Establishment of *Aloe*, *Gasteria*, and *Haworthia* Shoot Cultures from Inflorescence Explants

Arthur M. Richwine¹

The Holden Arboretum, 9500 Sperry Road, Mentor, OH 44060

Jimmy L. Tipton² and Gary A. Thompson³

Department of Plant Sciences, 303 Forbes Building, University of Arizona, Tucson, AZ 85721

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Abstract. Shoot cultures of *Aloe*, *Gasteria*, and *Haworthia* species were initiated directly from immature inflorescences. Explants placed on a modified MS medium containing 5.4 μM zeatin riboside initiated shoots within 8 to 12 weeks. Long-term shoot cultures were established and maintained on media containing either 5.4 μM zeatin riboside or 4 μM BA. Shoots easily rooted in vitro, and rooted plantlets were established in soil. Chemical names used: *N*-(phenylmethyl)-1*H*-purin-6-amine (BA); 6-[4-hydroxy-3-methyl-but-2-enylamino]purine riboside (zeatin riboside).

Some species from the genera *Aloe*, *Gasteria*, and *Haworthia* are grown commercially as ornamentals, while rare species are of interest to collectors (Bayer, 1982). These species can be propagated from seed, leaf cuttings, crown divisions, or proliferations produced on the inflorescence (Pilbeam, 1983). We tested the ability of several members of the Asphodelaceae [*Aloe barbadensis* Mill., *Aloe harlana* Reynolds, *Gasteria liliputiana* Poelln., *Gasteria* species, *Haworthia attenuata* Haw., *Haworthia coarctata* (Salm-Dyck) Haw., and *Haworthia limifolia* Marloth.] to initiate shoots in vitro from racemose inflorescence segments.

Materials and Methods

The general protocol and medium formulation were based on previous shoot culture initiation studies with red yucca [*Hesperaloe parviflora* (Torr.) J. Coult.] inflorescences (Richwine, 1995). Immature inflorescences from greenhouse-grown *Gasteria* and *Haworthia* and landscape *Aloe* plants were collected when they were ≈ 7 cm long (*Gasteria* and *Haworthia*) and ≈ 15 cm (*Aloe*) but before flower buds had begun to expand. Tissue was rinsed with tap water, and peduncles were cut

transversely into 1-cm sections. Only segments containing a minimum of one bract were used for this experiment. Segments were divided into two categories, region I and region II. Region I consisted of axils from the distal portion of the inflorescence; these axils typically produced flowers in vivo. Region II consisted of those axils from the proximal portion of the inflorescence that typically do not produce flowers. Explants were surface-sterilized in a solution of 10% commercial

bleach and 0.1% Tween 20 for 10 min and rinsed three times with sterile water. Explants were placed on a modified Murashige and Skoog medium [MS salts (Murashige and Skoog, 1962), Nitsch and Nitsch vitamins (Nitsch and Nitsch, 1967), pH 5.7, plus (g·liter⁻¹) 30 sucrose and 6 Phytagar] containing 5.4 μM zeatin riboside. Filter-sterilized zeatin riboside was added after the medium was autoclaved at 121°C at 250 kPa for 20 min. Explants were placed with the basal end in the medium and grown at 25°C under 24 h of illumination from cool-white fluorescent lamps (40 to 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Plant material was transferred to fresh medium every 6 weeks.

Results and Discussion

Within 8 weeks both species of *Aloe*, all three species of *Haworthia*, and the *Gasteria liliputiana* had initiated shoots from axils of bracts (Fig. 1B). By 12 weeks, shoots had initiated from axils of the unidentified *Gasteria* species (Fig. 1A). Shoots originated from bract axils in region II in *G. liliputiana* and all three species of *Haworthia*. Shoot initiation occurred in regions I and II in *A. barbadensis* hybrid but only occurred in region I of the unidentified *Gasteria* species. Shoots initiated in region II were not accompanied by floral development, but shoots initiated in region I originated from axils that also produced flowers. Floral development occurred simultaneously with shoot initiation. Richwine (1995) reported a similar response during shoot initiation from pedicel buds of red yucca. Several explants of *A. barbadensis* hybrid and *H. limifolia* also showed hypertrophy of the explant base, and sev-

Fig. 1. (A) Shoots initiated on region I inflorescence segment of *Gasteria* species. (B) Shoots initiated on region II inflorescence segment of *Gasteria liliputiana*. (C) Established shoot culture of *Aloe barbadensis* hybrid. (D) Established shoot culture of *Haworthia attenuata*.

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¹Corning Fellow for Horticultural Research.

²Associate Extension Specialist.

³Assistant Professor.

eral shoots initiated from the swollen areas.

Axillary shoots were maintained on initiation medium or transferred to a similar medium containing 4 μ M BA (Fig. 1 C and D). When subcultured shoots of the *Haworthia* species were longitudinally bisected, shoot proliferation occurred in most leaf axils, regardless of contact with the medium, suggesting that apical dominance had been reduced. *Haworthia* shoot cultures were maintained on these media for 9 months without apparent callus formation or a reduction in shoot proliferation. Shoots of *Aloe* and *Gasteria* species were maintained for 8 months. The average number of axillary shoots initiated from subcultured explants ranged from five to 10 for *H. attenuata*, *H. coarctata*, and *A. barbadensis*; three to five for *H. limifolia*, *A. harlana*, and

the two for *Gasteria* species. Shoots were easily rooted within 1 month of transfer to MS medium without plant growth regulators, and they subsequently were established in soil.

We have described a method for the direct initiation of shoot cultures from axils of bracts from *Aloe*, *Gasteria*, and *Haworthia* species. Our results suggest that immature inflorescences are a useful source of explants in several monocotyledonous genera, even if proliferations do not occur in vivo. This approach is particularly beneficial in those species with reduced or subterranean meristems, which are difficult to isolate without contamination. Cultures initiated directly from immature inflorescences likely have a lower frequency of somaclonal variation than shoots derived from callus. In addition, subsequent proliferation of

shoots from axillary buds contributes to the genetic stability of propagules.

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