COMPARATIVE STUDY ON REGENERATIVE AND ORGANOGENIC CAPACITY OF ECHINOCACTUS MIHANOVICHII AND ECHINOPSIS (ZUCC.) CHAMAECEREUS F. LUTEA CULTIVATION IN VITRO IN THE PRESENCE IN THE CULTURAL MEDIUM OF 3-INDUTYL BUTYRIC ACID (AIB) ADDED IN DIFFERENT CONCENTRATIONS

Vidican Iuliana Teodora*, Lazăr Andra Nicoleta, Iancu Carmen Violeta, Cărbunar Mihaela

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

Abstract

Cactuses with red epidermis Echinocactus mihanovichii or yellow Echinopsis chamaecereus f. lutea, are part of the group of chlorophyll cacti - deficient due to a mutation that occurs spontaneously in the culture, as a result, they are unable to synthesize chlorophyll and only if they are chlorophyll grafted.

In order to establish the in vitro culture of Echinocactus mihanovichii and Echinopsis chamaecereus f. lutea, we took explants represented by mini-cuttings (seedlings) from mother plants grown in the greenhouse. We inoculated the explants on a culture medium consisting of macroelements and Fe EDTA Murashige-Skoog (1962), Heller microelements (1953), supplementation with medium supplemented with 3-indolyl butyric acid (IBA) in different concentrations, respectively 1 mg/l IBA (V_1); 1,5 mg/l IBA (V_2) and 2 mg/l IBA (V_3).

The evolution of the explants was monitored for 90 days. At the end of the experiment, we observed that the response of Echinocactus mihanovichii and Echinopsis chamaecereus f. lutea explants differs depending on the composition of the culture medium, as follows: the presence in the culture medium of 2 mg/l AIB (V_3) was positively influenced both species, constant increase in diameter at the level of phytoinocles with an increase of 33,33% compared to the values of the same parameter recorded in the control group V_0 (medium without growth regulators) in Echinopsis (Zucc.) chamaecereus f. lutea also stimulated the process of callus formation in both species, their average number being 0,3 calluses/ variant in both cactus species, with an average diameter of 0,6 cm in Echinocactus mihanovichii and 0,5 cm la Echinopsis chamaecereus f. lutea.

It should be noted that, in this experiment, the process of caulogenesis and rhizogenesis did not take place in any variant and in any of the species used in the experiment

Keywords: cactus, vitro cultures, 3 indolyl butyric acid (IBA), callus, newly formed stems, roots.

INTRODUCTION

Deficient chlorophyll cacti such as *Gymnocalycium mihanovichii* and *Echinopsis chamaecereus f. lutea* are a cactus with colored epidermis (fig.1), lacking the ability to synthesize chlorophyll due to the small number of chloroplasts, about 1/3 of all plastids (Shemorakov, 2003).

Pigmentation is caused by the spontaneous appearance in cultures of mutations largely influenced by temperature and light (Shemorakov, 2003).

According to Skoulkin (2000), plants kept at a lower temperature than the optimal one and in the shade rarely or not at all develop such mutations.

Fig.1. Image with chlorophyll-deficient cacti.Where:
A- *Gymnocalycium mihanovichii*;
B- *Echinopsis chamaecereus f. lutea*

Russian researchers have shown a special interest in chlorophyll-deficient cacti species, so they have classified them according to the color of the epidermis (Shemorakov, 2003).

According to Shemorakov (2001), the reversible mutation of plastids during meiosis keeps the generative reproduction of these species to a minimum (Kornilov, 2008), thus concluding that plants can retain color only by cloning. This has led to the search for new and economically efficient methods (Son, 2000, Lee et al., 2003) for their rapid multiplication.

3-Indolyl butyric acid (IBA) is an auxin with great efficacy in callus formation and rhizogenesis. In callus culture, auxin provides high friability, facilitating the separation of cells into cell suspensions and somatic embryogenesis.

