STUDY ON REGENERATION CAPACITY PLANT *Echinocactus* (Pfiff.) *mihanovichii* GROWN SUPPLEMENTED CULTURE MEDIA WITH A MIXTURE OF EQUAL PARTS OF 3-INDOLILBUTIRIC (AIB), AND BENZYLADENINE (BA), ADDED IN VARIOUS CONCENTRATIONS

Vidican Iuliana Teodora*

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

Abstract.

Echinocactus mihanovichii is a cactus with exceptional decorative qualities, being highly appreciated appearance spectacular. Hostility is offset sharp spikes at the cactus chlorophyll-deficient skin coloring. This last feature is preserved only by cloning. Thus the establishment of in vitro cultures of Echinocactus mihanovichii I harvested coastal portions that have fragmented so that each hold 3-4 explant areola. The culture medium used was composed of macro-elements, Murashige-Skoog (1962) with the addition of growth regulators consists of a mixture of equal amounts of an auxin (3-indolilbutiric - IBA) and cytokinins (benzyladenine - BA), added at different concentrations, respectively, 1 mg/l IBA + 1 mg/l BA (V_1); 1,5 mg/l IBA + 1,5 mg/l BA (V_2) and 2 mg/l IBA + 2 mg/l BA (V_3).

Evolution vitro cultures was monitored for 90 days. At the end of the experiment to prove the reaction i Echinocactus mihanovichii plants differ from the new strains is their generating, based on the composition of the culture medium, we have obtained the best results in expalantele grown on a culture medium supplemented with 2 mg/l IBA + 2 mg/l BA (V_3). After 90m days of culture in vitro, in either experimental varintele i got roots or callus.

Keywords: cacti, vitrocultures, benzyladenine – BA, 3-indolilbutiric – IBA, buds.

INTRODUCTION

Echinocactus mihanovichii native plant from Paraguay (Copăcescu, 2001), is part of chlorophyll-deficient cacti. It is a cactus that fascinates not only the tenderness and beauty of flowers and red skin (Figure 1).

Fig. 1.Images of *Echinocactus mihanovichii*. Where: a-flower; b-decorative stems.

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 color is 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 multiplication as economically efficient these plants (Son, 2000, Lee et al., 2003).

In vitro cultures, the combination of growth regulators called organogenesis hormonal balance adjustment can be achieved within certain limits by changing the concentration or ratio of regulatory present in the growth medium. After the Cachiță et al. (2004), the existence of a culture medium of high concentrations of auxin, cytokinins with one, stimulate rooting process while an increase in the content promotes the formation of shoots cytokinins; in the culture medium in the presence of high concentrations, but equal, the two compounds will be driven with the morphogenesis process, so the generation of callus and its growth.

Hormonal balance in the culture medium can not be fully controlled, it is influenced to a large extent on the endogenous phytohormone ratio. The Rubluo et al. (1996), believes that in vitro cultures of cactus, rooting is the result of interaction between cytokinins and auxins added to the culture medium in the form of exogenous growth regulators, but which, in Taiz et al. (1998), are affected by the amount of light they are exposed vitro cultures.

This experiment was aimed at studying the way they react cactus explants in culture medium supplementation changed us - V0 - medium lacking growth regulators - with a combination of equal amounts between an auxin (3-indolilbutiric - IBA) and cytokinins (benzyladenine - BA), added in different concentrations, respectively, 1 mg/l IBA + 1 mg/l BA (V_1); 1,5 mg/l IBA + 1,5 mg/l BA (V_2) and 2 mg/l IBA + 2 mg/l BA (V_3).

MATERIAL AND METHODS

Disinfection plant material was achieved by submersarea explants for one minute in 96° alcohol, after which they were coated with a solution of 0.8% sodium hypochlorite mixed with water in a ratio of 1:2, and the surfactant is added three drops of Tween 20. During this 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 thereof. 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 (Dabenkaussen et 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) I removed tissue from the middle portion leaving to experts about 0,7 to 1 cm parenchymal tissue (Fig.2b), and moved to portioning future inoculated explant so that each hold 3-4 areola, and sizes to fit into already established (Fig.2c).

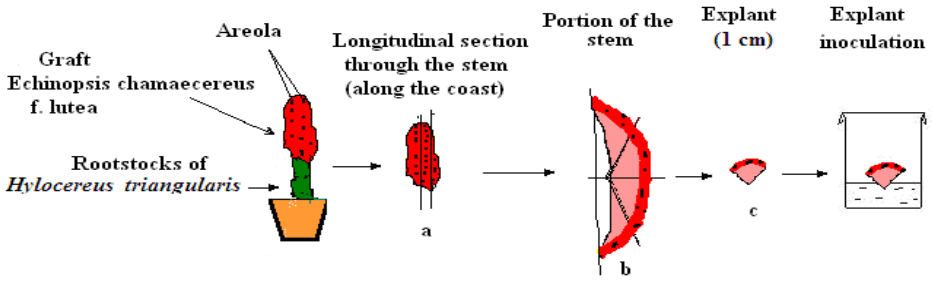

Fig. 2. Explantare schemetică representation of how the buds and fragments of *Echinocactus* (Pfiff.) *mihanovichii* and slicing their ribs to obtain inoculum.

