STUDIENS ON THE INFLUENCE OF ACID CONCENTRATION - β INDOLILBUTIRIC (IBA) ON REGENERATIVE CAPACITY AND ORGANOGENESIS OF EXPLANTS OF *Opuntia* (Tournef.) Mill. *fragilis var. fragilis*

Vidican Iuliana Teodora*

**University of Oradea, Faculty of Enviromental Protection,11 Borsecului St., 410571 Oradea, e-mail: iuliateodora68@yahoo.com*

Abstract.

Due to the economic importance of plants of the genus Opuntia growing needs of young plants than did vital to find a quick method îmultiplicare and safest method is in vitro micropropagation.To establish vitroculturii of Opuntia fragilis var, fragilis I cladodii harvested young, they were broken into segments of 1/1 cm, thickness 0.5 cm and were inoculated in a mineral medium - macro, Murashige-Skoog (1962), with added growth regulators, micronutrients Heller (1953), supplemented with IBA (β-indolilbutiric acid) in different concentrations.

The evolution of explants was monitored for 90 days. Their response was different depending on the concentration of β-indolilbutiric acid (IBA) present in the culture medium. Finally, it was shown that explants of Opuntia fragilis var fragilis, showed caulogenesis phenomena and rootedness, callus is absent.

Keywords: cacti, vitrocultures, β-indolilbutiric acid (IBA), rootedness, shoots.

INTRODUCTION

The economic importance of the family *Cactaceae* plant led to studies on the cultivation of "*in vitro*" them, to achieve since 1957. Since 1962, the cacti came to the attention of several researchers, such as: Steinhart (1662), Mauseth (1977-1979), were closely concerned micropropagation cacti "*in vitro*" and studied behavior of various explants their cultivation under aseptic environments. The 80s many researchers such as Starling (1985), Martinez-Vázquaz et al., (1998, 1993) obtained encouraging results in terms of cultivation "*in vitro*" of cacti.

Opuntia cactus genus are among the most studied in the world (Griffith, 2001a; Pinkava, 2002). This is due to the economic importance of this cactus, they are valuable not only for edible fruit, but also as valuable as vegetable or vegetable meal as feed (Casas and Barbera, 2002). This plant is recognized as a good indicator of the presence of pollutants (Nobel, 1994), is also regarded as an important tool to combat desertification (Flores-Valdez, 1994).

Fast and efficient multiplication of these plants is achieved by micropropragare *in vitro* (Escobar et al., 1986; Rubluo et al., 1996; Smith et al., 1991).

Our aim was to study how the *Opuntia fragilis* reacted lime fragilis explants cultured *in vitro* culture in the presence of different concentrations of β-indolilbutiric acid (IBA).

MATERIALS AND METHODS

To initiate *in vitro* cultures of *Opuntia fragilis* var *fragilis* I keep prelevet strains with mature areolas but with less thorns trainers, shorts and white. The material so obtained was secţiont transverse operation which resulted dished washers that were divided so that eventually fragments were inoculated following dimensions: about 1 cm long and 0.5 cm thick, yet have minimum 2-3 areola. After these operations we obtain the explants from mid dial and lateral (Fig. 1).

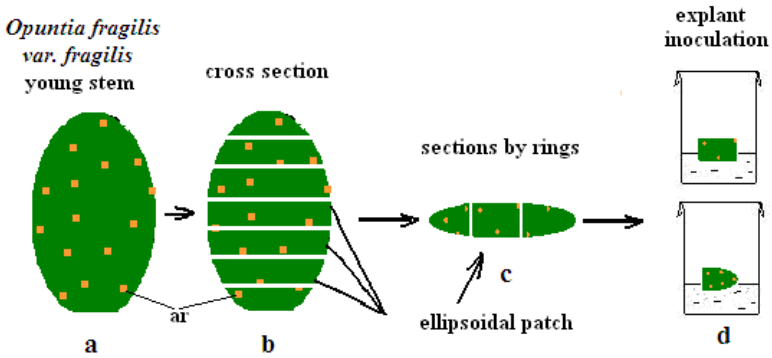

Fig. 1. Schemetic representation of *Opuntia fragilis* var *fragilis* young stems (a, b), and how slicing it into rings ellipsoid (c) and lateral explants inoculated on media centers and aseptic (d), where: ar - areola.

