In Vitro Rooting and Greenhouse Acclimatization of *Veltheimia bracteata* and *V. capensis* Shoots

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Additional index words. bush lily, forest lily, sand lily, micropropagation, tissue culture

Abstract. Shoot initiation and multiplication were obtained in vitro from immature flower bud and leaf explants of *Veltheimia bracteata* Bak. 'Lemon Flame' and from leaf explants of *V. bracteata* 'Rosalba' cultured on a Murashige and Skoog (MS) medium supplemented with sucrose at 30 g·L⁻¹, and either 8.87 μM BA plus 0.54 μM NAA or 8.87 μM BA plus 5.40 μM NAA. Shoot initiation and multiplication was obtained from a single leaf explant of *Veltheimia capensis* (L.) DC. on MS medium with 8.87 μM BA plus 0.54 μM NAA. Shoots of the three genotypes rooted on subculture to medium with 0.0, 4.14, or 8.29 μM K-IBA or 0.0, 4.46, or 8.92 μM K-NAA. Maximal rooting was 98% for *V. bracteata* 'Lemon Flame', 95% for *V. bracteata* 'Rosalba', and 98% for *V. capensis*, from medium with 4.46 μM K-NAA. Rooted shoots were acclimatized for 3 to 4 weeks. Overall survival percentage was 69% for *V. bracteata* 'Lemon Flame', 65% for *V. bracteata* 'Rosalba', and 83% for *V. capensis*. Chemical names used: 6-benzyladenine (BA); potassium salt of indole-3-butyric acid (K-IBA); potassium salt of 1-naphthaleneacetic acid (K-NAA); 1-naphthaleneacetic acid (NAA).

Veltheimia bracteata, the bush or forest lily, and *V. capensis*, the sand lily, are ornamental species of the Hyacinthaceae native to southern Africa (Du Plessis and Duncan, 1989). These bulbous plants are winter-growing, summer-dormant, and are not frost-tolerant. They are ideal greenhouse and home-window plants. They are 1) easy to cultivate, 2) pest-free, and 3) remain in flower for up to 6 weeks in winter and early spring. The attractive leaves have undulating margins and the racemes bear numerous cylindrical, pendulous flowers in shades of pink or chartreuse (Du Plessis and Duncan, 1989).

Veltheimias are propagated from seed or offsets; however, plants from seed take 3 or more years to flower (Du Plessis and Duncan, 1989), and offsets are slow to form (Doutt, 1994). *Veltheimia bracteata* is extremely variable in flower color (Du Plessis and Duncan, 1989). The superior forms should be vegetatively propagated for extensive horticultural use, but due to the propagation difficulties, selected clones are not readily available commercially. Tissue culture propagation could be a method of clonally increasing selected forms of these species. The objective of this study was to develop a protocol for shoot initiation, multiplication and rooting, and greenhouse acclimatization of tissue-culture propagated plants of *V. bracteata* 'Lemon Flame', *V. bracteata* 'Rosalba', and a compact form of *V. capensis* (received as *V. capensis*

var. *deasii*). To my knowledge, no other published report exists on the tissue-culture propagation of these plants. However, other geophyte species of the Hyacinthaceae have been tissue-culture propagated, including *Eucomis* (Ault, 1995), *Galtonia* (Drewes and van Staden, 1993), *Hyacinthus* (Hussey, 1975), and *Lachenalia* (Ault, 1995; Nel, 1983).

Materials and Methods

Plants of *V. bracteata* 'Lemon Flame' were obtained from a commercial nursery and from Kirstenbosch Botanical Garden in South Africa in 1986. Plants of *V. bracteata* 'Rosalba' were obtained from a commercial nursery in New Zealand in 1991. Plants of *V. capensis* were donated to Longwood Gardens in 1987. Five or fewer plants of each genotype were available for propagation at the initiation of this study. To avoid sacrificing plants, bulb tissue was not used for explants; instead, leaf tissue of all three genotypes and immature flower buds of *V. bracteata* 'Lemon Flame' were used as explants. Similar techniques were used for *Lachenalia*, a related genus from South Africa that has been tissue-culture propagated from leaf tissue (Ault, 1995; Klessner and Nel, 1976; Nel, 1983).

Leaves were excised between Nov. 1992 and Mar. 1993 from the bulbs of greenhouse-grown plants. Leaves of *Veltheimia* are borne on the above-ground portion of a partially subterranean-growing bulb. The individual plants were vegetative to ≈50% into floral anthesis at the time of leaf removal. One leaf per plant was removed. The proximal one-third of each leaf (≈10 to 15 cm × 15 to 20 cm for *V. bracteata* and 2 to 4 cm × 3 to 5 cm for *V. capensis*) was rinsed in running tap water for 5 min, surface-disinfected in a continu-

ously stirred solution of 1.0% sodium hypochlorite and 0.1% Tween 20 for 12 min, and rinsed once in sterile distilled water for 5 to 10 min. Individual leaf explants, each 5 × 10 mm, were placed horizontally, abaxial side down, in 25 × 150-mm culture tubes containing 10 mL of medium. Medium consisted of MS (Murashige and Skoog, 1962) basal salts and vitamins, sucrose at 30 g·L⁻¹, and either no plant growth regulators (PGRs) or the combinations of 4.44 μM BA or 8.87 μM BA with 0.54 μM NAA or 5.4 μM NAA. Medium pH was adjusted to 5.7 before adding agar at 7 g·L⁻¹ (Sigma A 1296, Sigma Chemical Co., St. Louis). Culture tubes were sealed with polypropylene caps and autoclaved at 121 °C for 15 min.

