Micropropagation of Members of the Cactaceae Subtribe Cactinae

Philip W. Clayton¹, John F. Hubstenberger, and Gregory C. Phillips²

Department of Agronomy and Horticulture, New Mexico State University, Las Cruces, NM 88003-0003

S. Ann Butler-Nance

Department of Experimental Statistics, New Mexico State University, Las Cruces, NM 88003-0003

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Abstract. Micropropagation of 11 rare or endangered cacti species belonging to the subtribe Cactinae was achieved by rooting of proliferated axillary shoots. Shoot tip explants were obtained from seedlings of *Escobaria missouriensis* D.R. Hunt, *E. robbinsorum* (Earle) D.R. Hunt, *Sclerocactus spinosior* (Engelm.) Woodruff & L. Benson, and *Toumeyia papyracantha* (Engelm.) Br. & Rose, and from mature plants of *Mammillaria wrightii* Engelm., *Pediocactus bradyi* L. Benson, *P. despainii* Welsh & Goodrich, *P. knowltonii* L. Benson, *P. paradinei* B.W. Benson, *P. winkleri* Heil, and *S. mesae-verdae* (Boissevain) L. Benson. Three or four species were used in each of a series of experiments investigating the effects of basal media and auxin and cytokinin types and concentrations on axillary shoot proliferation. Low or no auxin but moderate to high cytokinin concentrations were required for axillary shoot production. All species rooted spontaneously on hormone-free media; however, several species rooted better on media containing auxin. All species were re-established in the greenhouse.

The propagation of native cacti is usually done with seed and rooted offshoots; however, conventional propagation methods are inadequate for those species that exhibit no to few offshoots, seed dormancy, low germination rates, self-sterility, slow growth, or that require many years to mature. These difficulties are compounded by the limited availability of species that are threatened with extinction.

Tissue culture techniques can overcome certain limitations associated with conventional propagation of rare cacti. The establishment of callus cultures from several genera of cacti (King, 1957; Minocha and Mehra, 1974; Havel and Kolar, 1983) and studies on morphogenesis (Mauseth and Halperin, 1975; Kolar et al., 1976; Johnson and Emino, 1979a) have been reported. At least 24 species of cacti from 16 genera have been micropropagated by rooting of shoots proliferated through axillary branching (Mauseth, 1979; Johnson and Emino, 1979b; Vyskot and Jara, 1984; Starling, 1985; Ault and Blackmon, 1985, 1987; Escobar et al., 1986; Rubluo et al., 1986). Cacti micropropagated through axillary branching have shown a high degree of phenotypic uniformity within clones (Mauseth, 1979; Ault and Blackmon, 1987).

Of the cacti investigated in the present report, only *Pediocactus paradinei* has been evaluated previously for micropropagation potential. Successful in vitro axillary shoot proliferation and rooting were reported, but it is not clear whether plants were successfully re-established (Sluis and Wochok, 1980).

The objective of this investigation was to evaluate basal medium composition and requirements for hormone types, concentrations, and combinations for axillary shoot proliferation of 11 species of rare (*Escobaria missouriensis*, *Mammillaria wrightii*, *Sclerocactus spinosior*), candidate (*Pediocactus des-*

painii, *P. paradinei*, *P. winkleri*, *Toumeyia papyracantha*), threatened (*E. robbinsorum*, *S. mesae-verdae*), or endangered (*P. bradyi*, *P. knowltonii*) cacti. These cactus species are difficult to grow and are valued as collector's items. In addition, the potential for root development and plantlet re-establishment of these 11 species was assessed.

