COMPARATIVE STUDY ON THE REGENERATIVE AND ORGANOGENIC CAPACITY OF *ECHINOCACTUS (PFIFF.) MIHANOVICHII* EXPLANTS, IN THE PRESENCE IN THE CULTURE MEDIUM OF 2.5 mg/l OF 3-INDOLYLBUTYRIC ACID (AIB) AND 2.5 mg/l OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4D)

Iuliana Teodora VIDICAN[1#], Andra Nicoleta LAZĂR[1], Carmen Violeta IANCU[1], Mihai Marcel CĂRBUNAR[1], Oana Maria VIDICAN[2]

[1]University of Oradea, Faculty of Environmental Protection, Oradea City, Maghru 26, 410087, Romania Institution
[2] Student of the University of Oradea, Faculty of Environmental Protection, Oradea City, Maghru 26, 410087, Romania Institution

RESEARCH ARTICLE

Abstract

Echinocactus mihanovichii, cactus with a red epidermis is part of the group of chlorophyll-deficient cacti, the result of a spontaneous mutation, it survives only grafted being unable to synthesize chlorophyll.

In order to establish the in vitro culture of Echinocactus mihanovichii, we took explants, mini-shoots (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), microelements Heller (1953) representing the control sample V_0 and supplemented with 2.5 mg/l 3-indolyl butyric acid (IBA), variant V_1 and 2.5 mg/l 2.5 mg/l 2,4-D variant V_2.

The evolution of the explants was monitored for 90 days, after which we noticed that the response of the Echinocactus mihanovichii explants differed depending on the composition of the culture medium, as follows: the explants grown on medium without growth regulators (V_0) had an increase, at the increase in the diameter of the phytoinoculi, by 25% compared to the values of the same parameter recorded in the V_1 variant (medium supplemented with 2.5 mg/l AIB) and by 31% compared to the V_2 variant (medium supplemented with 2.5 mg/l 2, 4-D). Caulogenesis was manifested only in the explants of the control group V_0 (medium without growth regulators) recording an average number of 0.5 buds/variant with an average basal diameter of them of 0.4 cm.

Callus generation was determined by the presence in the culture medium of both 2.5 ml/l 2,4-D (V_2) with 0.5 callus/variant and a 50% increase compared to the control group V_0 (phytoinoculi grown on medium without growth regulators) and an average diameter of 2.0 cm which represents a 200% increase as well as 2.5 mg/l AIB (V_1) with 0.3 calluses/variant respectively a 30% increase and a diameter of 0.6 cm, so an increase of 60%.

It should be noted that, in this experiment, the rhizogenesis process did not occur in any variant.

Keywords: Explant, rhizogenesis, caulogenesis, callus, phytoinoculation.
#Corresponding author: iuliateodora68@yahoo.com

INTRODUCTION

Chlorophyll deficient cacti such as *Gymnocalycium mihanovichii* is a cactus with colored epidermis (fig.1), lacking the ability to synthesize chlorophyll due to the small number of chloroplasts, approximately 1/3 of all plastids (Shemorakov, 2003).

Figure.1. **Image representing chlorophyll deficient cactus *Gymnocalycium mihanovichii*.**

Pigmentation is caused by the spontaneous appearance of mutations in cultures largely influenced by temperature and light (Shemorakov, 2003). According to Skulkin (2000), cacti kept at a lower than optimal temperature and in the shade develop such mutations less often or not at all.

The classification of chlorophyll-deficient cacti species was made 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 at a minimum level (Kornilova L.P., 2008) thus plants can keep their color only by cloning, a fact that determined the
search for new and efficient methods of rapid multiplication and their economic (Son, 2000, Lee et al., 2003)

The aim of this experiment is to analyze the regenerative and organogenic capacity of the *Gymnocalycium mihanovichii* cactus grown in

vitro on culture medium without growth regulators (V_0), but also in the presence of 2.5 mg/l 3-indolyl acid in the culture medium butyric (IBA) variant V_1 and of 2.5 mg/l 2.4 D, variant V_2.

