STUDY ON THE IN VITRO CULTURE INCREASE AND DEVELOPMENT BY Echinocactus mihanovichii AT THE COMPOSITION OF THE ADDITIONAL CULTURAL ENVIRONMENT WITH DIFFERENT CONTAINS OF 3-indolyl butyric acid (IBA)

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Abstract

Gymnocalycium mihanovichii lacking chlorophyll has a different color compared to most cactus. The red color can only be preserved by grafting or cloning. From aesthetic point of view it is highly appreciated.

We harvested coastal portions that have fragmented so that eachhold3-4explant areola. The culture medium sed was composed of macro-elements, Murashige-Skoog (1962), supplemented with 3-indolyl butyric acid (IBA) at different concentrations, respectively 1 mg/l IBA (V_1); 1,5 mg/l IBA (V_2) and 2 mg/l IBA (V_3).

Evolution vitro cultures was monitored for 90days. At the end of the experiment, we noticed that the Gymnocalycium mihanovichii explants differs very little depending on the composition of the culture medium, as follows: Calusogenesis occurred only in inoculums cultured on medium supplemented with 2 mg/l AIB (V_3), the rhizogenesis or formation of strains did not occur at any time of the experimental variants studied in this experiment. A steady increase in diameter at all inoculums was noted for the experimental variants, regardless of the AIB concentration in the culture medium.

Keywords: cacti, vitrocultures, 3-indolyl butyric acid–IBA,stems, calluses

INTRODUCTION

Gymnocalycium mihanovichii is a South American cactus species, Paraguay (Copăcescu, 2001), commonly grown as an ornamental plant. The most popular varieties are mutants lacking chlorophyll, red, orange or yellow. It is a cactus that fascinates not only the tenderness and beauty of flower sand red skin (Fig.1).

a.  b. 

Fig. 1.Images of*Gymnocalycium mihanovichii*. Where: a-decorativestems; b-flower

The presence of this cactus is a highly appreciated appearance spectacular. The pigmentation is caused by the appearance of spontaneous, in cultures of mutations largely influenced by temperature and light (Shemorakov, 2003). Due to reversible mutation plastids during meiosis (Shemorakov, 2003) by generative reproduction chances that these plants to keep the coloris minimal (Kornilov, 2008), thus it was concluded that plants can retain color only reproduced by cloning. This has led, as now, to seek new technologies for rapid multiplicationas economically efficient these plants (Son, 2000, Leeet al., 2003).

Gymnocalycium mihanovichii is a deficiency of chlorophyll cactus, with red skin (Copăcescu, 2001), deprived of the opportunity to synthesize chlorophyll chloroplasts due to the small, about 1/3 of all plastids (Shemorakov, 2003).

The process of discoloration is caused by spontaneous mutations in culture (Shemorakov, 2001) greatly influenced by temperature and light. After Skulkin (2000) plants maintained at a temperature lower than normal and shadow, growings lowly, if at all, such mutations. Russian scientists showed great interest in the species chlorophyll - deficient cactus, so they made their classification based on the color of skin (Shemorakov, 2003), that, *Gymnocalycium mihanovichii* is part of a single color.

After Shemorakov (2001) reversible plastid mutation during meiosis makes the reproduction generation to *Gymnocalycium mihanovichii* have little chance for it to retain color (Kornilov 2008). thus it concluded that plants can retain this particular property only reproduced by cloning.

Plant hormones or growth regulators are organic compounds in concentrations much lower than those nutrients or vitamins, stimulates or inhibits growth and morphogenesis, respectively regulate different physiological processes in the tissues and organs of the plant.

3-indolyl butyric acid (AIB) is a class of synthetic auxin, but apparently can be found in nature, but only in some plant species (after, Cachiţă et al., 2004). Auxins are commonly used in tissue culture with stimulants rootedness process and with cytokinins, and they play a major role in the proliferation and growth of plant cells. Fito inoculated tissues synthesized, as well, auxin - or IBA - in the apical meristems, and by adding to the culture medium of the phytohormone produced by synthesis, are favored rootedness. At vitroculturile of *Opuntia ellisiana* on culture media with added 3-indolyl butyric acid (AIB), the percentage of rooting explants was 100%, considering that the cumulative effect of endogenous auxin in the intake of exogenous auxins leads getting a large number of roots (Juárez et al., 2002).