After explant placement, culture tubes were sealed with parafilm and placed upright in 40-tube racks in an incubator (model 818; Precision Scientific, Chicago). Cultures were maintained at 22 °C and given a 12-h photoperiod with a photosynthetic photon flux (PPF) of 30 to 44 μmol·m⁻²·s⁻¹ provided by two 40-W broad-spectrum fluorescent lamps (Verilux, Greenwich, Conn.). PPF was measured with a quantum sensor (LI-190SA; LI-COR, Lincoln, Neb.).

Precanthesis flower buds were removed from four plants of *V. bracteata* 'Lemon Flame'. Treatment and culture of flower buds was identical to that for leaf explants, except no running-water rinse was used, and explant orientation (abaxial or adaxial) was not monitored. Explants consisted of intact flower buds 7 to 15 mm long.

After 10 to 12 weeks of culture, explants exhibiting a morphogenetic response (roots, preshoots, or callus) were placed either intact or split in half before placement on an identical fresh medium. There were 24 to 40 explants per treatment after placement. After another 10 to 13 weeks of culture, explants with normal-appearing shoots were combined from all treatments for each genotype and explant source. They were placed on fresh medium with 8.87 μM BA plus 5.4 μM NAA for the *V. bracteata* cultivars and on fresh medium with 8.87 μM BA plus 0.54 μM NAA for *V. capensis*. Subsequently, individual shoots or clusters of small shoots were excised and placed on fresh medium at 8- to 15-week intervals for continued shoot multiplication. After the fourth culture sequence, shoots from the *V. bracteata* cultivars also were placed on medium with 8.87 μM BA plus 0.54 μM NAA. Shoots derived from the two explant sources for *V. bracteata* 'Lemon Flame' (leaves and flower buds) appeared identical and were combined for further shoot multiplication.

The shoot initiation and multiplication study was conducted once due to the limited availability of explant material. For shoot initiation, 40 explants, either leaf tissue or flower buds, were placed on each of the five treatments for each genotype. Percentage of morphogenetic response for the first culture period and the percentage of shoot initiation response for the second, third, and fourth culture periods were collected. The number of shoots per explant was not determined as it

Received for publication 16 Jan. 1996. Accepted for publication 30 Aug. 1996. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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was not possible to accurately count the number of shoots in each culture tube without sacrificing the material. The medium selected for subsequent shoot multiplication was based on percent shoot initiation and on visual observation of the initiation medium that yielded the most normal-appearing shoots for each genotype.

In 1993, nonrooted shoots 2 to 10 cm tall of *V. bracteata* 'Rosalba' were individually cultured on MS medium supplemented with sucrose at 30 g·L⁻¹ and 0.0 or 4.46 µM K-NAA. In 1994, nonrooted shoots 2 to 10 cm tall of *V. bracteata* 'Lemon Flame' and nonrooted shoots 2 to 5 cm tall of *V. capensis* were individually cultured on the same medium supplemented with 0.0, 4.14, or 8.29 µM K-IBA, or 0.0, 4.46, or 8.92 µM K-NAA. Rooting percentage was recorded after 11 to 12 weeks. Shoots with at least one root ≥1.0 cm were recorded as rooted. Rooted shoots of *V. bracteata* 'Rosalba' were removed from tissue culture in either Nov. 1993 or Jan. 1994, while rooted shoots of *V. bracteata* 'Lemon Flame' and *V. capensis* were removed from tissue culture in Oct. 1994. Rooted shoots were rinsed free of medium, and transferred to the greenhouse for acclimatization. Shoots were planted in 36-cell plug trays, each cell containing ≈160 cm³ medium of nonsterilized 3 sand : 1 peat (v/v). Trays were covered with clear plastic domes, then placed under 50% shade cloth in the greenhouse. The plastic domes were propped up ≈3 cm on one side after 2 to 3 weeks and completely removed after 3 to 4 weeks. Shoot survival was recorded 10 to 12 weeks after the shoots were removed from tissue culture.

The rooting study was conducted once. For *V. bracteata* 'Rosalba' 180 nonrooted shoots were placed on to the medium without K-NAA and 100 shoots on to the medium with K-NAA. For *V. bracteata* 'Lemon Flame' and *V. capensis*, 40 to 60 nonrooted shoots were used for each treatment. Rooting percentages were analyzed with the G statistic comparing the individual auxin treatments to the control. Differences in survival percentages between treatments were analyzed with Chi-square.