MATERIAL AND METHOD

The biological material used in our experiments consisted of regenerated mini-cuttings (seedlings) on strains of *Gymnocalycium mihanovichii* (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).

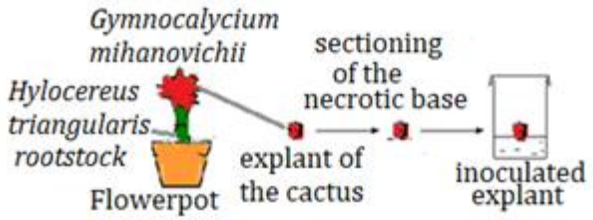

Figure.2. **Schematic representation of the sampling of *Gymnocalycium mihanovichii* fragments that will be inoculated on aseptic media.**

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 basic medium (MB) we added 2.5 mg/l 3-indolyl butyric acid and 2.5 mg/l 2,4 D; obtaining the following experimental variants: V_0 - control variant, medium without growth regulators, V_1 - 2.5 mg/l AIB and V_2 - 2.5 mg/l 2.4 D.

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 DISCUSSIONS

After 90 days of culture in vitro, at the level of *Echinocactus mihanovichii* explants, it can be seen that the growth rate is different depending on the composition of the culture medium, thus the growth of the average basal diameter of the main stem was found to be much faster (1.6 cm) in the phytoinocula belonging to the control sample V_0 (medium without growth regulators) (Fig.4A), compared to those of the variant V_1 (medium supplemented with 2.5 mg/l AIB) of 1.2 cm (Fig.4A) which represents a minus of 25% (Fig.5A) or 1.1 cm (Fig.) in the V_2 variant (medium supplemented with 2.5 mg/l 2,4-D), thus recording a deficit of 31% (Fig.5A).

These results, from a statistical point of view, are considered to be distinct significant (Table 1).

It is known that *Echinocactus mihanovichii* is a cactus that roots hard (Copăcescu V.S., 2001), although there are specimens that also live on their own roots; in the present experiment, it is noted that the phenomenon of rhizogenesis was not manifested in any variant studied, regardless of the composition of the

culture medium. These results are consistent with those obtained by Clayton et al. (1990), who, in a study of cockatiels of the genus *Mammillaria*, reported a complete lack of response to in vitro cultivation of *Mammillaria eichlamii Quehl*, a fact suggesting that each species of catfish may require a specific recipe vis-a-vis the composition of the culture medium (Johnson et al., 1981; Starling et al., 1983; Vyskot et al., 1984, Martinez-Vázquez et al., 1989).

The experimental variant V_0 (medium without growth regulators) was the only sample in which the caulogenesis phenomenon was detected, recording an average number of 0.5 buds/variant (Fig. 4B), and an average basal diameter of them of 0.4 cm (Fig. 4C), the new buds keeping the red color of the initial explant (Fig. 3A).

