Propagation

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Section Editor

Hardwood Cutting Propagation of Sequoyah™ Crape Myrtle

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Index Words Rooting, root-promoting compounds, auxin, IBA, NAA, wounding, *Lagerstroemia*

Significance to Industry Sequoyah™ crape myrtle cuttings dipped in Dip'N Grow® (DNG) at an IBA rate of 5000 ppm had greater root numbers compared to cuttings that received no auxin and higher root and cutting quality ratings compared to cuttings that received no auxin or basal wounding treatment. However, cuttings dipped in Hortus IBA Water Soluble Salts™ (Hortus IBA) or DNG, regardless of auxin concentration, had similar rooting percentages, root numbers, average length of three longest roots, root quality, cutting quality, and growth. These results suggest that hardwood cuttings of Sequoyah™ crape myrtle will root without wounding or use of auxin. However, a basal quick-dip in Hortus IBA or DNG at an IBA rate of 1000 or 5000 ppm does result in a better liner.

Nature of Work In many southern landscapes, crape myrtles provide a reliable source of color, often flowering for more than 100 days (2, 6). The cultivar palette is constantly expanding, and Mississippi State University has been active in the development of new crape myrtle selections, including Sequoyah™. Sequoyah™ is a hybrid resulting from the cross of *Lagerstroemia 'Arapaho'* (7) and an unknown pollen donor. *Sequoyah*™ has a clear, true red flower color and medium to large growth habit. Three-year-old plants in a research setting are 15+ feet and have flowered from early June through late August.

Propagation of crape myrtle via softwood or hardwood cuttings is widely described as easy (2, 4). Byers (2) reports using 8-inch (20-cm) hardwood cuttings taken after frost and stored overwinter. Dirr and Heuser (4) report early February hardwood cuttings rooted better (43%) than early January or early March cuttings when using bottom heat and peat:perlite or bark. The objective of this research was to evaluate ease of rooting and determine optimal commercial auxin formulation and concentration and possible value of a basal wounding for hardwood cutting propagation of Sequoyah™.

Five-inch (12.7-cm) medial cuttings were harvested from the parent plant and stuck to a depth of 1 inch (2.5 cm) on 1 Feb. 2017. Propagation medium was 100% perlite placed in 3-inch (7.6-cm) containers. Treatments included two basal wounding treatments (wounded or non-wounded), three auxin formulations [Hortus IBA (Hortus IBA Water Soluble Salts™), Dip'N Grow®, or Hortus IBA + KNAA (Hortus IBA Water Soluble Salts™ + NAA potassium salt)], and three levels of auxin (0, 1000, or 5000 ppm IBA). DNG and Hortus

IBA + KNAA formulations contained NAA at one-half the rate of IBA. KNAA is a research-only product, but was added to Hortus IBA in selected treatments for comparison with DNG. Experimental design was a randomized complete block design with five single cutting replications. Data collected after 60 days included rooting percentage, growth index (new shoots), cutting quality (0-5, with 0 = dead and 5 = transplant-ready cutting), total root number, average root length (of three longest roots), and root quality (0-5, with 0=no roots and 5=healthy, vigorous root system). Data were analyzed using linear mixed models and generalized linear mixed models with the GLIMMIX procedure of SAS (ver. 9.4; SAS Institute Inc., Cary, NC).

Results and Discussion Rooting percentage, average length of three longest roots, and growth index were similar among treatments (Table 1). Rooting percentage ranged from 80% to 100%, higher than those reported by Dirr and Heuser (4) for hardwood cuttings. However, rooting percentages were similar to those reported by Dirr (3) for summer-propagated 'Natchez' crape myrtle using 5000 ppm IBA or 95% ethanol, the solvent used for IBA. Similarly, Blythe *et al.* (1) reported greater than 90% rooting when using 1000 ppm DNG for 'Natchez' crape myrtle. Differences in rooting percentages may be due to differences in cultivars evaluated or cultural conditions of the parent material (5). Cuttings that were wounded and dipped in DNG 5000 ppm had more roots compared to cuttings wounded and dipped in DNG 1000 ppm or Hortus IBA(1000 ppm) + KNAA (500 ppm), non-wounded Hortus IBA (1000 ppm) + KNAA (500 pm), and cuttings receiving no auxin, regardless of basal wounding treatment. Total number of roots for Sequoyah™ were very similar to total number of root reported for 'Natchez' when 5000 ppm IBA was used, although the solvent differed (3). However, total number of root for Sequoyah™ dipped in DNG 1000 ppm averaged 10 more when compared to a previous study using 'Natchez' crape myrtle (1). Cuttings that were wounded and dipped in DNG 5000 ppm had higher root and cutting quality ratings compared to cuttings that were non-wounded and received no auxin, but were similar to all other treatments.