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. The basal medium (MB) added a mixture of equal amounts of 1 mg/l IBA and 1 mg/l BA (variant V_1), 1,5 mg/l IBA and 1,5 mg/l BA (variant V_2) or 2 mg/l IBA and 2 mg/l BA (variant V_3), obtaining the following experimental: V_0 - version control, medium without growth regulators; V_1 - 1 mg/l IBA and 1 mg/l BA; V_2 - 1,5 mg/l IBA and 1,5 mg/l BA and V_3 - 2 mg/l IBA and 2 mg/l BA.

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, 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 *Echinocactus mihanovichii* i noticed that the main stem basal medium showed only pluses compared with values recorded in the control group - V_0 (medium lacking growth regulators) - considered to be 100%, with 0,3 cm (Fig. 3A) to the explants embodiment V_1 (medium supplemented with a mixture of 1 mg/l IBA and 1 mg/l BA), which represents an increase of 27,27%, and 0,4 cm at V_2 (medium supplemented with a mixture of 1,5 mg/l IBA and 1,5 mg/l BA) by 0,6 cm V_3 (medium supplemented with a mixture of 2 mg/l IBA and 2 mg/l BA), ie an increase of 36,36% and 54,54% in the second case (Fig. 4A).

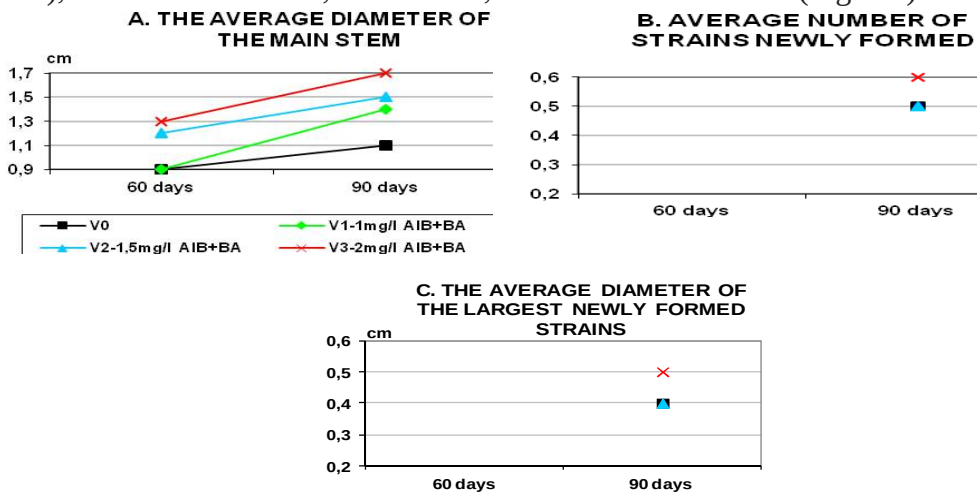

Fig. 3. Graphical presentation of the mean values corresponding to the monitored parameters vitro cultures of *Echinocactus* (Pfiff.) *mihano-vichii*, based on the aseptic environment as new - (variant V_0) - with the addition of a mixture consisting of equal amounts of 1 mg/l IBA and 1 mg/l BA (variant V_1), 1,5 mg/l IBA and 1,5 mg/l BA (variant V_2) or 2 mg/l IBA and 2 mg/l BA (variant V_3), data expressed in absolute values; (where: A-the average diameter of the main stem; B-strains, the average number of newly formed, the average diameter of the, C-strain newly formed higher).

This assessment noted the favorable composition of the culture medium on the process of generating new strains, such variant V_2 (medium supplemented with a mixture of 1,5 mg/l IBA and 1,5 mg/l BA) was recorded the average number 0,5 of the newly formed stem / variant mist led to the equalizer control V_0 at the same time the explants belonging to the variant V_3 (medium supplemented with a mixture of 2 mg/l IBA and 2 mg/l BA) have generated a number of new strains formed 0,65/variant (Fig. 3B) values relative to those recorded in the control group, represented an increase of 20% (Fig. 4B).

The average diameter of the strain newly formed basal leveling control V_0 (0,4 cm) in the case of the variant V_2 belonging expantelor (medium supplemented with a mixture of 1,5 mg/l IBA and 1,5 mg/l BA), but it above the 0,1 cm value at the variant V_3 (medium supplemented with a mixture of 2 mg/l IBA and 2 mg/l BA) (Fig. 3C) making an increase of 25% (Fig. 3C) .

