In Vitro Cell. Dev. Biol.—Plant 32:262–266, October–December 1996

1054-5476/96 $05.00 + 0.00

MICROPROPAGATION OF *HESPERALOE PARVIFLORA*

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(Received 17 January 1996; accepted 2 July 1996; editor M. A. L. Smith)

SUMMARY

We successfully micropropagated *Hesperaloe parviflora* from mature plants. Shoot cultures were directly initiated from mature plants using pedicel bud explants on a modified Murashige and Skoog medium containing Nitsch and Nitsch vitamins and 1 μ*M* zeatin riboside. Axillary shoot multiplication from established cultures was most responsive to changing concentrations of N^6-benzyladenine (BA) with the greatest production on 6 μ*M* BA. Growing shoots on a medium supplemented with 6 μ*M* BA for 6 wk and then transferring cultures to a 1 μ*M* BA medium for 6 more wk increased the number of transferable shoots, but not significantly. However, our data predicts that the maximum number of transferable shoots produced from a single microshoot would occur on media with 5.4 μ*M* zeatin riboside. Shoots rooted easily *in vitro* or *ex vitro* and rooted shoots were easily acclimatized. The methods described in this paper are being used to commercially micropropagate *H. parviflora*.

Key words: shoot culture; floral explants; Agavaceae; red yucca; zeatin riboside.

INTRODUCTION

Hesperaloe parviflora (Agavaceae) is a low-maintenance landscape ornamental in the arid Southwestern United States. Mature plants form acaulescent yuccalike clumps up to 1 m wide from evergreen semisucculent leaves. Bell-shaped flowers, typically red to pink or orange, are produced on 2-m-tall panicles primarily in the spring. Yellow-flowered mutants lacking anthocyanin are rare. These mutants are considered desirable by the nursery industry, but are difficult to propagate.

H. parviflora is commercially propagated from open-pollinated seed or by rhizome divisions. Yellow-flowered plants are largely self-incompatible and outcrossing results in wild-type progeny. Rhizome division produces only one to three new plants per year, not economically viable for commercial production. Micropropagation offers the potential to produce a large amount of clonal material in less time than traditional methods and could provide a means for enhancing availability of yellow-flowered *H. parviflora*.

Species in several genera of the Agavaceae, including *Hesperaloe*, have been successfully micropropagated. *Hesperaloe funifera*, a potential source of paper fiber (McLaughlin and Schuck, 1992), was regenerated from callus derived from seedling tissue (B. McHaud, Department of Plant Sciences, University of Arizona, personal communication). A variety of explants, including rhizome internode tissue (Robert et al., 1987; Bihn et al., 1990; Das, 1992) and leaves from proliferations produced on the inflorescence (Powers and Backhaus, 1989), have been used to initiate *Agave* shoot cultures. *Yucca* species have been micropropagated from shoot tips (Pierik and Steegmans, 1983; Bentz and Parliman, 1985), axillary buds (Litz and Conover, 1977), flower buds (Murashige, 1974; Durmishidze et al., 1983; Bentz et al., 1988), and rhizome tissue (Bentz et al., 1988). The purpose of this study was to develop a commercially viable protocol for micropropagation of yellow-flowered *H. parviflora*.

MATERIALS AND METHODS

Two populations of plants were used as sources of explant material for initiation experiments. The first population was composed of heterogeneous wild-type plants growing in the landscape at the University of Arizona campus. The second consisted of yellow-flowered mutants asexually reproduced from a single seedling.

Initiation and multiplication experiments were conducted with a basal medium consisting of MS salts (Murashige and Skoog, 1962), Nitsch and Nitsch vitamins (Nitsch and Nitsch, 1967), 30.0 g l^{-1} sucrose, pH 5.6, and solidified with 6.0 g l^{-1} Phytagar. Heat stable cytokinins [N^6-benzyladenine (BA) and kinetin] were added to the medium prior to autoclaving for 15 min at 121° C and KPa 103.4. Zeatin riboside was filter-sterilized through 0.2 μm syringe filters and added after autoclaved medium had cooled to 50° C. Thirty milliliters of medium were dispensed into large baby food jars (95 mm tall × 60 mm diameter) and cultures grown under 40 watt cool white fluorescent lights with 24-h illumination (50–60 μmol $m^{-2}s^{-1}$) 25 ± 4° C.