Results and Discussion

The procedure for surface-disinfection appeared optimum for the *V. bracteata* cultivars. Most of the flower bud and leaf explants (96%; 574 of 600) were noncontaminated and appeared to be living after 8 weeks of culture. Conversely, only 77% (154 of 200) of the *V. capensis* explants were noncontaminated and appeared to be living after 8 weeks of culture. The lower explant survival percentage for *V. capensis* was due to explant bleaching and death from the sodium hypochlorite treatment and was not due to contamination. Therefore, it appears necessary either to decrease the concentration of the sodium hypochlorite solution or to shorten the time in the disinfecting solution for optimum *V. capensis* explant survival.

Morphogenetic response of the explants after 10 to 12 weeks of culture ranged from 0% to 67%, depending on the genotype, explant

Table 1. Auxin effects on the rooting of *Veltheimia bracteata* 'Lemon Flame', *V. bracteata* 'Rosalba', and *V. capensis* recorded after 11 to 12 weeks of culture on rooting medium, and their subsequent shoot survival in the greenhouse.

Auxin (µM)		Species tested					
		<i>V. bracteata</i>				<i>V. capensis</i>	
		Lemon Flame		Rosalba			
K-IBA	K-NAA	Rooting ^a (%)	Survival ^b (%)	Rooting (%)	Survival (%)	Rooting (%)	Survival (%)
0.0	0.0	66	83	51	66	65	64
4.14	0.0	95**	63	---	---	84*	100
8.29	0.0	96**	63	---	---	93**	85
0.0	4.46	98**	77	95**	65	98**	79
0.0	8.92	86*	65	---	---	96**	83

^aSignificance of rooting percent within each species is based on the comparison of individual auxin treatments with the control (no hormone) using the G statistic.

^bSurvival percent within each species was not significantly different at $P=0.05$ according to Chi-square test. Number of surviving shoots per treatment ranged from 26 to 60.

*, **Significant at $P \leq 0.05$ or 0.01, respectively.

type, and PGR treatment; no clearly discernible shoots were evident on any of the explants. None of the explants on medium without PGRs exhibited a morphogenetic response and thus were discarded.

Shoot initiation became evident during the second culture period. Percentage of response ranged from 0% to 79%, depending on the genotype, explant type, and growth regulator treatment. Maximum percentage of explants with shoot formation was 79% for *V. bracteata* 'Lemon Flame' flower bud explants on medium with 4.44 µM BA plus 0.54 µM NAA, 39% for *V. bracteata* 'Lemon Flame' leaf explants on medium with 8.87 µM BA plus 5.4 µM NAA, and 54% for *V. bracteata* 'Rosalba' leaf explants on medium with 8.87 µM BA plus 5.4 µM NAA.

Many of the shoots for the *V. bracteata* explants exhibited deformed leaf morphology, vitrification, or callus production. The best overall medium for normal-appearing shoots was with 8.87 µM BA plus 5.4 µM NAA; hence, the selection of this medium for subsequent culture sequences. By selecting only normal appearing shoots for subculture, 87% or more of the subcultured shoots exhibited normal morphology by the end of the fourth culture sequence. Upon subculturing shoots to medium with 8.87 µM BA plus 0.54 µM NAA after the fourth culture sequence, there was a marked decrease in callus production with no apparent diminution in shoot quality or proliferation.

For *V. capensis*, only one explant (out of 200) initiated shoots. This was during the second culture sequence on medium with 8.87 µM BA plus 0.54 µM NAA; therefore, this medium was chosen for subsequent shoot multiplication. All shoots of *V. capensis* used in the rooting study were derived from this single initial explant. There was minimal or no callus production, and shoot morphologies were normal-appearing. *Veltheimia capensis* shoot multiplication and growth was markedly slower than for the *V. bracteata* cultivars, which is in agreement with the comparative growth rates of the two species from seed or divisions under greenhouse growing conditions.

Shoots of the three genotypes rooted on all rooting media. The four auxin treatments significantly increased rooting percentages in

comparison to the control (no PGR) for the three genotypes (Table 1). Maximum percent rooting exceeded 95% for all three genotypes on medium with 4.46 µM K-NAA. These rooting percentages are superior to those reported for tissue culture rooted shoots of the related genus *Lachenalia* (Ault, 1995; Klessner and Nel, 1976), and similar to those reported for the related genus *Eucomis* (Ault, 1985).

Survival of rooted shoots after removal from tissue culture did not differ significantly between the prior rooting treatments (Table 1). Percent survival was greater than 63% for all treatments. These survival percentages are overall lower than those reported for greenhouse acclimatization of tissue culture rooted *Lachenalia* shoots (Ault, 1995) and *Eucomis* shoots (Ault, 1995). However, these survival rates are similar to the 82% reported for *Galtonia candicans* (Drewes and van Staden, 1993), indicating the need for additional research to optimize shoot survival for *Veltheimia* tissue culture shoots.

By employing the appropriate combination of BA and NAA, it is possible to promote shoot initiation and multiplication, and shoot rooting in tissue culture from nonbulb tissue of *V. bracteata* and *V. capensis*. The use of explants from other than bulb tissue avoids the destruction of stock plants. An effective method for propagating selected *Veltheimia* genotypes should help enhance their horticultural appeal and commercial availability.

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