MATERIAL AND METHODS

Disinfection plant material was achieved by submers area explants for one minute in 96° alcohol, after which they were coated with a solution of 0,8%sodiumhypochlorite mixed with water in a ratio of1:2, and the surfactant is added three drops ofTween20.Duringthis operation, which was 20 minutes, the plant material was continuously stirred. After decanting disinfectant plant material was washed with sterile distilled water to remove chlorine, achieving five consecutive rinses, of five minutes each.

Plant material, such disinfect was deposited on filter paper discs (previously sterilized in the oven) in Petri capsules in a laminar flow hood, horizontal air sterile operation, operation followed by inocula future size and removing necrotic parts there of. Knowing that if in vitro cultures of cactus induction of roots, shoots or callus, gives the best results if you use large explants, which have at least three areola (Dabenkaussenet al., 1991), where.

The experimental current in inside the laminar flow hood, horizontal air sterile in operation, we cut along the ribs strain (longitudinal) (Fig.2a). After this operation we obtained a fragment of strain in a semicircle (which have side with nipples) We removed tissue from the middle portion leaving to experts about 0,7 to 1cm parenchymal tissue(Fig.2b), and moved to portioning future in oculated explants so that eachhold3-4areola, and sizes to fit in to already established(Fig.2c).

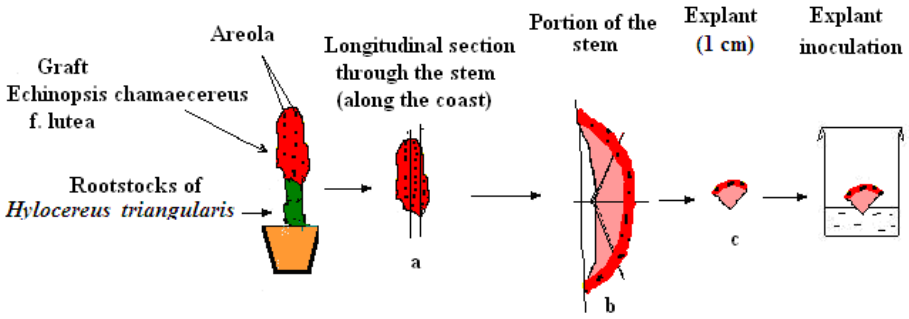

Fig. 2.Scheme representing how to cut Gymnocalycium mihanovichii buds to obtain inoculums

Culture medium used for growth explants consisted of: macro Murashige-Skoog EDTA and Fe (1962), Heller microelements (1953), mineral mixture to which was added vitamins: pyridoxine HCl, thiamine

HCl and nicotinic acid (containing 1 mg/l each), m-Inositol - 100 mg/l, sucrose - 20 g/l and agar 7 g/l the pH of the medium was adjusted to5.8, the first to autoclaving. In the base medium (MB) we added 3-indolyl butyric acid in different concentrations as follows: 1 mg/l IBA (V_1 variant), 1,5 mg/l IBA (V_2 variant) and 2 mg/l IBA (V_3variant), obtaining the following experiments: V_0 - control variant, environment without growth regulators; V_1 - 1 mg/l IBA; V_2 – 1,5 mg/l IBA and V_3 - 2 mg/l IBA.

Culture medium thus obtained was placed in a glass vial with a capacity of 15 ml (each container was placed 5 ml of medium). Medium vials were sterilized by autoclaving for 30 minutes at a temperature of 121°C. After cooling media proceeded to inoculate explants, aseptic room operation performed in a laminar flow hood with sterile air. To obstruction fitoinoculi containers we used polyethylene, immobilized with elastic. Containers were inoculated Transferred to room for growth, under the Following Conditions: temperature ranged from 24ºC in the range of light and 20°C during the phase of darkness and light was the regime fotoperiodic 16 hours with light / 24h, lighting Achieving cultures with the white light emitted by fluorescent lamps, the intensity of 1700 lux.

Explants and explants reaction progress was monitored for 90 days. In this time period were conducted periodic observations and reading severy 30 days. Values thus obtained in the control group (V_0, fitoinoculig row non basic medium, without growth regulators) were considered the reference as 100% beingreported- everytrait- allreading save ragedevery experimental variant part.

RESULTS AND DISCUSSION

After 90 days of vitro culture, *Gymnocalycium mihanovichii* explants showed an increase in the basal mean diameter of the main strain much accelerated in the phytoinocultures of the control sample V_0 (medium lacking growth regulators), compared to those grown on medium supplemented with auxin (AIB). The recorded data show that at a mean diameter of the 1,1 cm strain (Fig.3A).