Immature inflorescences 12–84 cm in length were collected from several wild-type landscape plants in March 1993 prior to pedicel expansion and rinsed with water. Pedicel bud explants were prepared by first removing the subtending bract and then excising the bud leaving a small portion of peduncle tissue attached. Explants were surface sterilized in a solution of 10% commercial bleach (vol/vol) and 0.1% Tween 20 for 10 min, then rinsed three times with sterile water.

Basal media was supplemented with 0, 0.1, 1.0, or 10.0 μ*M* BA, kinetin, or zeatin riboside to evaluate the effect of cytokinin type and concentration on shoot initiation. The bottom four pedicel buds from wild-type plants were placed basal end down in the media. There were two explants per container and five containers per treatment. Cultures were transferred onto fresh media every 2 wk and initiated shoots counted. The experiment was terminated after 6 wk.

The effect of bud position along the length of the inflorescence on shoot initiation was also studied. Pedicel buds were numbered one through seven and above with bud number one at the base of the inflorescence. Explant

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FIG. 1. Morphological responses *in vitro* from pedicel bud explants of *Hesperaloe parviflora*. Responses ranged from direct shoot initiation to continued flowering and combinations thereof.

material from clonal yellow-flowered plants was prepared as described and the buds placed basal end down on medium supplemented with 6 μ*M* zeatin riboside. Again, there were two explants per container and five containers per bud position. Explants were transferred every 2 wk to fresh media and the shoots counted. The experiment was terminated after 6 wk.

Stock cultures were maintained on basal medium with 6 μ*M* zeatin riboside and subcultured every 6 to 8 wk. A clonal wild-type shoot culture initiated from peduncle buds in a pilot study was used for all multiplication and rooting experiments. Shoots with a basal diameter of 3–5 mm were placed on basal medium containing 0, 2, 4, 6, 8, or 10 μ*M* BA, kinetin, or zeatin riboside to determine the effect of cytokinin type and concentration on shoot proliferation. One microshoot per container was placed on the media and allowed to grow for 8 wk. Single shoots were then subcultured onto fresh media in 10 containers and grown for another 8 wk. The total number of shoots and the number of shoots suitable for subculture were counted.

The effect of high followed by low concentrations of BA on shoot proliferation was tested. Single shoots with basal diameters of 3–5 mm were placed on media containing 6 μ*M* BA for 6 wk. Cultures were then transferred to media containing either 6 or 1 μ*M* BA for another 6 wk. The total number of shoots and the number of shoots suitable for subculture were counted at the time of transfer and again at the end of the experiment. There were 14 containers per treatment, each initiated with a single shoot.

The effect of three soilless rooting media (perlite, vermiculite, and 1:1 perlite:vermiculite) on *ex vitro* rooting was studied. The rooting media was thoroughly moistened and placed in clear plastic boxes (24 × 23 × 9 cm). Ten shoots with basal diameters of 3–6 mm were harvested from multiplication experiments and inserted into each media. Rooting boxes were placed in a growth chamber for 6 wk. After 3 wk, the shoots were fertilized with 4 g l^{-1} of a commercial water-soluble fertilizer (20-20-20). The number of rooted shoots per treatment, the number of roots per shoot, and the type of roots per shoot (tuberous or fibrous) were recorded. Root systems were ranked on a scale of one to five as follows: 1 = one root; 2 = two tuberous roots; 3 = one tuberous and one fibrous root; 4 = two fibrous roots; and 5 = three or more roots regardless of type. There were two replicates (rooting boxes) per treatment and the experiment was performed twice.