Materials and Methods

Initiation of cultures. A few mature plant specimens of *M. wrightii*, *P. bradyi*, *P. despainii*, *P. knowltonii*, *P. paradinei*, *P. winkleri*, and *S. mesae-verdae* provided shoot tip explants from primary or secondary stems. Excised shoot tips 1 to 2 cm in diameter were immersed in 95% ethanol for 1 rein, 2% sodium hypochlorite (diluted commercial bleach) for 7 rein, and rinsed three times in sterile water. Seeds of *E. missouriensis*, *E. robbinsorum*, *S. spinosior*, and *T. papyracantha* were surface-sterilized by the same procedure and germinated in vitro on hormone-free MS medium (Murashige and Skoog, 1962). Shoot tip explants =0.5 cm in diameter (2 to 6 months old) were excised. Explants were inoculated onto MS media containing one or more of the following combinations of hormonal supplements to initiate tissue cultures: 24.6 μM N-(methyl-2-butenyl)-1H-purin-6-amine (2iP) + 0.15 mM 1H-purin-6-amine (adenine sulfate); 11.4 μM 1H-indole-3-acetic acid (IAA) + 18.6 μM N-(2-furanylmethyl)-1H-purin-6-amine (kinetin) + 0.30 mM adenine sulfate; 0.3 μM 1-naphthaleneacetic acid (NAA) + 4.4 μM N-(phenylmethyl)-1H-purin-6-amine (BA); and 4.9 μM 1H-indole-3-butyric acid (IBA) + 49.2 μM 2iP. All media contained vitamins according to the L2 formulation of Phillips and Collins (1979), 87.6 mM sucrose and 8 g agar/liter (Carolina Biological, Burlington, N. C., plant tissue culture type). Cultures were transferred to fresh media every 4 weeks for proliferation of axillary shoots. To minimize potential carryover effects, these stock cultures were standardized by serial subculture on a common medium composition for 3 months before beginning each formal experiment. All cultures were grown in 100 $\times$ 25-mm polystyrene petri dishes and were incubated at $29 \pm 1^\circ\text{C}$ under continuous fluorescent light ($150 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$).

Experiments evaluating individual factors. The first three formal experiments evaluated basal medium formulations, auxin

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¹Present address: U.S. Fish and Wildlife Service, c/o Corpus Christi State Univ. Campus Box 338, Corpus Christi, TX 78412.

²To whom reprint requests should be addressed.

type and concentrations, or cytokinin type and concentrations as individual factors using a randomized complete block design. Three or four species were selected for each experiment, depending on the availability of shoots for inoculation. Four shoots (replications) of each species were inoculated into a petri dish, with four dishes (blocks) per treatment. At each monthly subculture, only newly developed axillary shoots were transferred to fresh medium (four per dish). In all experiments that evaluated axillary shoot production, data were collected monthly just before subculture on the number of axillary shoots per explant, for each of three consecutive months. Analyses of variance were calculated for each experiment over 3 months of data (SAS, 1982). Means were separated using the least significant difference test at $P = 0.05$. In addition, observations were made on shoot quality and callus production.

Table 1. Effect of five basal nutrient media on shoot production of four species of cacti during 3 months following micropropagation.

Basal medium treatment ^z	Mean shoot number per monthly passage				Treatment means ^y
	<i>Esco-baria missouri-ensis</i>	<i>Pedio-cactus bradyi</i>	<i>Pedio-cactus knowltonii</i>	<i>Sclero-cactus spino-sior</i>	
L2	6.7	5.9	3.6	5.8	5.5 a
SH	5.7	5.8	2.9	4.5	4.7 b
B5	6.7	4.5	3.7	2.8	4.4 b
MS	6.3	4.4	2.1	3.6	4.1 b
MMS	4.6	2.0	1.3	2.2	2.5 c
Species means ^y	6.0 a	4.5 b	2.7 d	3.8 c	
Source of variation	df		Mean squares		F value
Block	3		5.63		1.10 ^{NS}
Basal medium	4		85.70		16.80***
Species	4		114.42		22.44***
Basal medium × species	16		7.04		1.38 ^{NS}
Error	71		5.10		

^zAll media included 11.4 μM IAA + 18.6 μM kinetin + 0.30 mM adenine sulfate. L2 = Phillips and Collins (1979); SH = Schenk and Hildebrandt (1972); B5 = Gamborg et al. (1968) as modified by Dunstan and Short (1977); MS = Murashige and Skoog (1962); MMS = MS as modified by Gladfelter and Phillips (1987).

^yValues followed by different letters within a column or within a row differ at $P \leq 0.05$.

^{NS,***}Nonsignificant at $P \leq 0.05$ or significant at $P \leq 0.001$, respectively.

major salts proved least effective. The slowest-growing cactus, *P. knowltonii*, showed the lowest rate of axillary shoot production, while the fastest-growing cactus, *E. missouriensis*, showed the greatest production of axillary shoots.