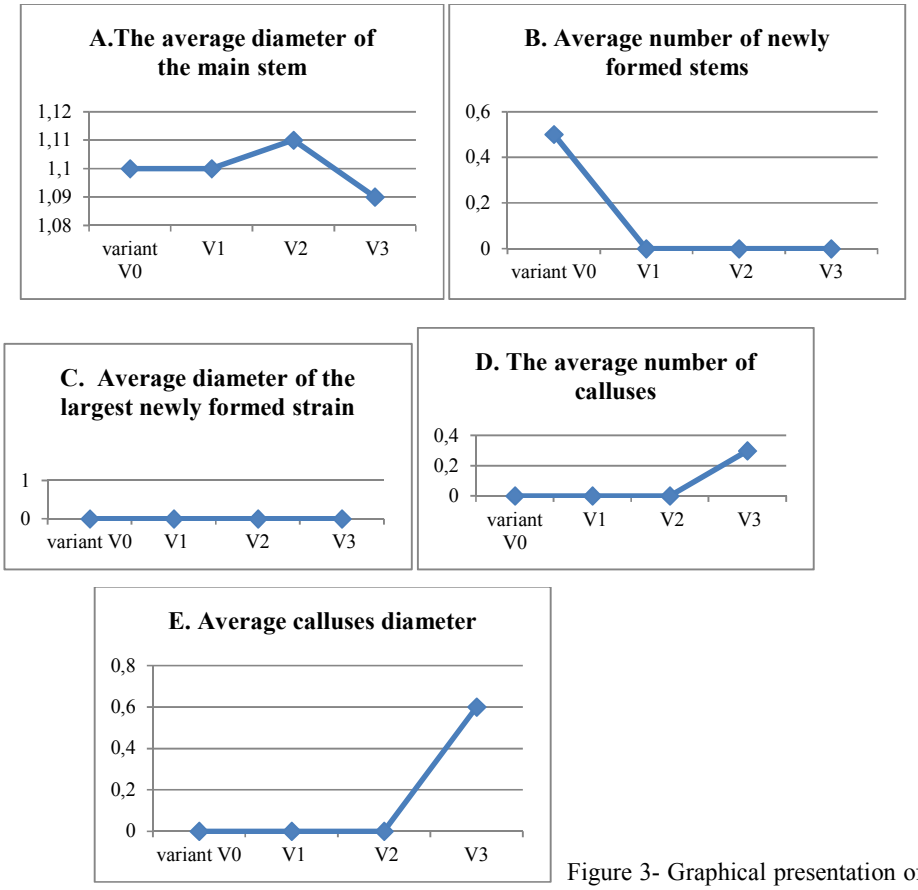

Figure 3- Graphical presentation of the mean values corresponding to the parameters observed at the level of the Gymnocalycium mihanovichii vitrocultures, on aseptic base modified by us - (variant V0) - with addition of 1mg / l AIB (variant V1), 1.5 mg / l AIB (variant V2) or 2 mg / l AIB (variant V3), data expressed in absolute values; (where: A - the average diameter of the main stem, B - average number of newly formed stems, C - average diameter of the largest newly formed strain, D – the average number of calluses, E - average calluses diameter).

The experimental variant V_0 (the medium lacking growth regulators) was the only sample found to trigger the caulogenesis phenomenon, averaging 0,5 buds/variant (Fig. 3B), and a mean basal diameter of these of 0,4 cm (Fig. 3C).

At this time only the explants of variant V_3 (medium supplemented with 2 mg/l AIB) noted callus generation; their average number being 0,3 calluses/variant (Fig.3E), while its average diameter was 0,6 cm (Fig.3F).

It is known that *Gymnocalycium mihanovichii* is a cactus that roots hard, although they are specimens that live on their own roots; In the present experiment, it is noted that the explants taken in the study had no reaction to

the presence in auxin culture medium - 3-indolyl butyric acid (AIB) - irrespective of the concentration used in the current experiment.