The aim of the experiment covered by this article is to analyze the reaction variability of vitro cultures of *Gymnocalycium mihanovichii* and *Echinopsis chamaecereus f. lutea,* in the presence in the culture medium of 3-indolyl butyric acid (IBA) in different concentrations, respectively 1 mg/l IBA (V_1), 1,5 mg/l IBA (V_2) and 2 mg/l IBA (V_3).

MATERIAL AND METHOD

The biological material used in our experiments consisted of regenerated mini-cuttings (seedlings) on strains of *Gymnocalycium mihanovichii* and *Echinopsis chamaecereus f. lutea* (fig.2). The explants were about 1 cm long, 0.5 cm thick and 0.5-1.5 cm in diameter, depending on the area from which they were harvested (Fig. 2).

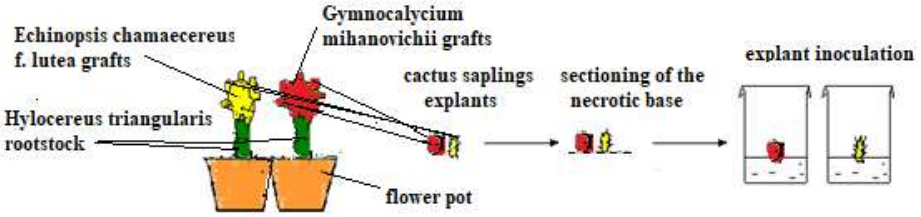

Fig.2. Schematic representation of the exploitation of the fragments of *Gymnocalycium mihanovichii* and *Echinopsis chamaecereus f. lutea*, which will be inoculated on aseptic media.

The vegetable material was asepticized by immersion, for one minute, in 96º ethyl alcohol, followed by its coating with 0,8% sodium hypochlorite solution, mixed with water in a ratio of 1:2; in the disinfectant solution was added - as a surfactant - three drops of Tween 20 each (Cachiţă et al., 2004).

During asepticization, the vegetative material was stirred continuously (Cachiţă et al., 2004). After 20 minutes, the disinfectant was removed and the plant material was washed with sterile distilled water, making five consecutive rinses, of five minutes each. Then, the vegetal material was deposited in aseptic conditions, in a hood with horizontal laminar flow, of sterile air, in operation, on the rounds of filter paper sterilized in the oven, introduced in aseptic Petri dishes. Subsequently, the necrotic parts of the future inocula were detached.

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 to 5.8, 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_3 variant), 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, 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 readings every 30 days. Values thus obtained in the control group (V_0, phyto inoculi 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

After 90 days of viticulture, both in the explants of *Echinocactus mihanovichii* and *Echinopsis (Zucc.) chamaecereus f. lutea*, there is a much faster increase in the mean basal diameter of the main stem in phytoinocles belonging to the control sample V_0 (Vidican et al, 2009), so in the first species of cactus the data recorded show that at an average value of stem diameter of 1,1 cm (Fig.3) , this parameter equaled the control (Fig.3), while in the second species of cactus this parameter equal to 1,2 cm (Fig.3) represents an increase of 33,33% (Fig.4), compared to the values of the same parameter recorded in the control group V_0 (environment without growth regulators).

These results are consistent with those reported by Corneanu et al., (1994), who reported that explants represented by fragments of *Dilochothele longimamma,* cultured "in vitro" on Murashige-Skoog medium (1962), without growth regulators, may manifest the phenomenon of morphogenesis.

Regarding the generation of new strains, in both species used in the experiment, it was shown that the presence in the culture medium of different concentrations of AIB inhibited caulogenesis. The presence of new strains is noticeable in both experiments in the control variant V_0 (medium without growth regulators) where in *Echinopsis (Zucc.) chamaecereus f. lutea* generated an average number of 0,3 buds/variant (Fig.3) these having an average basal diameter of 0,4 cm (Fig.3) while in *Echinocactus mihanovichii* the experimental variant V_0 recorded an average number larger than buds/variant, respectively, of 0,5 (Fig.3), with the same average basal diameter, of 0,4 cm (Fig.3).