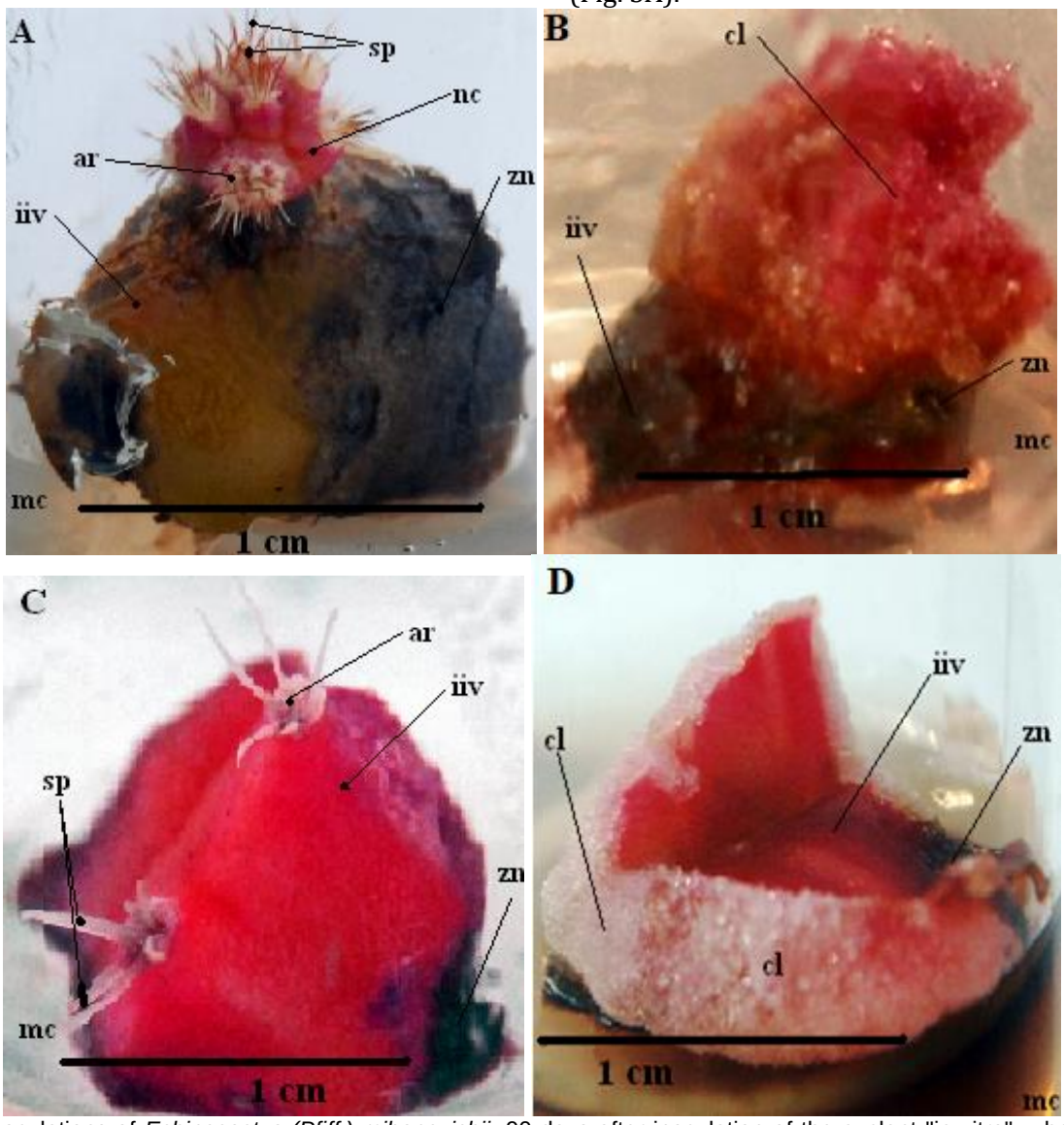

Fig.3 . Inoculations of *Echinocactus (Pfiff.) mihanovichii*, 90 days after inoculation of the explant "in vitro", where: A-on new modified basic medium without growth regulators (V_0); B,C-on basic medium with the addition of 2.5 mg/l AIB (V1); C- D-on basic medium with the addition of 2.5 mg/l 2,4-D(V_2); (iiv–initial viable inoculum; mc–culture medium; nc–caulinary neoformation; sp-thorns; ar-areola; cl–callus; zn-necrotic zone).