Auxin effects. The analysis of variance for this experiment indicated the block effect was not significant, but the main effects of auxin treatment and species and the interaction of auxin and species were significant (Table 2). Auxin treatment means were separated within species for data averaged over 3 months (Table 2). Two of the species showed discrimination for auxin type; e.g., NAA was a suitable auxin source for *P. knowltonii*, but not for *S. spinosior*, while IAA was a suitable auxin for *S. spinosior*, but not for *P. knowltonii*. *P. bradyi* performed well on one concentration of each of the auxin types. The auxin-free treatment and the treatments including 82.8 nM picloram or 1.0 μM IBA were equally effective with all three species.

Cytokinin effects. The analysis of variance for this experiment indicated the block effect was not significant, but the main effects of cytokinin treatments and species and their interaction were significant (Table 3). Cytokinin treatment means were separated within species for data averaged over the 3-month duration of the experiment. For the species evaluated, high concentrations of cytokinin (i.e., 22.8 to 49.2 μM) proved most effective. All four species performed optimally on the medium that included 22.8 μM zeatin. Only *P. bradyi* responded to BA at one of the concentrations tested. Kinetin at 46.4 μM was suitable for *P. bradyi* and *S. spinosior* axillary shoot proliferation. The 23.2-μM kinetin treatment was compared with and without auxin (IAA at 11.4 μM); *S. spinosior* showed a definitely better response to auxin with this cytokinin. The auxin-free version of this cytokinin treatment was compared with and without adenine sulfate as a supplement; *P. bradyi* produced more axillary shoots in the presence of adenine sulfate, but *S. spinosior* yielded more in the absence of adenine sulfate.

Table 2. Effect of four auxin types on shoot production of three species of cacti during 3 months following micropropagation.

Auxin ^z	Concn (μM)	Mean shoot number per monthly passage ^y			Treatment means
		<i>Pedio-cactus bradyi</i>	<i>Pedio-cactus knowltonii</i>	<i>Sclero-cactus spino-sior</i>	
Picloram	0.8	0.6 d	0.0 d	0.9 d	0.5
	0.0828	2.5 abc	1.8 ab	2.6 a	2.3
NAA	10.7	1.6 c	0.4 cd	1.1 cd	1.0
	1.1	2.2 abc	1.8 ab	1.4 bcd	1.8
	0.1	2.1 bc	2.1 a	1.2 cd	1.8
IBA	9.8	1.9 bc	1.8 ab	1.0 d	1.6
	1.0	2.6 ab	1.5 ab	2.0 abc	2.0
IAA	11.4	3.1 a	1.1 bc	2.3 ab	2.1
	1.1	1.6 c	0.9 bcd	2.0 abc	1.5
None	---	2.9 ab	1.8 ab	2.8 a	2.5
Species means		2.1	1.3	1.7	
Source of variation	df	Mean squares		F value	
Block	3	3.78		1.92 ^{NS}	
Auxin treatment	9	17.05		8.65***	
Species	2	23.80		12.08***	
Auxin treatment × species	18	3.40		1.73*	
Error	87	1.97			

^zAuxin + 24.6 μM 2iP + 0.15 mM adenine sulfate + MS basal medium.

^yValues followed by different letters within a column differ at $P \leq 0.05$.

^{NS,*,***}Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

Experiments evaluating combinations of factors. The fourth experiment further evaluated the interaction of auxin and cytokinin in cactus axillary shoot proliferation. Three auxin treatments and two cytokinin treatments identified in the previous experiments, plus a higher concentration of zeatin, were tested in combinations using the L2 basal medium. The analysis of variance for this experiment indicated the main effects of hormone treatments and species and their interaction effects were significant (Table 4). Hormone treatment means were separated within species averaged over the 3 months of the experiment, illustrating the strong interaction of species with hormone treatments. There was no single hormone treatment that proved optimal for all four species tested, but each treatment proved effective with at least one species. Treatments that included 22.8 μM zeatin with or without 1.1 μM NAA were most effective for the majority of the species.

The fifth experiment further evaluated the interaction of basal medium with hormone treatments. The MS and L2 media were tested in combinations with two auxin treatments and two cytokinin treatments. The analysis of variance for this experiment indicated the main effects of media and species and their interaction effects were significant (Table 5). Media means were separated within species for data averaged over 3 months. The use of either L2 or MS medium that included 45.6 μM zeatin + 1.1 μM NAA proved optimal for axillary shoot production from all four species.