TABLE 1

N⁶-BENZYLADENINE (BA), KINETIN (KIN), AND ZEATIN RIBOSIDE (ZR) EFFECTS ON SHOOT INITIATION FROM PEDICEL BUDS OF WILD-TYPE *HESPERALOE PARVIFLORA*

Cytokinin[1]	Concentration (μM)	n	Mean number of Shoots per Explant[2]
Control	0	17	0.1 a
BA	0.1	18	0.9 c
BA	1	17	1.1 cd
BA	10	15	1.3 d
KIN	0.1	19	0.1 ab
KIN	1	17	0.1 a
KIN	10	18	1.2 cd
ZR	0.1	18	0.4 b
ZR	1	17	1.7 e
ZR	10	19	1.2 cd

[1]BA = N⁶-benzyladenine, KIN = kinetin, ZR = zeatin riboside.
[2]Detransformed means reported. Mean separation by least significant difference (LSD) for unequal replications at $P = 0.05$.

TABLE 2

PEDICEL BUD POSITION EFFECTS ON SHOOT INITIATION OF YELLOW-FLOWERED *HESPERALOE PARVIFLORA* ON 6 μM ZEATIN RIBOSIDE

Bud Position[1]	n	Mean Shoots per Bud[2]
1	4	4.3 a
2	5	4.3 a
3	5	6.8 a
4	4	11.4 b
5	5	11.4 b
6	5	13.4 b
7⁺	11	13.2 b

[1]Bud position: 1 = lowest, 7⁺ = highest.
[2]Detransformed means reported. Mean separation by least significant difference (LSD) for unequal replications at $P = 0.05$.

All experiments were conducted in a completely randomized design. Counts and ranks were transformed by taking the square root after adding 0.5. Detransformed means are reported. Analysis of variance was performed by ANOVA procedure (Microsoft Excel, Microsoft Corp., Redmond, WA). Means were separated by least significant difference (LSD) for equal and unequal replications as described by Little and Hills (1978). Multiple linear regression with index variables was performed as described in Neter et al. (1983).

Results and Discussion

All meristems of acaulescent Agavaceae species are usually subterranean. Thus, traditional micropropagation protocols result in stock plant destruction to obtain explant material and frequent culture contamination. We sought to minimize these problems by initiating cultures from the inflorescence. In preliminary work, we initiated shoot cultures indirectly from inflorescence pieces, peduncle sections, pedicel buds, and bracts based on B. McHaud's (Department of Plant Sciences, University of Arizona, personal communication) unpublished protocol for micropropagation of *H. funifera* from callus of aseptically germinated seedlings. We further investigated methods for direct shoot initiation to reduce the possibility of somaclonal variation.

Several Monocotyledoneae families produce proliferations on their inflorescences (Stout, 1934; Gentry, 1982; Pilbeam, 1983; Dahlgren et al., 1985). This ability has been exploited to initiate microshoot cultures directly from inflorescence explants of orchids (Tanaka et al., 1988; Tokuhara and Mii, 1993), onions (Pike and Yoo, 1990), and several genera in the Asphodelaceae (Kaul and Sabaharwal, 1972; Richwine et al., 1995). Although inflorescence proliferations have not been reported in *Hesperaloe*, we attempted to initiate microshoots from pedicel bud explants.

Pedicel bud explants showed a variety of responses when placed on basal medium containing various cytokinins. Some buds did not respond, while others grew directly into shoots. In the most common response, explants formed rosettes that subsequently elongated (Fig. 1). Floral development continued with some explants blooming *in vitro*. After 4 wk, vegetative shoots initiated in the floral axils along the entire length of the expanded pedicel bud explants. Shoots initiated on all treatments (Table 1), with the best response on medium supplemented with 1 μM zeatin riboside.

