

**IN VITRO PROPAGATION OF *OBREGONIA DENEGRII* FRIČ. (CACTACEAE)**

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**Abstract**

The possibility of establishing an efficient *in vitro* technique for propagation and conservation of the endangered cactus, *Obregonia denegrii* Frič., was investigated. Disinfested seeds were incubated on filter paper wetted with distilled water; otherwise seeds were soaked in 1.4  $\mu\text{M}$  of  $\text{GA}_3$  or in distilled water (control), then disinfested and placed on solid MS medium. In the first experiment, seeds germination reached a maximum value of 72% after 28 days. Compared to control (22%),  $\text{GA}_3$  treatment significantly increased germination rate (85%) just after 7 days. Four week-old seedlings were subcultured on MS medium supplemented with different plant growth regulators. After 4 months the growth index was 1.62 in presence of 10.7  $\mu\text{M}$  of NAA and 0.74 with 4.4  $\mu\text{M}$  of BAP. MS medium containing 4.4  $\mu\text{M}$  of BAP and 10.7  $\mu\text{M}$  of NAA was used as control in the further experiments. Shoot multiplication was investigated for different explants (longitudinal dissections of apical explants, single tubercles, apical, basal and radical explants) on different variants of MS medium with 1 mM of putrescine, 10.2  $\mu\text{M}$  of  $\text{AgNO}_3$  or 12.1  $\mu\text{M}$  of CPPU. Independently from substrate composition, multiple shoot formation from areoles was achieved from longitudinal dissections of apical explants and single tubercles. CPPU gave the highest number of shoots/explant (5.0) followed by putrescine (2.4),  $\text{AgNO}_3$  and control treatment (1.1). The callus proliferation was evident only on 36.8% of explants cultured with CPPU while the spontaneous root formation (33%) was observed in medium containing putrescine.