The presence in the culture medium of 2.5 mg/l 2,4-D (V_2) led to predictable results in terms of callus induction at the level of phytoinoculi grown on this nutrient substrate, thus a number of 0.5 calluses/variant (Fig. 4D), with an average diameter (measured in the widest area) of 2.0 cm (Fig. 4E), compared to 0.3 calluses/variant (Fig.4D) at inocula grown on culture medium supplemented with 2.5 mg/l AIB (V_1) and an average diameter of 0.6 cm (Fig.4E). Percentage-wise, the data obtained show a 50% increase compared to the control in the number of calluses/variant in variant V_2 (the medium supplemented with 2.5 mg/l 2,4-D) and 30% in V_1 (the medium supplemented with 2.5 mg/l AIB) there is also a 200% increase in the average callus diameter in V_2 (medium supplemented with 2.5 mg/l 2,4-D) and 60% in V_1 (medium supplemented with 2, 5 mg/l AIB

Table 1. The results of the biometric evaluations of the vitroplants of *Echinocactus mihanovichii*, performed 90 days after the inoculation of the explants on basic aseptic media (variant V_0) with the addition of 2.5 mg/l AIB (variant V_1) and 2.5 mg/l 2.4 D (variant V_2)

Parameter / Alternative	The average length of the main stem	Standard deviation	Significance	Average number of newly formed stems +/- Standard deviation	Standard deviation	Significance	Average length of the largest newly formed stem+/-Standard deviation	Standard deviation	Significance	Average number of new roots +/- Standard deviation	Standard deviation	Significance	Average length of the largest newly formed root +/-Standard deviation	Standard deviation	Significance	Average number of calluses +/-Standard deviation	Standard deviation	Significance	The average diameter of calluses +/-Standard deviation	Standard deviation	Significance
90 days																					
V0	1,10±0,31	0,0947	**	0	0		0	0		0	0		0	0		0	0		0	0	
V1	1,10±0,33	0,1084	**	0	0		0	0		0	0		0	0		0,30±0,26	0,0658	NS	0,60±0,38	0,1432	NS
V2	1,10±0,24	0,0558	**	0	0		0	0		0	0		0	0		0,50±0,26	0,0658	***	2,00±0,84	0,7063	

Legend: *** very significant ** distinctly significant * significant NS insignificant

These results, from a statistical point of view, are considered to be distinct significant and very significant (Table 1).

Analyzing the images in figure 3, it can be seen that, after 90 days of in vitro culture, the *Echinocactus mihanovichii* inoculums, which remained alive, have grown and show well-developed areolae and spines, but the section areas are necrotic, a phenomenon manifested by a change in color - these becoming brown In the phytoinicules of the experimental variant V_0 (medium without growth regulators), the existence of some areas where their initial color has changed, from red turned to a brick, even orange with a yellow tinge, can be seen (Fig.3).