For a more thorough examination of treatment factors, selected treatment combinations were compared using the Shaffer-Simulated method for simultaneous comparisons. Treatment comparisons were as follows: wounded vs. non-wounded, Hortus IBA vs. no auxin, DNG vs. no auxin, Hortus IBA + KNAA vs. no auxin, Hortus IBA vs. DNG, DNG vs. Hortus IBA + KNAA, Hortus IBA vs. Hortus IBA + KNAA, Hortus IBA at 5000 vs. 1000 ppm IBA, DNG at 5000 vs. 1000 ppm IBA, and Hortus IBA + KNAA at 5000 ppm IBA vs. 1000 ppm IBA. Rooting percentages and length of three longest roots were similar regardless of treatment comparison (Table 2). Wounding did not increase number of roots and root or cutting quality. However, cuttings treated with Hortus IBA, DNG, or Hortus IBA + NAA (averaged over auxin concentration and basal wounding treatment) had more roots compared to cuttings receiving no auxin treatment. Blythe et al. (2003) reported that 'Natchez' crape myrtle cuttings receiving DNG 1000 ppm as a basal dip had more roots compared to cuttings receiving no auxin from a foliar spray, but 'Natchez' cuttings receiving K-IBA as a basal dip had similar root numbers compared to cuttings receiving no auxin. Cuttings treated with Hortus IBA or DNG (averaged over auxin concentration and basal wounding treatment) had higher root quality when compared to plants receiving no

auxin. These results are similar to those reported by Dirr and Heuser (4) indicating use of auxin increased root number and improved quality of 'Natchez', 'Tuscarora', and 'Muskogee' crape myrtle cuttings. Cuttings dipped in Hortus IBA or DNG (averaged over auxin concentration and basal wounding treatment) had a higher root quality when compared to cuttings dipped in Hortus IBA + NAA (averaged over auxin concentration and basal wounding treatment). Cuttings dipped in Hortus IBA or DNG (averaged over auxin concentration and basal wounding treatment) had a higher cutting quality compared to cuttings that received no auxin. Cuttings dipped in Hortus IBA or DNG (averaged over auxin concentration and basal wounding treatment) had higher cutting quality compared to cuttings dipped in Hortus IBA + NAA. Cuttings dipped in Hortus IBA, averaged over auxin concentration and basal wounding treatment, had a higher growth index compared to cuttings dipped in Hortus IBA + NAA or cuttings receiving no auxin.

Literature Cited

1. Blythe, E.K., J.L. Sibley, K.M. Tilt, and J.M. Ruter. 2003. Foliar application of auxin for rooting stem cuttings of selected ornamental crops. J. Environ. Hort. 21:131-136.
2. Byers, D. 1983. Selection and propagation of crape myrtle. Comb. Proc. Int. Plant Prop. Soc. 33:542-545.
3. Dirr, M.A. 1990. Effects of P-ITB and IBA on the rooting response of 19 landscape taxa. J. Environ. Hort. 8:83-85.
4. Dirr, M.A. and C.W. Heuser, Jr. 1987. The Reference Manual of Woody Plant Propagation. Varsity Press, Inc. Athens, Georgia.
5. Hartmann, H.T., D.E. Kester, F.T. Davies, Jr., and R.L. Geneve. 2011. Hartmann & Kester's Plant Propagation: Principles and Practices, 8th ed. Prentice Hall, Boston, Massachusetts.
6. Knight, P.R., J.M. Anderson, D. Lee, M. Lee, L. DeJean, and D. Murchison. 2006. Crape myrtle evaluations in south Mississippi. HortScience 41:512.
7. Pooler, M.R. 2006. 'Arapaho' and 'Cheyenne' *Lagerstroemia.* HortScience 41:855-856.