**Key words:** CPPU, germination, micropropagation, *Obregonia denegrii*, putrescine, silver nitrate

**REFERENCES**

- AKASAKA-KENNEDY Y., YOSHIDA H., TAKAHATA Y. (2005). Efficient plant regeneration from leaves of rapeseed (*Brassica napus* L.): The influence of  $\text{AgNO}_3$  and genotype. *Plant Cell Reports*, 24: 649-654.
- ANDERSON E. F. (2001). *The Cactus Family*. Timber Press, Portland, 73 pp.
- ASHRAF M., WAHID S. (2000). Time-course changes in organic metabolites and mineral nutrients in germinating maize seeds under salt (NaCl) stress. *Seed Science and Technology*, 28: 641-656.
- BAIS H. P., SUDHA G. S., RAVISHANKAR G. A. (2000). Putrescine and silver nitrate influences shoot multiplication, *in vitro* flowering and endogenous titers of polyamines in *Cichorium intybus* L. cv. Lucknow local. *Journal of Plant Growth Regulation*, 19: 238-248.
- BRASIL J. N., JEREISSATI E. S., SANTOS M. R. A., CAMPOS F. A. P. (2005). *In vitro* micropropagation of *Nopalea cochenillifera* (Cactaceae). *Journal of Applied Botany and Food Quality*, 79: 160-162.
- CHI G. L., LIN W. S., LEE J. E. E., PUA E. C. (1994). Role of polyamines on de novo shoot morphogenesis from cotyledons of *Brassica campestris* ssp. *pekinensis* (Lour) Olsson *in vitro*. *Plant Cell Reports*, 13: 323-329.
- COUÉE I., HUMMEL I., SULMON C., GOUESBET G., EL AMRANI A. (2004). Involvement of polyamines in root development. *Plant Cell, Tissue and Organ Culture*, 76: 1-10.
- DABEKAUSSEN M. A. A., PIERIK R. L. M., VAN DER LAKEN J. D., HOEK S. J. (1991). Factors affecting areole activation *in vitro* in the cactus *Sulcorebutia alba* Rausch. *Scientia Horticulturae*, 46: 283-294.
- DENO N. C. (1994). The critical role of gibberellins in germination and survival of certain cacti. *Cactus and Succulent Journal*, 66: 28-30.
- DIVYA K., SWATHI ANURADHA T., JAMI S. K., KIRTI P. B. (2008). Efficient regeneration from hypocotyl explants in three cotton cultivars. *Biologia Plantarum*, 52: 201-208.
- FEIRER R. P., MIGNON G., LITVAY J. D. (1984). Arginine decarboxylase and polyamines required for embryogenesis in the wild carrot. *Science*, 223: 1433-1435.
- GIUSTI P., VITTI D., FICCHETTI F., COLLA G., SACCARDO F., TUCCI M. (2002). *In vitro* propagation of three endangered cactus species. *Scientia Horticulturae*, 95: 1-14.
- GUO D. P., ZHU Z. J., HU X. X., ZHENG S. J. (2005). Effect of cytokinins on shoot regeneration from cotyledon and leaf segment of stem mustard (*Brassica juncea* var. *tsatsai*). *Plant Cell, Tissue and Organ Culture*, 83: 123-127.
- GUTIÉRREZ MICELI F. A., ESTUDILLO A. D., ABUD ARCHILA M., DEL ROSARIO AYORA TALAVERA T., DENDOOVEN L. (2008). Optimization of *Renalmia mexicana* (Klotzsch ex. Petersen) cultivation *in vitro*. *In Vitro Cellular and Developmental Biology-Plant*, 44: 33-39.
- HUBSTENBERGER J. F., CLAYTON P. W., PHILLIPS G. C. (1992). Micropropagation of cacti (Cactaceae). In: Bajaj Y. P. S. (Ed.), *Biootechnology in Agriculture and Forestry*, vol. 20, High-tech and Micropropagation IV. Springer, Berlin: 49-68.
- IVANOVA M., NOVÁK O., STRNAD M., VAN STADEN J. (2006). Endogenous cytokinins in shoots of *Aloe polyphylla* cultured *in vitro* in relation to hyperhydricity, exogenous cytokinins and gelling agents. *Plant Growth Regulation*, 50: 219-230.
- JUÁREZ M. A., PASSERA C. B. (2002). *In vitro* propagation of *Opuntia ellisiana* Griff. and acclimatization to field conditions. *Biocell*, 26: 319-324.
- KAPCHINA-TOTEVA V., VAN TELGEN H.-J., YAKIMOVA E. (2000). Role of phenylurea cytokinin CPPU in apical dominance release in *in*