Rooting and re-establishment of micropropagated cacti. After termination of the shoot multiplication experiments, axillary shoots were collected and evaluated for rooting and re-establishment potential. The varied histories and treatments to which these shoots had been exposed appeared to influence the subsequent rooting and re-establishment responses. Therefore, only general observations were made with respect to the rooting and re-es-

Table 3. Effect of four cytokinin types and adenine sulfate on shoot production of four species of cacti during 3 months following micropropagation.

Cytokinin ^z		Mean shoot number per monthly passage ^y				Treatment means
Compound	Concn (μM)	<i>Pediocactus bradyi</i>	<i>Pediocactus knowltonii</i>	<i>Sclerocactus spinosior</i>	<i>Toumeyia papyracantha</i>	
2iP	1.0	1.8 e	0.5 cd	0.0 e	0.0 e	0.6
	4.9	2.7 de	0.4 cd	0.0 e	0.0 e	0.8
	24.6	3.6 cd	2.2 b	1.0 bcde	0.5 de	1.8
	49.2	5.6 b	0.6 cd	2.0 ab	1.9 bc	2.5
BA	0.9	3.8 c	0.3 d	0.0 e	0.0 e	1.0
	4.4	0.7 f	1.0 cd	0.1 de	0.0 e	0.4
Zeatin	4.6	6.1 ab	2.4 b	0.8 cde	2.6 ab	3.0
	22.8	7.1 a	3.9 a	2.3 a	3.6 a	4.2
Kinetin	23.2	4.3 c	0.7 cd	1.1 bcd	1.6 bc	1.9
	46.4	7.1 a	1.4 bc	1.8 abc	1.6 bc	3.0
Kinetin, no auxin	23.2	3.3 cd	0.1 d	0.0 e	1.7 bc	1.3
Kinetin, no auxin or adenine	23.2	1.8 e	0.4 cd	1.7 abc	1.1 cd	1.3
Species means		4.0	1.1	0.9	1.2	
Source of variation		df	Mean squares		F value	
Block		3	0.88		0.38 ^{NS}	
Cytokinin treatment		11	85.49		37.33 ^{***}	
Species		3	408.27		178.28 ^{***}	
Cytokinin treatment × species		33	13.73		6.00 ^{***}	
Error		141	2.29			

^zCytokinin + 0.30 mM adenine sulfate + 11.4 μM IAA + MS basal medium, except as indicated.

^yValues followed by different letters within a column differ at $P \leq 0.05$.

^{NS,***}Nonsignificant at $P \leq 0.05$ or significant at $P \leq 0.001$, respectively.

Table 4. Effect of combinations of auxins and cytokinins on shoot production of four species of cacti during 3 months following micropropagation.

Auxin and cytokinin ^z		Mean shoot number per monthly passage ^y				Treatment means
Compounds	Concn (μM)	<i>Mammillaria wrightii</i>	<i>Pediocactus despainii</i>	<i>Pediocactus winkleri</i>	<i>Sclerocactus mesae-verdae</i>	
Picloram	0.0828 +					
Zeatin	45.6	4.0 a	3.4 d	9.8 a	1.0 d	4.5
Picloram	0.0828 +					
Kinetin	46.4	0.6 b	4.4 cd	4.6 c	4.3 a	3.5
NAA	1.1 +					
Zeatin	22.8	3.8 a	6.5 ab	6.9 b	3.0 abc	5.1
NAA	1.1 +					
Zeatin	45.6	2.9 a	5.2 bc	6.9 b	1.4 cd	4.1
NAA	1.1 +					
Kinetin	46.4	0.7 b	4.4 cd	4.0 c	3.2 ab	3.1
No auxin +						
Zeatin	22.8	3.3 a	6.9 a	8.6 a	2.7 BC	5.4
No auxin +						
Zeatin	45.6	3.8 a	3.3 d	6.5 b	0.4 d	3.5
Species means		2.7	4.9	6.8	2.3	
Source of variation		df	Mean squares		F value	
Hormone treatment		6	48.08		9.12 ^{***}	
Species		3	477.53		90.61 ^{***}	
Hormone treatment × species		18	39.19		7.44 ^{***}	
Error		84	5.27			

^zAuxin + cytokinin + 0.30 mM adenine sulfate + L2 basal medium.