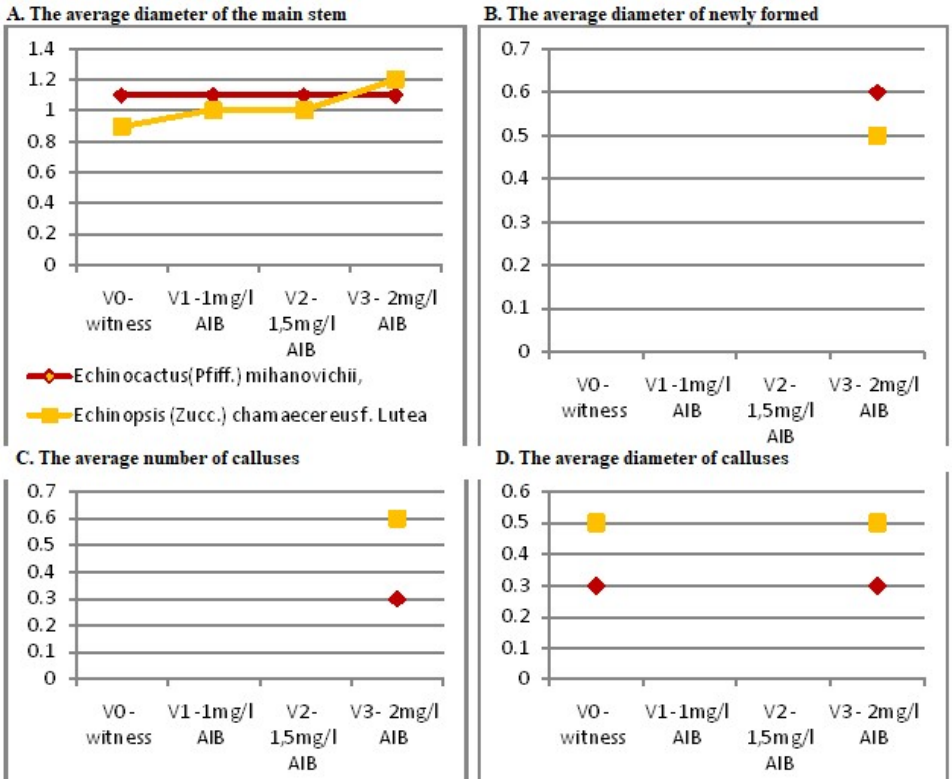

Fig 3.Graphic presentation of the average values corresponding to the in vitro parameters of *Echinocactus (Pfiff.) mihanovichii* and *Echinopsis chamaecereus f. lutea* cultures on basic aseptic medium modified by us - (variant V_0) - with the addition of 1mg / l AIB (variant V_1), 1,5 mg/l AIB (variant V_2) or 2 mg/l AIB (variant V_3), data expressed in absolute values; (where: A-the average diameter of the main stem; B-the average diameter of newly formed; C-the average number of calluses; D-the average diameter of calluses)

These results are consistent with those obtained by Clayton et al. (1990), which following a study of cacti of the genus *Mammillaria grown* "in vitro" suggests that each species of catus may require a specific recipe for the composition of the culture medium (Johnson et al., 1979a and b Starling et al., 1983; Vyskot et al., 1984, Martinez – Vázquez et al., 1989).

Callus induction was observed in both species at the level of explants of variant V_3 (average supplemented with 2 mg/l AIB) their average number being 0,3 calluses/variant (Fig.3), in both species of cactus, with a average diameter of 0,6 cm (Fig.3) in *Echinocactus mihanovichii* and 0,5 cm in *Echinopsis chamaecereus f. lutea* (Fig.3).