Knowing that *in vitro* cultures of naturally occurring cacti - the areola - some long hairs and bristles, host parties for a variety of organisms (Garcia-Saucedo et al., 2005), sanitized of plant material was achieved by submersare for one minute at 96 º alcohol, followed by the coating process it with a solution of 0.8% sodium hypochlorite mixed with water in a ratio of 1:2, which were added three drops of Tween 20 as surfactant (Cache et al., 2004). Sanitized lasted 20 minutes, during which 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.

After sterilization, the plant material was deposited in Petri capsules on filter paper discs (previously sterilized in the oven) in a laminar flow hood, horizontal air sterile operation, followed by sizing operation and future inocula removal of necrotic parts thereof.

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 pH of the medium was adjusted to a value of 5.8, its first autoclaving.

In basic medium (MB) presented, we added different concentrations of β-indolilbutiric acid (IBA), obtaining the following experiments: V_0 - version control, medium without growth regulators; V_1 - medium supplemented with 1 mg / l IBA (acid β -indolilbutiric); V_2 - medium supplemented with 1.5 mg / l IBA (acid β -indolilbutiric);V_3 - medium supplemented with 2 mg / l IBA (acid β -indolilbutiric).

The culture medium was placed in a glass vial with a capacity of 15 ml (each container was placed 5 ml of medium). Medium vials were sterilized for 30 minutes, by autoclaving 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 inocula were transferred to room for growth, under the following conditions: temperature ranged from 24ºC in peroada light and 20° during the phase of darkness and light was the regime fotoperiodic 16 hours lumină/24h, lighting cultures achieving is the white light emitted by fluorescent lamps, the intensity of 1700 lux.

Reaction and evolution of explants was monitored for 90 days. In this time period were conducted periodic observations and readings every 30 days. Values recorded biometric control group (V_0, fitoinoculi grown on basic medium, without growth regulators) were considered the reference as 100% being reported - every trait - all readings averaged every experimental variant part.

RESULTS AND DISCUSSION

At 90 days after inoculation explants of *Opuntia fragilis* var. *fragilis* in total explain only 78-80% survived, due to phenolic oxidation phenomenon (necrosis), which in in vitro cultures of cacti is very severe (George et al., 1984; Debergh et al., 1991), manifested visually in the first instance by changing the color of media, now followed by darkening inoculated and their entry into senescence.

Comparing the biometric measurement results at the end of the experiment, reported in version mers V0, there was a slowdown of growth in the average length of the main stem, the variants V_1 (medium supplemented with 1 mg/l IBA) and V_3 (medium supplemented with 2 mg/l IBA) that the average absolute value of 1.1 cm (Fig. 2A), respectively, of 1.3 cm recorded a deficit of 31.24% and 18.75% in the second case (Fig.

3A), while the variant V_2 (medium supplemented with 1.5 mg/l IBA) with the parameter value of 1.6 cm was matched control.

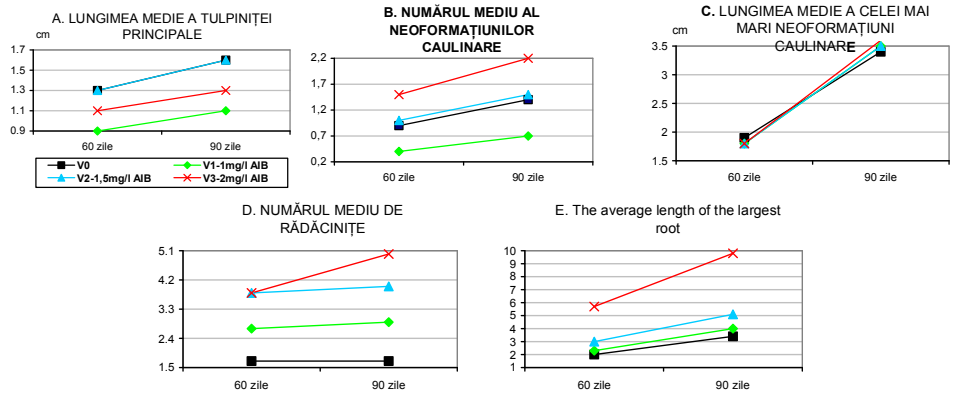

Fig. 2. Graphical presentation of mean values corresponding to the parameters follow vitroculturilor of *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis*, the basic aseptic environment changed us - (V_0 version) - with the addition of 1 mg/l IBA (V_1), 1.5 mg/l IBA (variant V_2) or 2 mg/l IBA (variant V_3) , data expressed in absolute values, (where A, the average length of main stem, B - average number of new formations caulinare, C - average length of the largest formations caulinare, D - average number of roots, E - average length of largest root).