^yValues followed by different letters within a column differ at $P \leq 0.05$.

^{***}Significant at $P \leq 0.001$.

establishment of these 11 species. Some shoots of each species rooted spontaneously on hormone-free MS medium; however, most species showed increased rooting frequencies after exposure to auxin (Table 6). Axillary shoots rooted in vitro are illustrated in Fig. 2 for *P. knowltonii*.

Re-establishment of all 11 species in the greenhouse was successfully achieved during the root development treatments described (Table 6). No significant phenotypic variation was observed among the propagules after 6 to 12 months of growth in the greenhouse. The size and quality of the re-established

Table 5. Effect of combinations of auxins, cytokinins, and basal media on shoot production of four species of cacti during 3 months following micropropagation.

Auxin and cytokinin ^z		Mean shoot number per monthly passage ^y				Treatment means
Compounds	Concn (μM)	<i>Escobaria robbinsorum</i>	<i>Pediocactus bradyi</i>	<i>Pediocactus paradinei</i>	<i>Toumeyia papyracantha</i>	
L2 basal medium						
Picloram	0.0828 +					
Zeatin	45.6	2.3 b	3.3 b	6.3 a	2.8 bc	3.6
Picloram	0.0828 +					
BA	44.4	3.3 ab	4.6 ab	2.8 c	1.5 c	3.0
NAA	1.1 +					
Zeatin	45.6	3.1 ab	4.5 ab	6.5 a	5.0 a	4.8
NAA	1.1 +					
BA	44.4	3.8 ab	4.9 a	3.5 bc	2.5 c	3.7
MS basal medium						
Picloram	0.0828 +					
Zeatin	45.6	4.3 a	5.4 a	5.7 a	2.7 bc	4.5
NAA	1.1 +					
Zeatin	45.6	2.9 ab	5.0 a	5.1 ab	4.3 ab	4.3
Species means		3.3	4.6	5.0	3.1	
Source of variation		df	Mean squares		F value	
Medium treatment		5	28.04		5.40***	
Species		3	82.78		15.95***	
Medium treatment × species		15	17.57		3.39***	
Error		72	5.19			

^zAuxin + cytokinin + 0.30 mM adenine sulfate + basal medium as indicated.

^yValues followed by different letters within a column differ at $P \leq 0.05$.

***Significant at $P \leq 0.001$.

Table 6. Rooting of micropropagated shoots of 11 species of cacti and re-establishment of plantlets in the greenhouse in response to hormone treatment.

Species ^z	Shoots rooting (mean % ± SE)		Plantlets re-established (mean % ± SE)
	No auxin ^y	Auxin and concn (μM) ^x	
<i>Escobaria missouriensis</i>			
<i>Escobaria robbinsorum</i>	71 ± 8	NAA (27) or IBA (25)	90 ± 3
<i>Mammillaria wrightii</i>			
<i>Pediocactus knowltonii</i>	36 ± 4	NAA (27) or IBA (25)	64 ± 9
<i>Pediocactus winkleri</i>			
<i>Pediocactus bradyi</i>	13 ± 8	NAA (27) or IBA (25)	45 ± 4
<i>Pediocactus paradinei</i>		or NAA (54) + IAA (57)	
<i>Pediocactus despainii</i>			
<i>Sclerocactus mesae-verdae</i>			
<i>Sclerocactus spinosior</i>	3 ± 2	IBA (25) or	7 ± 2
<i>Toumeyia papyracantha</i>		NAA (54) + IAA (57)	51 ± 8

^zSpecies are grouped for convenience of description of treatments and responses, and are listed in order of relative performance from best (top) to worst (bottom).

^yRooting percentage after three 1-month passages on hormone-free MS medium.

^xRooting percentage after 1 month on MS medium + auxin treatment as indicated, followed by 2 months on hormone-free MS.

plants is illustrated in Fig. 3 for *P. bradyi*. Micropropagated cactus plants appeared comparable in size and maturity to seed-grown plants several years old; e.g., *M. wrightii* flowered in the greenhouse during the summer following re-establishment, whereas seed-grown plants of this species normally require 3 to 5 years to reach maturity.