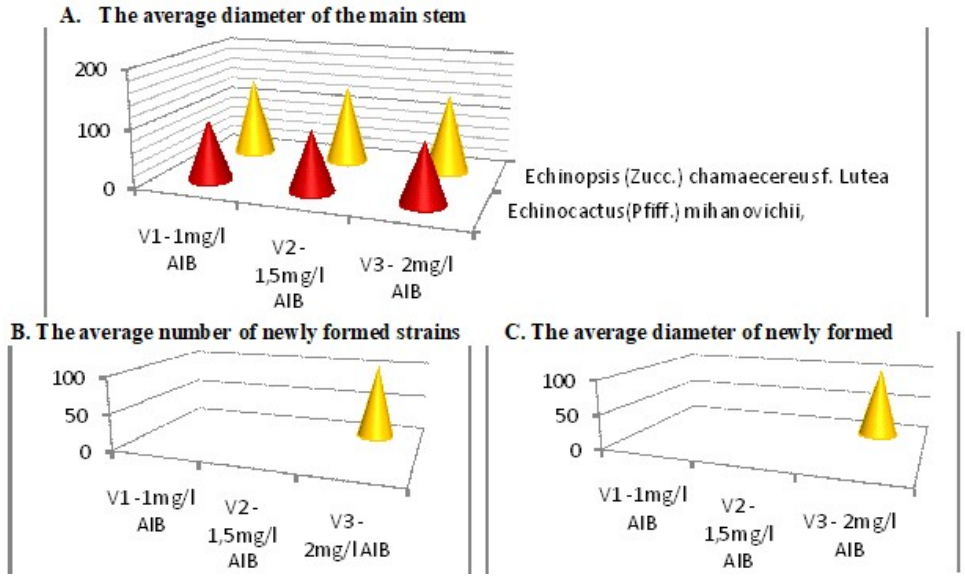

Fig 4.Graphic presentation of the average values corresponding to the parameters in vitro cultures of *Echinocactus (Pfiff.) mihanovichii* and *Echinopsis chamaecereus f. lutea* on aseptic medium modified by us - with the addition of 1mg/l AIB (variant V_1), 1,5 mg/l AIB (variant V_2) or 2 mg/l AIB (variant V_3), data expressed as a percentage, obtained after reporting the values read at the results recorded at the respective parameters in the control group (V_0), without growth regulators, values considered to be 100%;(where: A-the average diameter of the main stem; B-the average number of newly formed strains; C-the average diameter of newly formed)

In the present experiment it is noted that the cactus species and the variants studied have no reaction, in terms of new root formation, to the presence in the culture medium of auxin - 3-indolylbutyric acid (AIB) - regardless of the concentration used. in the current experiment.

The results are consistent with those reported by Copacescu, after Corneanu, 2001, who reports that in most vitro-cultivated species the process of rhizogenesis is easy on MS environment, supplement with endogenous auxins, but species that have a slow growth rate, create problems special at rooting.

Analyzing the images in figure 5, it is observed that, after 90 days of viticulture, the surviving *Echinocactus mihanovichii* inocula have grown and have well-developed areolas and spines, but the section areas are necrotic, a phenomenon manifested by color change - they become brown (Vidican et al, 2018).

In the phytoiniculates of the experimental variant V_0 (medium without growth regulators) and in V_1 (medium supplemented with 1 mg/l AIB) it is found the existence of some areas in which their initial color changed, from

red turned to a brick, even orange with yellow tint (Fig. 5A and B). In the case of explants grown on medium supplemented with 2 mg/l AIB (V_3), both the lack of spines and a red callus are noticeable (Fig.5D).