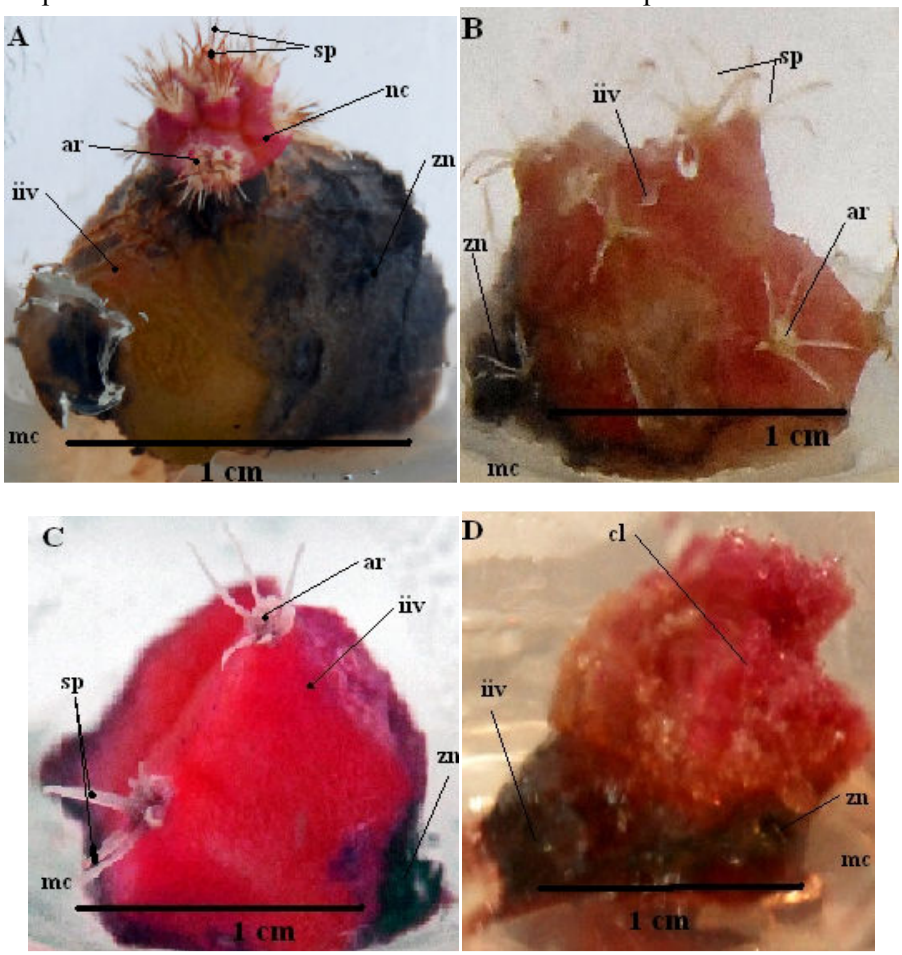

Fig.4. Inoculums of *Gymnocalycium mihanovichii*, 90 days after in vitro explant inoculation, where: A-on a modified base medium of the non-growth regulator (V_0); B-base medium with 1 mg/l AIB (V_1); C-on a basic medium with 1,5 mg/l AIB (V_2); D-on basic medium with addition of 2 mg/l AIB (V_3); (iiv–the initially viable inoculum; mc-culture medium; ar-areolas; sp-thorns; cl-callus; zn-necrotic area).

From a morphogenetic point of view - rhizogenesis, caulogeneis and calusogenesis - it is observed that from the four variants taken into study (V_0, V_1, V_2, V_3) only V_0 (medium lacking growth regulators) while at the explant level inoculated and grown on culture medium supplemented with 2 mg/l AIB (V_3), the calusogenesis phenomenon was stimulated. In the case of V_1 variants (medium supplemented with 1 mg/l AIB) and V_2 (medium supplemented with AIB 1,5 mg/l), there was no morphogenetic reaction at

the explants of these vitro cultures. These results are consistent with those obtained by Clayton et al. (1990), which reported a complete lack of reaction to the in vitro cultivation of *Mammillaria eichlamii Quehl* in a study of cacti in the genus *Mammillaria*, which suggests that each catus species might require a specific recipe (Johnson et al., 1979a and b;Starling et al., 1983**;** Vyskot et al., 1984,Martinez-Vasquez et al., 1989).

Analyzing the images in Figure 4, it is observed that after 90 days of vitro culture, *Gymnocalycium mihanovichii* innoculums remaining alive have grown and exhibited well-developed arioles and spines, but sectional areas are necrotic, a phenomenon manifested by color change - these become brown. The phytochemicals of experimental variant V_0 (medium lacking growth regulators) and V_1 (medium supplemented with 1 mg/l AIB) reveal the existence of areas where their initial color changed, from red turned to a brick, even orange with yellow tint (Fig.4A,B). At the level of explants increased on medium supplemented with 2 mg/l AIB (V_3), there is noticeable both the lack of spines and a red callus (Fig.4D). According to Cachita, (2004), red coloring or red nuances of callus is due to a very high content in anthocyanins, which accumulates in its cells due to its growth regime, species, origin and age; In the present case, this pigmentation may also be influenced by the red color of the epidermis of the chlorophyll-deficient cactus Echinocactus mihanovichii. A red, compact red callus, formed on the cut explant surface, was also obtained in some *Mammillaria* species, the color being due to the presence of beta alanine in its cells (Pérez et al., 1998).

