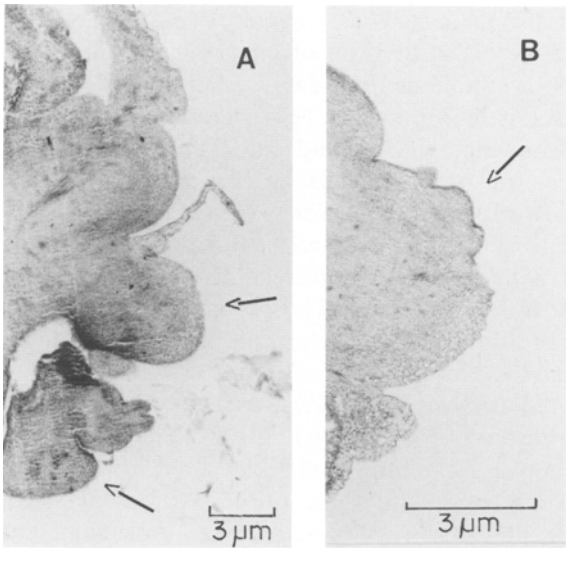

Fig. 1. Longitudinal sections of seedlings of *V. fosteriana* showing (*A*) new protuberances being formed (arrows), and (*B*) budlike primordia arising from a protuberance (arrow), after 3 months of culture on gelled KC medium supplemented with 2.7 μM NAA and 8.9 μM BA.

tested treatments (Table 2). These protuberances formed at a position corresponding to the second internode; after 3 months of culture, buds were formed (Fig. 1). The combination of 2.7 μM NAA and 8.9 μM BA induced the highest percentage of explants regenerating buds and number of buds per explant (Table 2). After subsequent transfer, the number of buds doubled when the same medium was used in liquid state and a further increase of nearly 20% in number of buds was obtained after one further transfer of the cultures to a liquid medium of equal composition. Although adventitious buds formed readly on *V. fosteriana* seedlings, no lateral bud development was noted.

Regeneration was also observed in leaf explants incubated with auxin and cytokinin. After 20 days of culture, protuberances were formed at the basal end of leaves and shoot-buds appeared after 1 month of culture in all treatments. The frequency of regeneration in leaf explants and number of shoots formed per explant were lower than in seedling explants (Table 2). The optimal combination for bud proliferation was 2.7 μM NAA and 8.9 μM BA. The number of leaf-derived buds can be duplicated by means of a subculture into liquid medium of the same composition. Young adventitious shoots can be regularly cut up thus providing explants for further large number of adventitious shoot production. Hosoki & Asahira (1980) suggested that intercalary meristems distributed in the leaf base of some bromeliad species may have a high potential for bud formation.

Addition of 0.54 μM NAA was necessary to stop adventitious proliferation as well as to re-establish apical growth of the shoots. When shoots attained ca. 2 cm in height (3 months of culture), rooting was easily induced, and the shoots were transferred to a medium supplemented with 1.1 μM NAA. After 2 months of culture every shoot formed 3-4 roots (ca. 2 cm length).

After a year of culture in pots in a greenhouse, all regenerated plants survived and looked phenotypically normal. No variation in plant morphology as a whole, nor in the characteristic pigmentation pattern of the leaves, was observed. In contrast, micropropagated plants of *Aechmea fasciata*, another variegated bromeliad species, included a high percentage of variants (Jones & Murashige 1974).

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