While the average number of shoots (Fig. 2B) was higher than the values recorded in the control sample (V_0), with a difference of 0.1 shoots/variant, the explants inoculated and grown in culture medium supplemented with 1.5 mg/l IBA (V_2) and 0.8 shoots/variant to those of variant V_3 (medium supplemented with 2 mg/l IBA), representing an increase of 7.14% respectively 57.14%. In variant V_1 (medium supplemented with 1 mg/l IBA), this parameter with an absolute average of 0.7 shoots/variant, was 50% (Fig. 3B) as recorded in witness V_0 values (culture medium without growth regulators). According to results published by Medeiros et al., (2006), who concluded that the regeneration of shoots Notocactus magnificus was possible environment AIB added in different concentrations in our experiment by adding in the culture of β-indolilbutiric acid (IBA) in explants of *Opuntia fragilis* var. *fragilis* was obtained regeneration of new shoots.

The average length of the largest shoot (Fig. 2C), was - in absolute terms - more than 0.2 cm explants witness variant V_3 (medium supplemented with 2 mg/l IBA) and 0.1 cm in those belonging variants V_1 (medium supplemented with 1 mg/l IBA) and V_2 (medium supplemented with 1.5 mg/l IBA), representing an additional 5.88%, respectively 2.94% (Fig. 3C) .

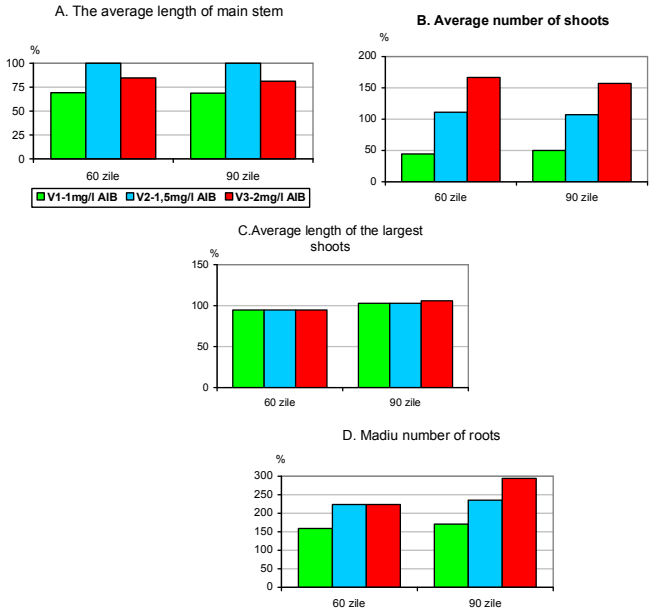

Fig. 3. Graphical presentation of mean values corresponding track parameters in the in vitro cultures of *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis*, the new modified basic aseptic environment with the addition of 1 mg/l IBA (V_1), 1.5 mg/l IBA (variant V_2) or 2 mg/l IBA (variant V_3), data expressed as a percentage, processed values obtained from reporting the results achieved in those parameters from the control group (V_0) without growth regulators, values considered as 100%, (where: A - the average length of main tulpiniței, B - average number of shoots, C - average length of the largest shoots, D - average number of roots, E - highest average length of the roots).

At this time, it was noted that shoots generated from explants inoculated on culture media supplemented with IBA, have dark green color and a fluffy appearance - gave the young spines, which are thin and white - maintained throughout the 90 days the experiment lasted (Fig. 4B). At the same time stand explants variant V_3 (medium supplemented with 2 mg/l IBA), where the shoots are emerging adventitious roots, light brown color (Fig. 4E).

Regarding the average number of roots, it exceeded all variants experimantale witness such an addition of 1.2 roots/varied registered explants variant V_1 (medium supplemented with 1 mg/l IBA) to consemanat an increase of 70.58% compared to the control group V_0 (culture medium without growth regulators). Increasing the amount of growth regulator auxinic - IBA - the culture medium caused an increase in direct proportion to the average number of roots/range, thus fitoinoculii variant V_2 (medium supplemented with 1.5 mg/l IBA) and V_3 (medium supplemented with 2 mg/l IBA) was recorded with one set of 2.3 roots /range (Fig. 2D),

respectively, of 3.3 roots/range (Fig. 4C) compared with the control V_0 (1.7 root/range), representing an increase of 135.29% and 194.11% in the second case (Fig. 3D).