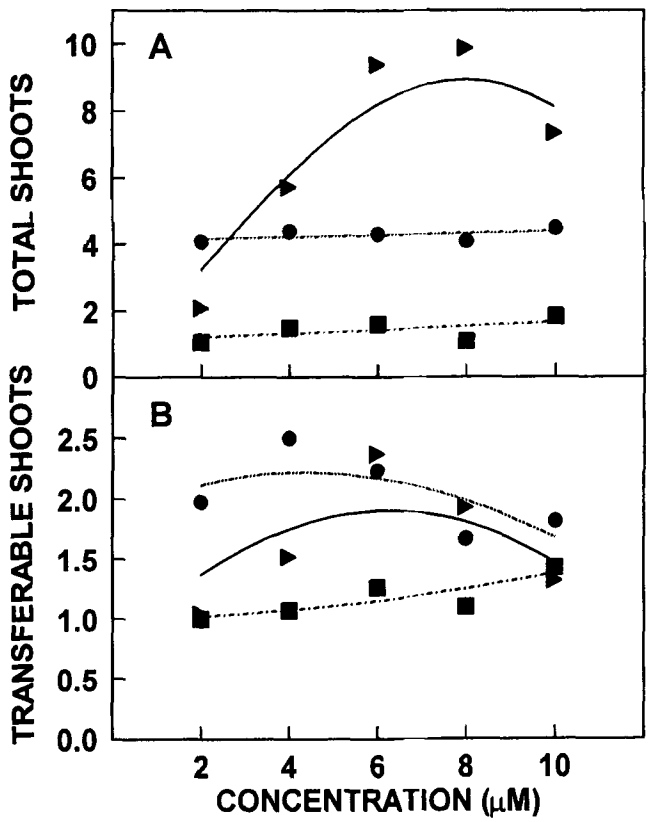

FIG. 2. Benzyladenine (BA – ▲), kinetin (KIN – ■), and zeatin riboside (ZR – ●) effects on total (*A*) and transferable (*B*) microshoot production by wild-type *Hesperaloe parviflora* after 8 wk in culture. Reduced regression equations for *A* are: $Y = 1.03 + 0.5X - 0.03X^2$ (BA), $Y = 1.27 + 0.02X + 0.0003X^2$ (KIN), and $Y = 2.15 + 0.005X - 0.0002X^2$ (ZR) where Y is the square root of shoot counts plus 0.5 and X is cytokinin concentration. Reduced equations for *B* are: $Y = 1.15 + 0.13X - 0.01X^2$ (BA), $Y = 1.22 + 0.004X + 0.001X^2$ (KIN), and $Y = 1.54 + 0.05X - 0.006X^2$ (ZR). Multiple correlation for the full model, after removing pure error sum of squares, was 0.8092 for *A* and 0.3337 for *B*.

TABLE 3

ANALYSIS OF VARIANCE AND MEANS FOR THE EFFECT OF BENZYLADENINE (BA) PULSING ON THE PRODUCTION OF MICROSHOOTS OF WILD-TYPE *HESPERALOE PARVIFLORA*.

Source	df	Mean Square and Means: Total Shoots	Mean Square and Means: Transferable Shoots
6 wks 6 μM BA vs. 6 weeks 6 μM BA then 6 weeks 6 μM BA			
Treatment	1	7.0*	8.7*
Error	26	1.4	0.4
Means[1]			
6 wks 6 μ*M* BA		8.1	0.4
6 wks 6 μ*M* BA then 6 wks 6 μ*M* BA		15.0	3.8
6 wks 6 μM BA vs. 6 wks 6 μM BA then 6 wks 1 μM BA			
Treatment	1	1.9 NS	19.4**
Error	26	1.6	0.7
Means			
6 wks 6 μ*M* BA		8.9	0.4
6 wks 6 μ*M* BA then 6 wks 1 μ*M* BA		12.3	6.2
6 wks 6 μM BA then 6 wks 6 μM BA vs. 6 wks 6 μM BA then 6 wks 1 μM BA			
Treatment	1	0.9 NS	2.0 NS
Error	26	1.9	0.9
Means			
6 wks 6 μ*M* BA then 6 wks 6 μ*M* BA		15.0	3.8
6 wks 6 μ*M* BA then 6 wks 1 μ*M* BA		12.3	6.2

[1]Detransformed means reported.

NS, *, **—Nonsignificant or significant at $P = 0.05$ and $P = 0.01$, respectively.