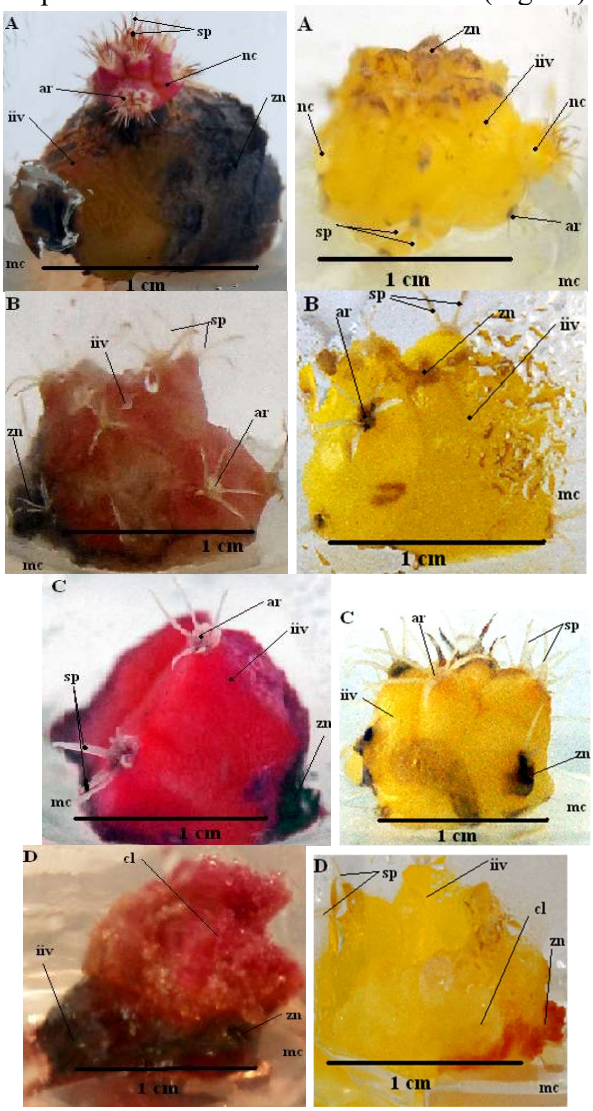

Fig. 5**.** Inoculi de *Echinocactus*(Pfiff.) *mihanovichii* și de *Echinopsis* (Zucc.) *chamaecereus* f. *lutea,* la *90 de zile* de la inocularea explantului „*in vitro",* unde: **A**-pe mediu de bază modificat de noi lipsit de regulatori de creştere(V_0); **B**-pe mediu de bază cu adaos de 1 mg/l AIB (V_1); C-pe mediu de bază cu adaos de 1,5 mg/l AIB (V_2); D-pe mediu de bază cu adaos de 2 mg/l AIB (V_3); (iiv–inocul iniţial viabil; mc–mediu de cultură; ar-areole; sp-spini; cl–calus; zn–zonă necrozată).

According to Cachiţă, (2004), the coloration in red or shades of red of the 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 this case, this pigmentation may also be influenced by the red color of the epidermis of the chlorophyll-deficient cactus *Echinocactus mihanovichii*. A compact red callus, formed on the cut surface of the explants, was also obtained in some species of *Mammillaria*, the color being due to the presence of beta alanine in its cells (Pérez et al., 1998). Unlike *Echinocactus mihanovichii*, in the inocula of *Echinopsis chamaecereus f. lutea* there are no significant changes in terms of morphological characteristics or color regardless of the culture medium used (Vidican et al, 20017).

CONCLUSIONS

Following, for 90 days, the reaction and evolution of phytoinocles of *Echinocactus mihanovichii* and *Echinopsis chamaecereus f. lutea* grown on culture medium improved with AIB in different concentrations, it is noted that: the values recorded both in control group V_0 of growth regulators) and considered as a reference, as being 100%, as well as of the other experimental variants, no existence of significant differences in their reaction mode was found.

1. There was a steady increase in diameter at the level of phytoinoculi, especially in those grown on medium supplemented with 2 mg/l AIB (V_3) and in the control sample V_0, thus in *Echinocactus mihanovichii* at an average value of stem diameter of 1,1 cm this parameter equaled the control, while in *Echinopsis (Zucc.) chamaecereus f. lutea* this parameter equal to 1,2 cm represents an increase of 33,33% compared to the values of the same parameter registered in the control group V_0 (average without growth regulators)

2. Caulogenesis was inhibited by the presence in the culture medium of different concentrations of AIB, a phenomenon that manifested itself only in the explants of the control group V_0 (environment without growth regulators); where in *Echinopsis (Zucc.) chamaecereus f. lutea* generated an average number of 0,3 buds/variant, and *Echinocactus mihanovichii* recorded an average number of buds/variant, of 0,5 in both cases they had an average basal diameter of 0,4 cm .