- in vitro* cultured *Rosa hybrida* L. Journal of Plant Growth Regulation, 19: 232-237.
- KUCHARSKA D., GRUCHALA A., ORLIKOWSKA T. (2006). *In vitro* propagation of four rose rootstocks. Propagation of Ornamental Plants, 6: 44-50.
- KUMAR V., SHARMA A., NARISIMHA PRASAD B. C., GURURAJ H. B., GIRIDHAR P., RAVISHANKAR G. A. (2007). Direct shoot bud induction and plant regeneration in *Capsicum frutescens* Mill.: influence of polyamines and polarity. Acta Physiologiae Plantarum, 29: 11-18.
- LI C., BANGERTH F. (2003). Stimulatory effect of cytokinin and interaction with IAA on the release of lateral buds of pea plants from apical dominance. Journal of Plant Physiology, 160: 1059-1063.
- LIN H. S., DE JEU M. J., JACOBSEN E. (1997). Direct shoot regeneration from excised leaf explants of *in vitro* grown seedlings of *Alstroemeria* L. Plant Cell Reports, 16: 770-774.
- MACHADO M. F. P. S., PRIOLI A. J. (1996). Micropropagation of *Cereus peruvianus* Mill. (Cactaceae) by areole activation. In Vitro Cellular and Developmental Biology-Plant, 32: 199-208.
- MALDA G., BACKHAUS R. A., MARTIN C. (1999a). Alterations in growth and crassulacean acid metabolism (CAM) activity of *in vitro* cultured cactus. Plant Cell, Tissue and Organ Culture, 58: 1-9.
- MALDA G., SUZÁN H., BACKHAUS R. A. (1999b). *In vitro* culture as potential method for the conservation of endangered plants possessing crassulacean acid metabolism. Scientia Horticulturae, 81: 71-87.
- MARTINEZ-VAZQUEZ O., RUBLUO A. (1989). *In vitro* mass propagation of the near-extinct *Mammillaria san-angelensis* Sánchez-Mejorada. Journal of Horticultural Science, 64: 99-105.
- MAYOR M. L., NESTARES G., ZORZOLI R., PICARDI L. A. (2003). Reduction of hyperhydricity in sunflower tissue culture. Plant Cell, Tissue and Organ Culture, 72: 99-103.
- MOEBIUS-GOLDAMMER K. G., MATA-ROSAS M., CHÁVEZ-AVILA V. (2003). Organogenesis and somatic embryogenesis in *Ariocarpus kotschoubeyanus* (Lem.) K.Schum. (Cactaceae), an endemic and endangered mexican species. In Vitro Cellular and Developmental Biology-Plant, 39: 388-393.
- MORÁN G. P., COLQUE R., VILADOMAT F., BASTIDA J., CODINA C. (2003). Mass propagation of *Cyrtanthus clavatus* and *Cyrtanthus spiralis* using liquid medium culture. Scientia Horticulturae, 98: 49-60.
- MURASHIGE T., SKOOG F. (1962). A revised medium for rapid growth and bioassays with tobacco cultures. Physiologia Plantarum, 15: 473-497.
- NOLASCO H., VEGA-VILLASANTE F., ROMERO-SCHMIDT L., DÍAZ-RONDERO A. (1996). The effects of salinity, acidity, light and temperature on the germination of seeds of cardón (*Pachycereus pringlei*) (S.Wats) Britton & Rose (Cacataceae). Journal of Arid Environments, 33: 87-94.
- OLVERA-CARRILLO Y., MÁRQUEZ-GUZMÁN J., BARRADAS V. L., SÁNCHEZ-CORONADO MA E., OROZCO-SEGOVIA A. (2003). Germination of the hard seed coated *Opuntia tormentosa* S.D., a cacti from the México Valley. Journal of Arid Environments, 55: 29-42.
- ORTEGA-BAES P., ROJAS-ARÉCHIGA M. (2007). Seed germination of *Trichocereus terscheckii* (Cactaceae): light, temperature and gibberellic acid effects. Journal of Arid Environments, 69: 169-176.
- OZDEN-TOKATLI Y., OZUDOGRU E. A., AKCIN A. (2005). *In vitro* response of pistachio nodal explants to silver nitrate. Scientia Horticulturae, 106: 415-426.
- PADILLA I. G. M., ENCINA C. L. (2003). *In vitro* germination of cherimoya (*Annona cherimola* Mill.) seeds. Scientia Horticulturae, 97: 219-227.
- PÉREZ-MOLPHE-BALCH E., DÁVILA-FIGUEROA C. A. (2002). *In vitro* propagation of *Pelecyphora aselliformis* Ehrenberg and *P. strobiliformis* Wedermann (Cacataceae). In Vitro Cellular and Developmental Biology-Plant, 38: 73-78.
- PÉREZ-MOLPHE-BALCH E., PÉREZ-REYES M. E., DÁVILA-FIGUEROA C. A., VILLALOBOS-AMODOR E. (2002). *In Vitro* Propagation of columnar cacti from the Sonoran Desert. HortScience, 37: 693-696.
- PUA E. C., SIM G. E., CHI G. L., KONG L. F. (1996). Synergistic effect of ethylene inhibitors and putrescine on shoot regeneration from hypocotyl explants of Chinese radish (*Raphanus sativus* L. var. *longipinnatus* Bailey) *in vitro*. Plant Cell Reports, 15: 685-690.
- QIN Y., ZHANG S., ZHANG L., ZHU D., SYED A. (2005). Response of *in vitro* strawberry to silver nitrate (AgNO<sub>3</sub>). HortScience, 40: 747-751.
- RAMIREZ-MALAGON R., ANGUIAR-RAMIREZ I., BORODANENKO A., PEREZ-MORENO L., BARRERA-GUERRA J. L., NUÑEZ-PALENIUS H. G., OCHOA-ALEJO N. (2007). *In vitro* propagation of ten threatened species of *Mammillaria* (Cactaceae). In Vitro Cellular and Developmental Biology-Plant, 43: 660-665.
- REYES S. J. (1994). Propagación de Cactáceas Mexicanas: una alternativa para la conservación de especies amenazadas y en peligro de extinción. In: Encuentro Internacional sobre el Impacto de la Biotecnología en el Desarrollo Sustentable. PROMESUP, OEA: 108-119.
- RODRIGUEZ-GARAY B., RUBLO A. (1992). *In vitro* morfogenetic responses of the endangered cactus *Aztekium ritteri* (Boedeker). Cactus and Succulent Journal, 64: 116-119.
- ROJAS-ARÉCHIGA M., CASAS A., VÁZQUEZ-YANES C. (2001). Seed germination of wild and cultivated *Stenocereus stellatus* (Cactaceae) from the Tehuacán-Cuicatlán Valley, Central México. Journal of Arid Environments, 49: 279-287.
- ROMERO-SCHMIDT H. L., VEGA-VILLASANTE F., NOLASCO H., MONTAÑO C. (1992). The effect of darkness, freezing, acidity and salinity on seed germination of *Ferocactus peninsulare* (Cactaceae). Journal of Arid Environments, 23: 389-395.
- RUBLUO A., CHÁVEZ V., MARTÍNEZ-VÁZQUEZ O. (1993). Strategies for the recovery of endangered orchids and cacti through *in-vitro* culture. Biological Conservation, 63: 163-169.
- RUBLUO A., MARÍN-HERNÁNDEZ T., DUVAL K., VARGAS A., MÁRQUEZ-GUZMÁN J. (2002). Auxin induced morphogenetic responses in long-term *in vitro* subcultured *Mammillaria san-angelensis* Sánchez-Mejorada (Cactaceae). Scientia Horticulturae, 95: 341-349.
- SANTOS-DÍAZ M. S., MÉNDEZ-ONTIVEROS R., ARREDANDO-GÓMEZ A., SANTOS-DÍAZ M. L. (2003). *In vitro* organogenesis of *Pelecyphora aselliformis* Erhenberg (Cactaceae). In Vitro Cellular and Developmental Biology-Plant, 39: 480-484.
- SCHOLTEN H. J. (1998). Effect of polyamines on the growth and development of some horticultural crops in micropropagation. Scientia Horticulturae, 77: 83-88.