To determine if there was a relationship between pedicel bud position and shoot initiation, individual buds from the full length of the inflorescence from clonal plants were placed on basal medium containing 6 μ*M* zeatin riboside. A higher concentration of zeatin riboside was used in this experiment based on unreported data from preliminary initiation experiments and production of transferable shoots in culture. Overall morphological changes were similar to those previously observed, but the lower three buds produced half as many shoots as the more distal buds (Table 2). The first leaves of many shoots were variegated yellow and green. Although a cytological study was not performed to verify the origin of these shoots, this suggests that floral meristems became vegetative since the variegation corresponded to the tepal pigmentation pattern.

A clonal line of stabilized shoot cultures was used to test the affect of cytokinins on shoot multiplication. These cultures did not produce callus, and new shoots originated from axillary meristems. Cytokinin type and concentration affected quantity and quality of shoots produced (Fig. 2 *A*). Significantly more shoots were produced on media supplemented with BA than kinetin, or zeatin riboside. There was a quadratic relationship between shoot production and BA concentration with a peak of 9.9 shoots on 8 μ*M* BA. Cultures grown on zeatin riboside produced four times as many shoots as those on kinetin, but shoot production was essentially unresponsive to increasing concentration of either of these cytokinins.

Not all of the shoots produced on multiplication media were suitable for further culture. High BA concentrations produced stunted axillary shoots that could not be separated. Significantly more transferable shoots, defined as having bulbous bases with diameters of 2–6 mm, were produced on media containing zeatin riboside than BA or kinetin (Fig. 2 *B*). Lower concentrations of zeatin riboside (less than 6 μ*M*) were the most effective followed by moderate concentrations of BA (5 to 7 μ*M*). Cultures grown on media containing kinetin produced few transferable shoots. Regression analysis predicts an average of 2.2 transferable shoots would be produced on medium containing 5.4 μ*M* zeatin riboside and 1.9 on medium containing 6 μ*M* BA.

Because cultures grown on media supplemented with high concentrations of BA produced more total shoots, we hypothesized that transferring these cultures to a medium with less BA might allow further growth of poorly developed shoots and increase the number of transferable shoots. As expected, there was a significant increase in the number of transferable shoots produced between the first and second growth period regardless of BA concentration (Table 3). However, BA concentration did not significantly influence the number of transferable shoots produced during the second growth period. Cultures grown on high BA medium for 12 wk produced a mean of 3.8 transferable shoots, but cultures grown on high BA medium for 6 wk and then on low BA medium for another 6 wk produced a mean of 6.2 transferable shoots per culture.

H. parviflora microshoots root easily *in vitro* so we tested the feasibility of *ex vitro* rooting to reduce production costs. Preliminary studies indicated exogenous auxin applications were unnecessary and possibly detrimental to rooting (data not shown), so auxins were not used. Rooting medium did not have a significant effect on the quantity or quality of roots. All treatments had 100% rooting after 6 wk. Shoots grown in perlite or vermiculite produced an average of two roots while those in the perlite:vermiculite mix produced an average of 1.8 roots. The mean rank of roots produced in perlite, vermiculite, and the perlite:vermiculite mix was 3.1, 2.9, and 2.4, respectively.

We successfully micropropagated *H. parviflora* from mature stock plants. Shoot cultures can be directly initiated from mature plants using pedicel bud explants on a modified MS media containing zeatin riboside. Total shoot multiplication from established cultures was the greatest on 6 μ*M* BA. However, regression analysis predicts the maximum number of transferable shoots would be produced on media with 5.4 μ*M* zeatin riboside. Growing shoots on a medium supplemented with 6 μ*M* BA for 6 wk and then transferring to a 1 μ*M* BA medium for 6 more wk increased the number of transferable shoots, but not significantly. Shoots rooted easily *in vitro* or *ex vitro* and rooted shoots were easily acclimatized. The methods described in this paper are being used to commercially micropropagate *H. parviflora*, and will be useful in micropropagating *Hesperaloe* hybrids for use in the landscape and cloning superior fiber-producing *Hesperaloe* and *Yucca* plants.

ACKNOWLEDGMENTS

This work was supported by Desert Tree Farm, Phoenix, Arizona. The authors would like to acknowledge the contributions of B. McHaud and S. McLaughlin.