Table 1. Influence of basal wounding treatment, auxin concentration, auxin source on rooting percentage, root number, average length of three longest roots, root quality, cutting quality, and growth of Sequoyah™ crape myrtle.

Treatment[z]	Rooting (%)	Roots (no.)	Avg. length of 3 longest roots (inches)	Root quality rating[y]	Cutting quality rating[x]	Growth index[w]
Wounded control	100a[v]	6.9cd	3.9a	3.0ab	3.1ab	14.0a
Non-wounded control	100a	4.3d	2.4a	2.6b	2.8b	13.2a
Wounded Hortus IBA™ 1000 ppm	100a	11.8abcd	4.0a	3.3ab	3.5ab	17.2a
Non-wounded Hortus IBA™ 1000 ppm	80a	11.1abcd	3.7a	3.5ab	3.7ab	17.6a
Wounded Hortus IBA™ 5000 ppm	100a	15.8abc	4.0a	3.7ab	3.7ab	15.3a
Non-wounded Hortus IBA™ 5000 ppm	100a	14.3abcd	3.7a	3.4ab	3.5ab	15.4a
Wounded Dip'N Grow® 1000 ppm	100a	8.2bcd	4.1a	3.1ab	3.4ab	14.2a
Non-wounded Dip'N Grow® 1000 ppm	100a	11.3abcd	4.2a	3.2ab	3.5ab	15.3a
Wounded Dip'N Grow® 5000 ppm	100a	27.7a	3.8a	3.9a	3.9a	15.5a
Non-wounded Dip'N Grow® 5000 ppm	100a	23.3ab	3.4a	3.6ab	3.7ab	13.6a
Wounded Hortus IBA™ 1000 ppm KNAA 500 ppm	100a	8.2bcd	3.8a	2.7ab	3.3ab	14.4a
Non-wounded Hortus IBA™ 1000 ppm KNAA 500 ppm	100a	8.7bcd	2.8a	2.8ab	3.0ab	13.3a
Wounded Hortus IBA™ 5000 ppm KNAA 2500 ppm	100a	16.7abc	4.1a	3.1ab	3.2ab	12.9a
Non-wounded Hortus IBA™ 5000 ppm KNAA 2500 ppm	100a	16.9abc	3.9a	3.2ab	3.4ab	13.0a

[z]Dip'N Grow and Hortus IBA + KNAA formulations contained NAA at one-half the rate of IBA.
[y]Root quality (0-5, with 0=no roots and 5=healthy, vigorous root system).
[x]Cutting quality (0-5, with 0=dead and 5=transplant ready cutting).
[w]Growth index=(width1+width2+height)/3.
[v]Means followed by the same letter are similar according to Holm-Simulated method for simultaneous comparisons (α = 0.05).

Table 2. Direct comparisons of selected treatment combinations on rooting percentage, root number, average length of three longest roots, root quality, cutting quality, and growth of Sequoyah™ crape myrtle.

Comparison	Rooting (%)	Roots (no.)	(Length of 3 longest roots)/3 (inches)	Root quality rating[z]	Cutting quality rating[y]	Growth index[x]
Wounded vs. non-wounded	NS[w]	NS	NS	NS	NS	NS
Hortus IBA™ vs. no auxin	NS	**	NS	**	**	*
Dip'N Grow® vs. no auxin	NS	**	NS	**	**	NS
Hortus IBA™ + NAA vs. no auxin	NS	**	NS	NS	NS	NS
Hortus IBA™ vs. Dip'N Grow®	NS	NS	NS	NS	NS	NS
Dip'N Grow® vs. Hortus IBA™ + NAA	NS	NS	NS	*	*	NS
Hortus IBA™ vs. Hortus IBA™ + NAA	NS	NS	NS	*	*	**
Hortus IBA™ 5000 ppm vs. Hortus IBA™ 1000 ppm	NS	NS	NS	NS	NS	NS
Dip'N Grow® 5000 ppm vs. Dip'N Grow® 1000 ppm	NS	**	NS	NS	NS	NS
Hortus IBA™ + NAA 5000ppm vs. Hortus IBA™ + NAA 1000 ppm	NS	**	NS	NS	NS	NS