- SINGH S. K., SYAMAL M. M. (2001). A short pre-culture soak in thidiazuron or forchlorfenuron improves axillary shoot proliferation in rose micropropagation. *Scientia Horticulturae*, 91: 169-177.
- SPOMER L. A., SMITH M. A. L. (1996). Direct measurement of water availability in gelled plant tissue culture media. *In Vitro Cellular and Developmental Biology-Plant*, 32: 210-215.
- SRISKANDARAJAH S., GOODWIN P. (2005). *In vitro* propagation of *Tasmannia stipitata* AC Smith. *Propagation of Ornamental Plants*, 5: 95-99.
- SUNAGAWA H., AGARIE S., UMEMOTO M., MAKISHI Y., NOSE A. (2007). Effect of urea-type cytokinins on the adventitious shoots regeneration from cotyledonary node explant in the common ice plant, *Mesembryanthemum crystallinum*. *Plant Production Science*, 10: 47-56.
- TARENGHI E., CARRÉ M., MARTIN-TANGUY J. (1995). Effects of inhibitors of polyamine biosynthesis and of polyamines on strawberry microcutting growth and development. *Plant Cell, Tissue and Organ Culture*, 42: 47-55.
- TIAN C. E., LI R. G., GUAN H. (1994). Relationship between polyamines and morphogenesis in cotyledons of *Cucumis melo* L. cultured *in vitro*. *Acta Botanica Sinica*, 36: 219-222.
- VASUDEVAN A., SELVARAJ N., GANAPATHI A., KASTHURIRENGAN S., RAMESH ANBAZHAGAN V., MANICKAVASAGAM M., CHOI C. W. (2008). Leucine and spermidine enhance shoot differentiation in cucumber (*Cucumis sativus* L.). *In Vitro Cellular and Developmental Biology-Plant*, 44: 300-306.
- Zhu C., Chen Z. (2005). Role of polyamines in adventitious shoot morphogenesis from cotyledons of cucumber *in vitro*. *Plant Cell, Tissue and Organ Culture*, 81: 45-53.