Discussion

In a micropropagation system, shoot number is the single most important variable to measure. However, the presence of callus may introduce the opportunity for spontaneous genetic

mutation (Lee and Phillips, 1988), and callus production may represent a loss of shoot proliferation potential (Clayton, 1987). Shoot quality may also be an important measure of subsequent competency for re-establishment potential, as well as potential product value. Studies (Clayton, 1987) related to those reported here indicated that shoot quality, but not callus formation, was highly correlated with shoot number when used as response variables; assessments using combinations of these response variables may be useful for optimizing micropropagation systems for some species of cactus. Only the number of axillary shoots formed from each explant per passage is discussed in the

Fig. 2. Root development on hormone-free MS medium, following auxin treatment (27 μ M NAA) in the previous passage, from micro-propagated shoots of *Pediocactus knowltonii*.

Fig. 3. Root system of re-established *Pediocactus bradyi* plant derived from tissue culture after 9 months of growth in the greenhouse.

present report. The highest rate of axillary shoot formation was observed for *P. paradinei* (9.8 shoots per explant per passage in the fourth experiment); while the lowest rate was observed for *P. knowltonii* (3.9 shoots per explant in the third experiment).

The MS basal medium has been widely used for cactus tissue cultures (Kolar et al., 1976; Johnson and Emino, 1979b; Escobar et al., 1986). However, researchers in cactus micropropagation had not evaluated this factor in detail. In the first experiment reported here, the most effective basal medium was L2, which differs from the other basal media tested in having the highest total major and minor salts concentrations, and in having a higher concentration of magnesium and calcium ions, which may be important for cactus nutrition. In contrast, the MMS medium containing half-strength major salts yielded the least satisfactory results, compared to the other four media typically categorized as high-salt media. However, the results of the fifth experiment indicated MS as well as L2 basal media can give satisfactory shoot proliferation cultures of cactus when the hormone combinations have been optimized. These results support the suggestion by Mauseth (1979) that any of the commonly used high-salt basal media may be suitable for establishing cactus micropropagation systems.

In evaluating auxin type and concentration as factors for axillary shoot proliferation in the second experiment, we found that treatments with no or low concentrations of auxin were more effective than those with high concentrations. However,

results of the fourth experiment indicated the presence of low levels of auxin with cytokinin enhanced axillary shoot production in some species. To the best of our knowledge; cactus researchers had not previously evaluated this factor in detail for axillary shoot production. Some shoots of all 11 species subsequently rooted spontaneously on hormone-free medium; however, auxin treatments enhanced subsequent root induction frequencies for most species. These results confirm those of Mauseth (1979) and Ault and Blackmon (1987) concerning the role of auxin in cactus root development.

Zeatin was the most effective cytokinin type for axillary shoot proliferation from all four species in the third experiment. There was a strong interaction between the other cytokinin sources with species. Cytokinin is considered essential for development of cactus axillary shoots (Mauseth, 1979; Johnson and Emino, 1979b; Escobar et al., 1986).

In four of the five experiments conducted in this study, interaction effects between species and tissue culture factors were significant. In the fifth experiment, the treatments including 45.6 μ M zeatin + 1.1 μ M NAA, with either L2 or MS, were proven most effective for all four species evaluated. However, in the fourth experiment evaluating four different species, this same treatment using L2 was most effective for only one species. The treatments including L2 + 22.8 μ M zeatin, with or without 1.1 μ M NAA, were each effective for three of the four species in the fourth experiment. Thus, a single culture medium may not be suitable for optimal shoot proliferation of several genera of cacti. These results support the suggestion made by Johnson and Emino (1979a; 1979b) that the optimal hormone combination may be unique for each cactus species. However, the results of the fourth and fifth experiments of the present study, considered together, suggest that a group of three related media can be used to elicit optimal shoot proliferation responses from a number of cactus species.

The cacti micropropagated in this study are among those more difficult to propagate using conventional procedures. Micropropagated cacti can supply the commercial trade with difficult-to-propagate species. Conservation efforts involving outplantings of threatened or endangered cacti can be facilitated through micropropagation of several discrete clones sampled from native populations. Micropropagation also can be used in germplasm preservation for future study and use of rare, threatened, or endangered cacti.

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