CONCLUSION

1. Following the reaction and evolution of *Gymnocalycium mihanovichii*inoculations for 90 days of the values recorded for both the control group V_0 (culture medium lacking growth regulators) and considered as 100% as the reference for the other experimental variants did not find any significant differences in their reaction mode.
2. A steady increase in inoculum diameter was noted for all experimental variants irrespective of the AIB concentration in the culture medium.
3. Caulogenesis occurred only at the explants cultivated on medium supplemented with 2 mg/l AIB (V_3).
4. The phenomenon of rhizogenesis did not manifest in any of the experimental variants studied in this experiment.

REFERENCES

1. Cachiţă C.D., Deliu C., Tican R.L., Ardelean A., 2004, Tratat de biotehnolo-gie vegetală. Vol.I, Editura Dacia, Cluj-Napoca, p. 29-154.
2. Cachiţă C.D., Ardelean A., 2004, Vitroculturile vegetale în fitopatologie. In: Fiziologia celulei vegetale în regim de vitrocultură. Al XII-lea Simpozion National de Culturi de Tesuturi si Celule Vegetale, Jibou 5, Ed.Daya, Satu Mare, p. 18-29.
3. Clayton P.W., Hubstenberg J.F., Phillips G.C., Butler–Nance S.A., 1990, Micropropagation of members of the Cactaceae subtribe Cactinae. Journal of the American Society for Horticultural Science, vol. 115, nr. 2, p. 337 – 343.
4. Copăcescu V.S., 2001, Cactuşii, monografie; Ed. Ceres, Bucuresti, p. 11-517.
5. Dabekaussen R., Pierik R., Van der Laker J., Hoek J., 1991, Factors affecting areole activation in vitro in the cactus Sulcorebutia alba. Rausch. Scientia Horticulturae, vol. 46, p. 283 – 294.
6. Heller H., 1996, Labour, Science, and Tehnology in France, 1500-1620. Cambridge and New York: Cambridge University Press, p. 112, 376, 453-454.
7. Johnson J., Emino E., 1979a, Tissue culture propagation in the Cactaceae. Cactus and Succulent Journal (U.S.A.), vol. 51, p. 275 - 279.
8. Johnson J., Emino E., 1979b, In vitro propagation of Mammillaria elongate. HortScience, vol. 14, nr. 5, p. 605 – 606.
9. Juarezi, M.C., Passera C.B., 2002, In vitro propagation of Opuntia ellisiana Griff. and acclimatization to field conditions. Biocell, vol. 26, p. 319–324.
10. Kornilov L.P., 2008, Grafting on Pereskiopsis, Cultivar, publicat online: 20 decembrie.
11. Lee J.M., Oda M., 2003, Grafting of herbaceous vegetable and ornamental crops. Hort. Rev., vol. 28, p. 61-124
12. Martines –Vázquez O., Rubluo A., 1989, In vitro mass propagation of the near extinct Mammillaria san –angelensis Sánchez Mejorada. Journal of Horticultural Science, vol. 64, nr. 1, p. 99 – 105.
13. Murashige T., F. Skoog, 1962, A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473 –497.
14. Pérez E., Perez M., Villalobos E., Meza E., Morones L., Lizalde H., 1998, Micropropagation of 21 species of mexican cacti by axillary proliferation. In vitro Cellular Development Biology Plant, vol. 34, p. 131– 135.
15. Shemorakov, N., 2001, Plastid mutations. Cultivar, vol. 2, nr. 3, p. 11-20.
16. Shemorakov N., 2003, Cultivar's classification by stem color, Cultivar 2(18), Published Online: aprilie.
17. Starling R., Dodds J., 1983, Tissue culture propagation of cacti and other succulents. Bradleya, vol. 1, p. 84 – 90.
18. Son B.K., 2000, The culture of cacti & succulents (in Korean).Gyeonggi Province, Korea, vol. 28, p. 61-124
19. Vyskot B., Jara Z., 1984. Clonal propagation of cacti through axillary buds in vitro. Journal of Horticultural Science, vol. 59, nr. 3, p. 449 – 452.