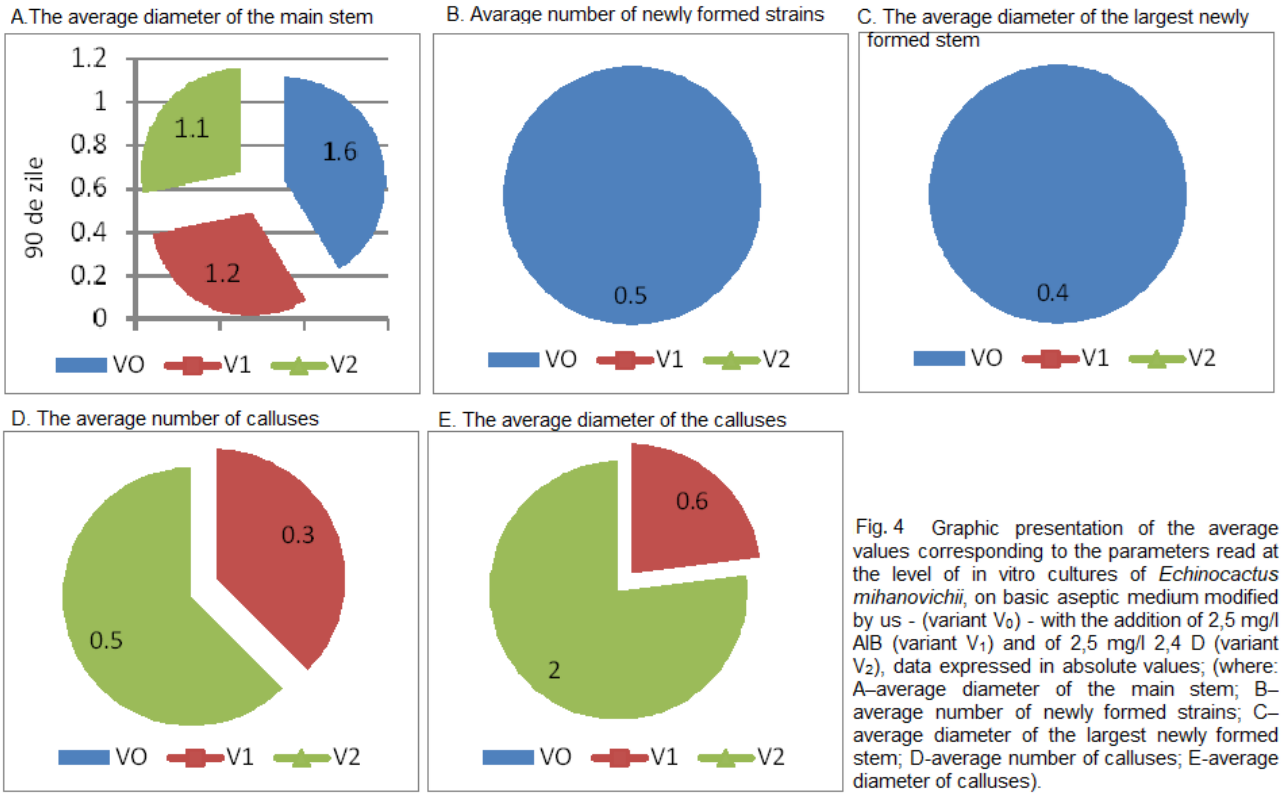

Fig. 4 Graphic presentation of the average values corresponding to the parameters read at the level of in vitro cultures of *Echinocactus mihanovichii*, on basic aseptic medium modified by us - (variant V_0) - with the addition of 2,5 mg/l AIB (variant V_1) and of 2,5 mg/l 2,4 D (variant V_2), data expressed in absolute values; (where: A–average diameter of the main stem; B–average number of newly formed strains; C–average diameter of the largest newly formed stem; D-average number of calluses; E-average diameter of calluses).

It can be observed (fig.3) that the callus generated by the inocula grown on medium supplemented with 2.5 mg/l 2.4 D (V_2) is located only on their surface, is white in color, and appears in the form of crystals, similarly, the total absence of thorns is seen, a fact probably due to the "defoliating" action of 2,4-D auxin (Vidican I.T., 2012).

At the level of explants grown on medium supplemented with 2.5 mg/l AIB (V_1), both the absence of spines and a red callus can be noted (Fig.3). According to Cachiţă et al, (2004), the red coloration or shades of red of the callus is due to a very high content of anthocyanins, which accumulate 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* (Vidican, et al., 2018). A red, compact 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., 2002).