[z]Root quality (0-5, with 0=no roots and 5=healthy, vigorous root system).
[y]Cutting quality (0-5, with 0=dead and 5=transplant ready cutting).
[x]Growth index=(width1+width2+height)/3.
[w]NS=Not significant or significant at α = 0.01 (**) or 0.05 (*) using the Shaffer-Simulated method for simultaneous comparisons,

Responses of *Astrophytum asterias* (Zucc.) Lem (Cactaceae) to Cytokinins in the Activation of Areoles During Mass Propagation Under *In Vitro* Conditions

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Index Words *Astrophythum*, biznaga algodoncillo de estrella, star cactus, tissue culture, *in vitro* propagation, micropropagation

Significance to Industry: We performed this research to develop an *in vitro* micropropation system for *Astrophytum asterias*, which is an endemic cactus of Mexico that naturally grows in the states of Tamaulipas and Nuevo León (1, 2). This plant species has serious reproductive problems. Its natural habitat is being degraded and decimated by overcollection; consequently, the Mexican government (NOM-059-SEMARNAT-2010) (3) and international institutions including CITES had classified native populations endangered. To contribute to the rescue of this cactus, the conditions to activate the axillary buds and shoot proliferation were set in order to establish the basis for an efficient and reliable micropropagation protocol. The establishment of a scheme to propagate massively this plant species may produce benefits to both the nursery and ornamental industries because the micropropagation protocol described here can be used with commercial purposes, however, most importantly to rescue and restore the native populations in natural habitats in Mexico. After one year of research, we found that breaking dormancy of the axillary meristems located in the areoles can be achieved by the cytokinins KN and BAP at different concentrations. The best treatment produced 26 shoots per explant. Whole plants can easily be produced if the regenerated shoots are cultured in a medium free of growth regulators; however, the supplementation of the culture medium with K-IBA (1.2 mg/L^{-1}) significantly improved the responses.

Nature of Work To establish a reliable massive micropropagation system for *Astrophytum asterias*, we conducted varies experiments covering the four micropropagation steps. Before starting these experiments and because our plant model is a threatened species, we focused in germinate seeds aseptically to produce the explants for the propagation process. The seeds were initially cleaned through immersion in an ethanol solution for 5 min, immersion in a Clorox solution (30% v/v) plus 0.1% of Tween-20 for 20 min and rinsed 5 times in deionized sterile water, and then inoculated in a half strength Murashige and Skoog (MS), (4) medium. Initially, the effect of several cytokinins including 6-benzyl-aminopurine (BAP) [0, 4, 8, 12, 16, 20, 24 mgL-1], Kinetin (KN) [0, 6, 9, 12, 15, 18, 21 mg L-1] and 2-isopentenyl-aminopurine (2ip) [0, 6, 9, 12 mg L-1] was evaluated in breaking dormancy of axillary buds and the production of shoots. For the proliferation subcultures, we performed a series of

experiments to optimize the conditions to increase shoot production in which the best treatments from the induction step (BA 16 and KN 18 mgL-1) were re-evaluated to increase the rate of multiplication. Four treatments (0, 0.6, 1.2 and 2.4 mg/L^{-1}) of the auxin indole butyric acid (K-IBA) were tested on adventitious root formation in the production of complete plantlets. The plants obtained were used to conduct an experiment of acclimatization, in which the effect of the presence of endomycorrhizal fungi and fertilization level of P (0, 22, 44, 66 ppm) was evaluated. In the experiment, eighty micropropagated plantlets were transplanted and acclimatized for 30 days in a bench with low light conditions (400 μmol m^{-2} s^{-1} of photosynthetic photon flux density, PPFD) prior the transfer to a greenhouse with 800 μmol m-2 s^{-1} of PPFD to evaluate plant performance and survival. After a year of culture, plant survival, growth and root colonization by the mycorrhizaal fungi were estimated. In all experiments of micropropagation, we use the MS (3) culture media, which was prepared with an adjusted pH= 5.8, agar= 7gL^{-1}, and sucrose= 3%. The cultures were incubated in a room with a photoperiod of 16 h of light, and light conditions of 400 μmol.m^{-2}.s^{-1} of photosynthetic photon flux density. Experimental variables included data on % calli production, number of shoots per explant, root number, total root length (mm), mean root length (mm), and % plantlet survival. All data were subjected to ANOVA and Tukey test ($\propto$= 0.05) for mean separation.