REFERENCES

Bentz, S. E.; Parliman, B. J. Sources of explants for *in vitro* propagation of *Yucca glauca* Nutt. Hortscience 20:540; 1985.

Bentz, S. E.; Parliman, B. J.; Talbott, H. J., et al. Factors affecting the *in vitro* culture of *Yucca glauca*. Plant Cell Tissue Organ Cult. 14:111–120; 1988.

Bihn, L. T.; Muoi, L. T.; Oanh, H. T. K., et al. Rapid propagation of agave by *in vitro* tissue culture. Plant Cell Tissue Organ Cult. 23:67–70; 1990.

Dahlgren, R. M. T.; Clifford, H. T.; Yeo, P. F. The families of the monocotyledons: structure, evolution, and taxonomy. Berlin: Springer-Verlag; 1985.

Das, T. Micropropagation of *Agave sisalana*. Plant Cell Tissue Organ Cult. 31:253–255; 1992.

Durmishidze, S. V.; Gogoberidze, M. K.; Mamaladze, M. N. Regeneration of plants from callus tissue of *Yucca gloriosa* buds. Z. Pflanzenphysiol. 11:179–182; 1983.

Gentry, H. S. Agaves of continental North America. Tucson: University of Arizona Press; 1982.

Kaul, K.; Sabharwal, P. S. Morphogenic studies on *Haworthia*: establishment of tissue culture and control of differentiation. Am. J. Bot. 59:377–386; 1972.

McLaughlin, S. P.; Schuck, S. M. Intraspecific variation in fiber properties in *Yucca elata* and *Hesperaloe funifera* (Agavaceae). Econ. Bot. 46:181–186; 1992.

Little, T. M.; Hills, J. F. Agricultural experimentation: design and analysis. New York: John Wiley & Sons; 1978.

Litz, R. E.; Conover, R. A. Tissue culture propagation of some foliage plants. Proc. Fla. State Hortic. Soc. 90:301–303; 1977.

Murashige, T. Plant propagation through tissue culture. Ann. Rev. Plant Physiol. 25:135–166; 1974.

Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–479; 1962.

Neter, J.; Wasserman, W.; Kutner, M. H. Applied linear regression models. Homewood, IL: Richard D. Irwin Inc.; 1983.

Nitsch, C.; Nitsch, J. P. The induction of flowering *in vitro* in stem segments of *Plumbago indica* L. Planta 72:355–370; 1967.

Pierik, R. L. M.; Steegmans, H. H. M. Vegetative propagation of a chimeral *Yucca elephantipes* Regel *in vitro*. Sci. Hortic. 21:267–272; 1983.

Pike, L. M.; Yoo, K. S. A tissue culture technique for the clonal propagation of onion using immature flower buds. Sci. Hortic. 45:31–36; 1990.

Pilbeam, J. *Haworthia* and *Astroloba*: a collectors guide. London: B. T. Batsford Ltd.; 1983.

Powers, D. E.; Backhaus, R. A. *In vitro* propagation of *Agave arizonica* Gentry & Weber. Plant Cell Tissue Organ Cult. 16:57–60; 1989.

Richwine, A. M.; Tipton, J. L.; Thompson, G. A. Establishment of *Aloe, Gasteria*, and *Haworthia* shoot cultures from inflorescence explants. HortScience 30:1443–1444; 1995.

Robert, M. L.; Herrara, J. L.; Contreras, F., et al. *In vitro* propagation of *Agave fourcroydes* Lem. (Henequen). Plant Cell Tissue Organ Cult. 8:37–48; 1987.

Stout, A. B. Daylillies: the wild species and garden clones both old and new of the genus *Hemerocallis*. New York: Macmillan Publishing Co.; 1934.

Tanaka, M.; Kumara, M.; Goi, M. Optimal conditions for shoot production from *Phalaenopsis* flower stalk cuttings cultured *in vitro*. Sci. Hortic. 35:117–126; 1988.

Tokuhara, K.; Mii, M. Micropropagation of *Phalaenopsis* and *Doritaenopsis* by cutting shoot tips of flower stalk buds. Plant Cell Rep. 13:7–11; 1993.