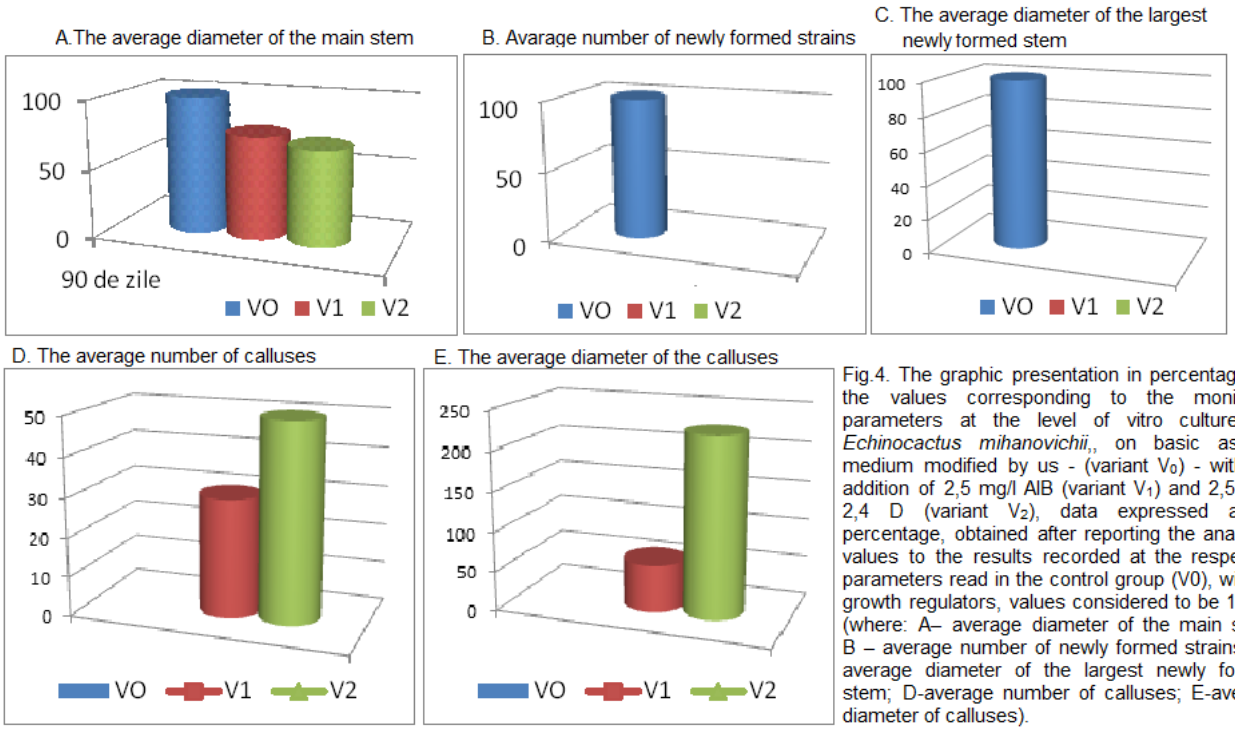

Fig.4. The graphic presentation in percentages of the values corresponding to the monitored parameters at the level of vitro cultures of *Echinocactus mihanovichii*,, on basic aseptic medium modified by us - (variant V_0) - with the addition of 2,5 mg/l AIB (variant V_1) and 2,5 mg/l 2,4 D (variant V_2), data expressed as a percentage, obtained after reporting the analyzed values to the results recorded at the respective parameters read in the control group (V0), without growth regulators, values considered to be 100%; (where: A– average diameter of the main stem; B – average number of newly formed strains; C– average diameter of the largest newly formed stem; D-average number of calluses; E-average diameter of calluses).

CONCLUSIONS

Following the reaction and evolution of *Echinocactus mihanovichii* phytoinoculi, for 90 days, from the values recorded both in the control batch V_0 (medium without growth regulators) and considered as reference, as regulators (V_0) with an absolute value of 1.6 cm compared to 1.2 cm in V_1 (medium supplemented with 2.5 mg/l AIB) which represents a minus of 25% and 1.1 cm at V_2 (medium supplemented with 2.5 mg/l 2,4-D), where a deficit of 31%

Caulogenesis was manifested only in the explants of the control group V_0 (medium without growth regulators).

Callus generation was determined by the presence in the culture medium of both 2.5 ml/l 2,4-D (V_2) with 0.5 callus/variant and a 50% 100%, as well as in the other experimental variants the existence of significant differences in their way of reaction was found.

A constant increase in the diameter of the inoculums was noted in the case of all the experimental variants, especially those cultivated on medium without growth increase compared to the control group V_0 (phytoinoculi grown on medium without growth regulators) and an average diameter of 2.0 cm, which represents a 200% increase as well as 2.5 mg/l AIB (V_1) with 0.3 calluses/variant, respectively, an increase of 30% and with a diameter of 0.6 cm, so an increase of 60%.

The phenomenon of rhizogenesis was not manifested in any of the studied experimental variants, within this experiment.

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