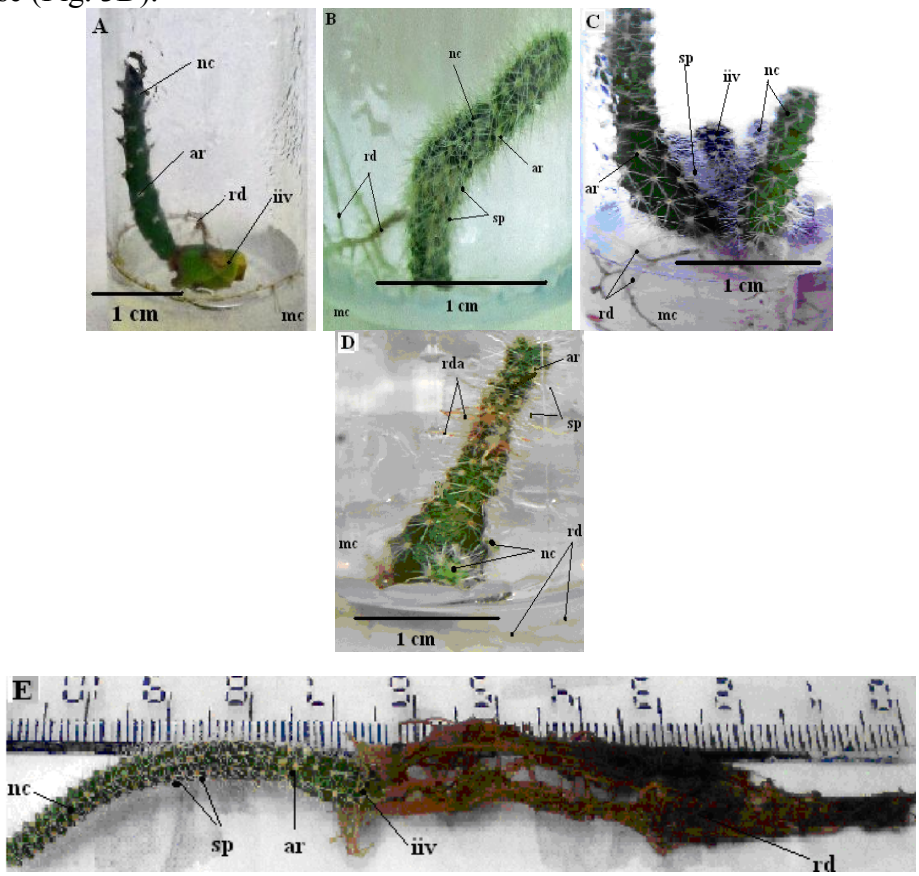

Fig. 4. Explants of *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis*, 90 days after explant inoculation "in vitro", where: A - the basic medium without growth regulators (V0), B - the basic medium containing 1 mg/l IBA (V_1), C - the basic medium containing 1.5 mg/l IBA (V_2), D, E, - the basic medium with the addition of 2 mg/l IBA (V_3) (iiv-initial inoculum viable, MC culture medium; nc- young shoots, rd-root, ar-areola, sp-thorns, RDA, adventitious roots).

Rootedness in vitro culture of *Opuntia fragilis* var. *fragilis* was manifest in the explants inoculated on medium lacking growth regulators (V_0), reaction often reported in the literature, so Escobar et al., (1986), were obtained in cultures in vitro root of cactus, the average auxinic without exogenous, probably due to excess endogenous auxine produced by explants of cactus (Clayton et al., 1990). In the case studied by us with increasing concentration in the culture medium added auxinic was a progressive increase rootedness process, from this point of view the most effective medium of culture - both in terms of number and length rădăcinițelor

neoformate - *Opuntia fragilis* var. *fragilis* in fitoinoculii of lime, has proven to be environmentally suplimentet with 2 mg/l IBA (V_3).

The average length of the largest root has seen the greatest growth in all the explants variant V_3 (medium supplemented with 2 mg/l IBA) that the average absolute value of this parameter is 9.8 cm (Fig. 2E), the registered an increase of 188.23% (Fig. 3E) compared with control V_0 (Fig. 38A). Significantly lower values were recorded in variants V_2 (medium supplemented with 1.5 mg/l IBA) and V_1 (medium supplemented with 1 mg/l IBA), which were recorded in this parameter an average of 5.1 cm , 4.0 cm respectively (Fig. 4B and C), which represents an increase of 50% in the first case and 17.64% in the second case. These results show the effect rhyzogene AIB great that you have on this species of cactus in vitrocultură regime, and especially added to the culture medium at a concentration of 2 mg/l.