Results and Discussion The aseptic germination started 25 days after seed inoculation and by day 70 we registered 98% of total seed germination. At this time, the seedlings had reached between 5 to 10 mm in height and 10 to 12 mm in diameter, which had developed between 10 to 20 areoles each. This was the optimal size to get the explants to start the experimentation for the micropropagation. The results obtained after one year of research revealed that from the cytokinins evaluated, BAP and KN were able to break the dormancy of the buds at any of the dosages tested but the best treatment during the induction step was KN 18 mg L-1 with 23 shoots produced per explant after 90 days of culture, which was significantly higher as compared to rest rest of treatments evaluated (Table 1). For the proliferation subcultures KN (18 mg L-1) produced 26 shoots in average, which were significantly higher than BAP (16 mg L-1) that produced 18 shoots per explant (Table 2). The best time to produce optimal growth of the new shoots during subculture was sixty days. Rhizogenesis was achieved after sixty days in a half-strength MS medium without supplementation of growth regulators, however, the addition of auxins, in particular K-IBA to the medium substantially improved the number and length of roots. 1.2 mg L-1 was the best concentration because it produced 12 roots of 14 mm in length (Table 3). One hundred percent of the micropropagated plants survived successfully to the acclimation conditions we provided and after one year of growing under greenhouse conditions we observed that plants inoculated with mycorrhizal fungi had higher rates of growth in comparison to the uninoculated.

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Literature Cited

1. Hernández-Oria, J. G., Chávez-Martínez, R. J., Sánchez-Martínez, E. 2007. Factores de riesgo en las cactáceas amenazadas de una región semiárida en el sur del Desierto Chihuahuense, México. Interciencia 32: 728-734.
2. Gómez-Sánchez, A. 2006. Enciclopedia Ilustrada de los Cactos y Otras Suculentas (Descripción de las Especies, Hábitat y Cuidados de Cultivo). Volumen II. Editorial Mundi-Prensa, Espana. 151 pp.
3. Norma Oficial Mexicana (NOM-059-SEMARNAT-2010). Diario Oficial de la Federación. Órgano del Gobierno Constitucional de los Estados Unidos Mexicanos. México, D. F. jueves 30 de diciembre de 2010.
4. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Phys. Plant. 15: 473-497.

Table 1. Effect of different cytokinins and concentration on shoot number during induction stage of *Astrophytum asterias* (Zucc.) Lem (Cactaceae) after 90 days of culture.

Treatment (mgL-1)	Shoot number
KN 18	22.833a*
BA 16	14.167b
BA 20	14.083b
KN 21	12.917bc
KN 15	12.250bc
BA 12	11.083c
BA 24	7.667d
BA 8	7.333d
KN 12	7.333d
BA 4	3.417e
KN 6	2.917e
KN 9	2.917e
BA 0	0.000f
KN 0	0.000f

*values with the same letter are statistically equal according to the Tukey Multiple Comparison Test ($\propto$= 0.05).

Table 2. Effect of different cytokinins on shoot number during proliferation subcultures of *Astrophytum asterias* (Zucc.) Lem (Cactaceae) after 40 days of culture.

Tratamiento (mgL-1)	Valor medio (NBE)
KN 18	26.185a*
BA 16	18.556b

*values with the same letter are statistically equal according to the Tukey Multiple Comparison Test ($\propto$= 0.05). n= 4.

Table 3. Effect of K-IBA on adventitious root formation of regenerated shoots *Astrophytum asterias* (Zucc.) Lem (Cactaceae) after 60 days of culture.

Treatment (mgL-1)	Root number
AIB 1.2	12.444a*
AIB 2.4	10.167b
AIB 0.6	8.000c
AIB 0	4.167d

*values with the same letter are statistically equal according to the Tukey Multiple Comparison Test ($\propto$= 0.05). n= 4 .