3. The presence in the culture medium of 2 mg/l AIB (V_3) stimulated the callus formation process in both species, their average number being 0,3 calluse /variant in both cactus species, with an average diameter of 0,6 cm in Echinocactus mihanovichii and 0,5 cm in *Echinopsis chamaecereus f. lutea*.

4. The phenomenon of rhizogenesis has not manifested, until this date, in any of the cactus species and the experimental variants studied.

REFERENCES

1. Cachiţă C.D., C. Deliu, R.L. Tican, A. Ardelean, 2004, *Tratat de biotehnolo-gie vegetală*. Vol.I, Editura Dacia, Cluj-Napoca, p. 29-154.
2. Cachiţă C.D., A. Ardelean, 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. Corneanu M., Corneanu G.C., 1994, *"In vitro"* culture of *Cactaceae* through different explant tipes. In: Proceedings of the 8th Natonal Szmposium of Industrial Microbiology and Biotehnology, Bucharest (I. Anghel, Ed.), p. 443-445.
6. Kornilova L.P., 2008, *Grafting on Pereskiopsis*, Cultivar, publicat online: 20 decembrie.
7. Heller R., 1953, Rescherches sur la nutrition minérale des tissus végétaux cultives *in vitro* Ann.Sci. Nat. Bot. Veg. Ser.,vol. II, p. 1-5.
8. Johnson J., Emino E., 1979a, Tissue culture propagation in the *Cactaceae.* Cactus and Succulent Journal (U.S.A.), vol. 51, p. 275 - 279.
9. Johnson J., Emino E., 1979b, In vitro propagation of *Mammillaria elongate.* HortScience, vol. 14, nr. 5, p. 605 – 606.
10. Lee J.M., Oda M., 2003, Grafting of herbaceous vegetable and ornamental crops. Hort. Rev., vol. 28, p. 61-124.
11. 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.
12. Shemorakov, N., 2001, Plastid mutations. Cultivar, vol. 2, nr. 3, p. 11-20.
13. Shemorakov N., 2003, Cultivar's classification by stem color. Cultivar, vol. 2, nr. 18, p. 68-76.
14. Starling R., Dodds J., 1983, Tissue culture propagation of cacti and other succulents. Bradleya, vol. 1, p. 84 – 90.
15. 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.
16. Murashige T., F. Skoog, 1962, A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, vol. 15, p. 473 –497.
17. Skulkin I. M., 2000, The History of Biological Discoveries, Yekaterinburg.
18. Son B.K., 2000, The culture of cacti & succulents (in Korean). Gyeonggi Province, Korea, vol. 28, p. 61-124.
19. Vidican I.T., D. Cachiţă, J.E. Romocea, 2009, The initiation of *Echinocactus mihanovichii, Echinopsis chamaecereus f. lutea* and *Aylostera heliosa* vitrocultures. Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii, Arad, vol. 19, nr.2, p. 351-358

20. Vidican I. T., Lazăr A. N., Stanciu A. Ș., 2017, Study on the Regenerative and Organogenic Capacity of *Echinopsis (Zucc.) chamaecereus f. lutea* in vitro Culture on an Addition Medium of 3-indolilbutyric (AIB), Analele Universităţii din Oradea, Fascicula Protecţia Mediului, Simpozion Internaţional, Vol. XXIX, anul 22, ISSN: 1224-6265, p.119-127
21. Vidican I. T., Lazăr A. N., Stanciu A. Ș., 2018, 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), Analele Universităţii din Oradea, Fascicula Protecţia Mediului, Simpozion Internaţional, Vol. XXXI, anul 23, ISSN: 1224-6265, p.83-91
22. 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