Comparing the data from the *Opuntia fragilis* var *fragilis* in vitro culture, we found that explants inoculated and grown on medium without growth regulators behaved unexpectedly well, they remarked at the end of the experiment by forming root and stem.

CONCLUSION

1. The evolusion *Opuntia fragilis* var *fragilis* explants was varied according to the concentration of IBA in the culture medium.
2. At 90 days after initiation of Opuntia fragilis var *fragilis* in vitro culture, we have found that explants cultured on MS medium variant with the addition of 2 mg/l IBA (V_3), have generated most branches caulinare, 57% above the values recorded in the control group. Their length marked the highest values respectively added 5.88%.
3. Cultures in vitro *Opuntia fragilis* var. *fragilis* grown on MS medium with the addition of 2 mg/l IBA (V_3) showed active phenomenon of rootedness, explants giving birth to most roots, marking an increase of 194.11%, they have increased in length most, this parameter been added 188.23%.
4. It should be noted that by the end of this experiment, callus phenomenon not seen in any of the variants tested.

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. Casas A., Barbera G., 2002, Mesoamerican domestication and diffusion. In P. S. Nobel [ed.], Cacti: biology and uses, University of California, Berkeley, California, USA, p. 143–162.
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. Debergh P., Read P., 1991, *Micropropagation*. In: Deberg, P y Zimmerman, R. (eds). *Micropropagation, Technology and Aplication*, Doordrecht, Nether-lands, Kluwer Academic Publishers, p. 1 – 13.
5. Escobar H.A., Villalobos V.M., Villegas A., 1986, *Opuntia* micropropagation by axillary proliferation. Plant Cell Tissue Org. Cult., vol. 7, p. 269–277.
6. Flores-Valdez C.A., 1994. Nopalitos" production, processing and marketing. In: Barbera et al., (ed.), *Agroecology cultivation and uses of cactus pear*, FAO International Technical Cooperation Network on Cactus Pear, p. 92-99.
7. Garcıa-Saucedo P.A., Valdez-Morales M., Valverde M.E., Cruz-Herna-ndez A., Paredes-Lopez O., 2005, Regeneration of three *Opuntia* genotypes used as human food. Plant Cell, Tissue and Organ Culture, vol. 80, p. 215–219.
8. George E., Sherrington P., 1984, *Plant propagation by tissue culture*. Hand-book and directory of commercial laboratories. London, England. Eastern Press, p. 709.
9. Griffith M. P., 2001a, A new Chihuahuan Desert prickly pear, *Opuntia x rooneyi*. Cactus and Succulent Journal (U.S.A.), vol. 73, p. 307-310.
10. 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.
11. Mauseth J.D.,1977, Cytokinin and gibberelelic acid induced effects on the determitation and morfogenesis of leaf primordial in *Opuntia polyacantha (Cactaceae*). American Journal of Botany, vol. 64, nr. 3, p. 337 – 346.
12. Medeiros L., Ribeiro R., Gallo L., Oliveira E., Demattê M., 2006; *In vitro* propagation of *Notocactus magnificus*. Plant Cell, Tissue and Organ Culture, Springer, vol. 84, nr. 2, p. 100147-100151.
13. Nobel P.S., 1994, Remarkable agaves and cacti. Oxford University Press, New York Environmental biology, p. 36-48.
14. Pinkava D.J., 2002, On the evolution of continental North American *Opuntioideae*. Succulent Plant Research, vol. 6, p. 59-98.
15. Rubluo A., Reyes J., Rodriguez-Garay B., Pimienta-Barrios E., Brunner I., 1996, *Métodos de propagación biotecnológicos y convencionales en cactáceas para zonas áridas. In: Técnicas Convencionales y Biotecnológicas para la Propagación de Plantas de Zonas Áridas*, J Izquierdo, G Palomino (eds). Santiago, Chile, vol 9, p. 345.
16. Smith R., Burdick P., Anthony J., Reilley A., 1991, In vitro propagation of *Coryphantha macromeris*. HortScience, vol. 26, nr. 3, p. 315.
17. Starling R., 1985a, *In vitro* micropropagation of the ornamental prickly pear cactus *Opuntia lanigera Salm–Dyck* and effects of sprayed GA_3 after transplantation to ex vitro conditions. Cactus & Succulent Journal (U.S.A.), vol. 57, p. 114–115
18. Steinhart C. E., 1962, Tissue cultures of a cactus. Science, vol. 137, p. 545 – 546.