Massive plantlet propagation of *Mammillaria hernandezii* (Glass et Foster) through tissue culture

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Index Words *Mammillaria*, biznaguita, micropropagation, *in vitro* propagation, tissue culture.

Significance to Industry We conducted this study to develop an *in vitro* micropropation system for *Mammillaria hernandezii*. The establishment of a successful micropropagation scheme may consequently produce a positive impact to the nursery and ornamental industries because the procedure described here can be used with commercial orientation for massive propagation and to rescue native populations and recover natural habitats.

Nature of Work *Mammillaria* is one of the largest cacti genera of the Cactaceae family with more than 350 recognized species. Most of the species of *Mammillaria* are endemic of the Chihuahuan desert (USA and México), the Antilles and Venezuela (1). *Mammillaria hernandezii* naturally grows in the state of Oaxaca in the Tehuacán-Cuicatlán valley (1, 2, 4). It is a beautiful cactus of small size that produce dark pink flowers. The Mexican government through the NOM-059-SEMARNAT-2010 and some international institutions including CITES have classified the wild populations under endanger due to over collection, serious reproductive problems and degridation and disturbance of its natural habitat (3). Because of this, we have been working for several years in an institutional program to contribute to the study and rescue this and other cacti species. Through the establishment of tissue culture methods our main objective for this plant species was to set up the conditions for an efficient protocol of micropropagation. In order to do this, we focused first in producing the explants, which corresponded to aseptic shoots obtained from aseptic germinated seedlings. The seeds were initially cleaned through immersion in an ethanol solution for 5 min, immersion in a Clorox solution (30% v/v) plus 0.1% of Tween-20 for 20 min and rinsed 5 times in deionized sterile water, and then treated for 0, 4, 6, 8, and 10 weeks (0, 672, 1008, 1344, and 1680 h) to a stratification treatment of 4° C. After the treatment, the seeds were inoculated in a half strength Murashige and Skoog (MS), (5) medium.

For the initial cultures (step I of micropropagation), we ran a simple experiment with a randomized design to assess the effects of four auxin:cytokinins concentrations (NAA= 1 mgL^{-1}) and (BAP= 0, 3, 4.5, 6 mg/L^{-1}) on breaking dormancy of axillary buds. For the proliferation subcultures (step II of micropropagation), we performed a simple

experiment with a randomized design to compare the best two treatments from step I (NAA= 1 mgL^{-1}) and (BAP= 4.5, 6 mg/L^{-1}) to optimize de conditions to increase shoot production. For the rooting stage (Step III), four treatments (0, 0.6, 1.2 and 2.4 mg/L^{-1} of K-IBA) were established in a simple experiment with a randomized design to test their effect on adventitious root formation. In all experiments, we use the MS culture media (5), which was prepared with an adjusted pH= 5.8, agar= $7gL^{-1}$, and sucrose= 3%. The cultures were incubated in a room with a photoperiod of 16 h of light, and light conditions of (400 $\mu mol.m^{-2}.s^{-1}$ of photosynthetic photon flux density, PPFD). Experimental variables included data on % calli production, number of shoots per explant, root number, total root length (mm), mean root length (mm), and % plantlet survival. All data were subjected to ANOVA and Tukey test ($\propto$= 0.05) for mean separation.

Results and Discussion: The process of seed germination started 14 days after inoculation and by day 60 after the cold treatment we registered 37.8 % of total seed germination for the best treatment (6 weeks of stratification). At this time, the seedlings from this treatment produced higher number of roots (6.17) and number of areoles (2.99) per seedlings h Table 1). In the stage I of micropropagation, we observed after 40 days of culture that all the treatments evaluated induced the production of two responses: production of undifferentiated rapid growing calli, which particularly turned red as they growth and differentiation of organogenic structures (shoots) regardless the concentration added to the culture medium. Both, callogenesis and organogenesis were initiated in the base of the explant (shoot without roots from the seedlings), however, calli were produced from cells at the cut surface while shoots were originated through activation of axillary meristems, which were located in the areole of each tubercle. Data of calli production varied according to the concentration of cytokinin in combination to $1mgL^{-1}$ of NAA. The combination (NAA $1mgL^{-1}$ and BAP 4.5 mgL^{-1}) produced the higher calli formation in the explants (66 %), however, the mean separation test did not show significance in comparison to the data of the other trreatments that only 50% of explants produced calli. In contrast to this, the control media that lack growth regulators showed no calli production. In regarding to the shoot production, this treatment also produced the higher values for shoot number (7.2 per explant), which resulted statistically significant as compared to the other treatments (Table 2).

Similar to what happened in the induction cultures, during sub-cultures (stage II of micropropagation), the explants produced two general responses: proliferating un-differientiated calli and shoots. Shoots, in general, were produced in the two treatments evaluated. However, treatment including NAA $1mgL^{-1}$ and BAP 4.5 mgL^{-1} produced higher averages (27.75) per explant, which resulted significantly different to the 17.50 shoots produced by the combination (NAA $1mgL^{-1}$ and BAP 6 mgL^{-1}). Interestingly, the propagation rate was significantly increased in comparison to what was observed in the induction cultures. In this experiment, we observed that calli were also produced by the two treatments regardless the concentration used, however, the data ranged from 75 to 92 %. The ANOVA of these data showed no statistical significance between the treatments (Table 3).

Whole plantlets were easily obtained through adventitious root formation since rooting of the regenerated shoots was produced on half-strength MS medium free of auxins, however, the supplementation of indole-3-butyric acid to the culture medium substantially improved the number and length of roots, however, the best treatment that was statistically higher to the rest of treatment was 2.4 mg L-1 because it produced 6.6 roots of 18.5 mm in lenght (Table 4).

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Literature Cited

1. Gómez-Sánchez, A. 2006. Enciclopedia Ilustrada de los Cactos y Otras Suculentas(Descripción de las Especies, Hábitat y Cuidados de Cultivo). Volumen II. Editorial Mundi-Prensa, Espana. 151 pp.
2. Mandujano-Sánchez, M. C. 2003. Ficha técnica de Mammillaria hernandezii. Clasificación de tres especies endémicas de *Mammillaria* dentro del PROY-NOM-ECOL-059-2000. Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México. Bases de datos SNIB-CONABIO. Proyecto No. W031. México, D.F.
3. Norma Oficial Mexicana (NOM-059-SEMARNAT-2010). Diario Oficial de la Federación. Órgano del Gobierno Constitucional de los Estados Unidos Mexicanos. México, D. F. jueves 30 de diciembre de 2010.
4. Pilbeam, J. and Weightman, B. 2006. Ariocarpus et cetera. British Cactus and Succulent Society, 276 pp.
5. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Phys. Plant. 15: 473-497.

Table 1. Effect of auxin and cytokinin mix on calli production and shoot number during culture induction of *Mammillaria hernandezii* **(**Glass et Foster**)** after 80 days of culture.

Treatment (mgL-1)	Shoot number	Calli production (%)
ANA 0 : BAP 0	0d*	0b*
ANA 1 : BAP 3	2.417c	50.0a
ANA 1 : BAP 4.5	7.167a	66.6a
ANA 1 : BAP 6	4.33bb	50.0a

*values with the same letter are statistically equal according to the Tukey Multiple Comparison Test ($\propto$= 0.05).

Table 2. Effect of auxin and cytokinin mix on calli production and shoot number during proliferation subcultures of *Mammillaria hernandezii* (Glass et Foster) after 40 days of culture.

Treatment (mgL-1)	Shoot number	Calli production (%)
ANA 1 : BAP 4.5	27.75a*	91.67a*
ANA 1 : BAP 6	17.50b	75.00a

*values with the same letter are statistically equal according to the Tukey Multiple Comparison Test ($\propto$= 0.05).

Table 3. Effect of K-IBA on adventitious root formation of regenerated shoots *Mammillaria hernandezii* (Glass et Foster) after 40 days of culture.

Treatment (mgL-1)	Root number	Root Length (mm)
AIB 0	2.16c*	8.01b*
AIB 0.6	2.33	9.35b
AIB 1.2	4.08b	8.29b
AIB 2.4	6.58a	18.54a

*values with the same letter are statistically equal according to the Tukey Multiple Comparison Test ($\propto$= 0.05).