Following the evolution of *Echinocactus mihanovichii* explants in this experiment, it is observed that in none of the variants studied were not generated root nor callus induction phenomenon occurred; one side of which is the formation of new shoots from explants belonging to the variant V_2 (medium supplemented with a mixture of 1,5 mg/l IBA and 1,5 mg/l BA) or V_3 (medium supplemented with a mixture of 2 mg/l IBA and 2 mg/l BA), while the explants grown in medium supplemented with a mixture of 1 mg/l IBA and 1 mg/l BA (V_7) remain impervious to the composition of the nutrient substrate; probably due to the restrictive effect that I had two growth regulators on each other. These results are consistent with those reported by Bustamante et al. (1990), who concluded that the culture "in vitro" of cacti, the combination of an auxin and cytokinins - in various concentrations - may be a limiting factor for the formation of shoots .

Reaction different explants of *Echinocactus mihanovichii* composition of the culture medium, manifested by the formation of a variable number of new shoots, or conversely, the complete absence of any response was signaled by Corneanu et al. (1994).

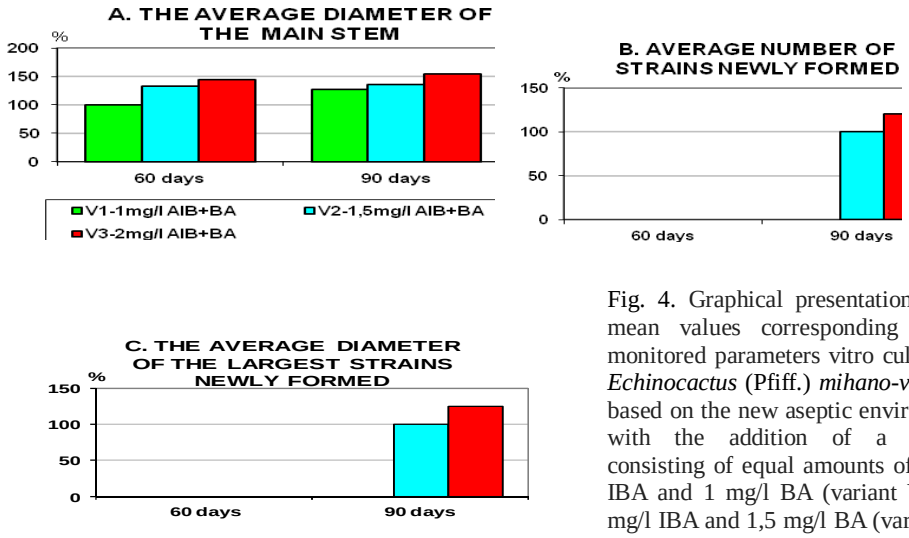

Fig. 4. Graphical presentation of the mean values corresponding to the monitored parameters vitro cultures of *Echinocactus* (Pfiff.) *mihano-vichii*, as based on the new aseptic environment, with the addition of a mixture consisting of equal amounts of 1 mg/l IBA and 1 mg/l BA (variant V_1), 1,5 mg/l IBA and 1,5 mg/l BA (variant V_2) or 2 mg/l IBA and 2 mg/l BA (variant V_3), data expressed as a percentage, obtained by reporting the values pursued the results recorded in the respective parameters in the control group (V_0), lacking growth regulators, values considered as 100%; (where: A-the average diameter of the main stem, B- strains, the average number of newly formed, the average diameter of the; C-strain newly formed higher).

Looking at the images in figure 5, it can be seen that V_0 explants control group (medium lacking growth regulators) kept their original color (Fig. 5A) - red - compared to the inoculated and grown in medium with a mixture of party suplimantate equal 3-indolilbutiric (AIB) and benzyladenine (BA), regardless of the added concentration (Fig. 5B,C and D). This phenomenon is also present in the shoots generated from explants inoculated and grown on suplimantat 1,5 mg/l IBA + 1,5 mg/l BA (V_2) or with 2 mg/l IBA + 2 mg/l BA (V_3), which are colored in different shades of orange to yellow, there is also, areola large that are provided five spines and white fluffy glohide protected (Fig. 5C and D). Characteristic is the fact that the area of contact with the culture medium, and the sectional area, the necrosis of explants. Looking at the images in figure 5, it can be seen that V_0 explants control group (medium lacking growth regulators) kept their original color (Fig. 5A) - red - compared to the inoculated and grown in medium with a mixture of party suplimantate equal 3-indolilbutiric (AIB) and benzyladenine (BA), regardless of the added concentration (Fig. 5B,C and D). This phenomenon is also present in the shoots generated from explants inoculated and grown on suplimantat 1,5 mg/l IBA + 1,5 mg/l BA (V_2) or with 2 mg/l IBA + 2 mg/l BA (V_3), which are colored in different shades of orange to yellow, there is also, areola large that are provided five spines and white fluffy glohide protected (Fig. 5C and D). Characteristic is the fact that the area of contact with the culture medium, and the sectional area, the necrosis of explants.

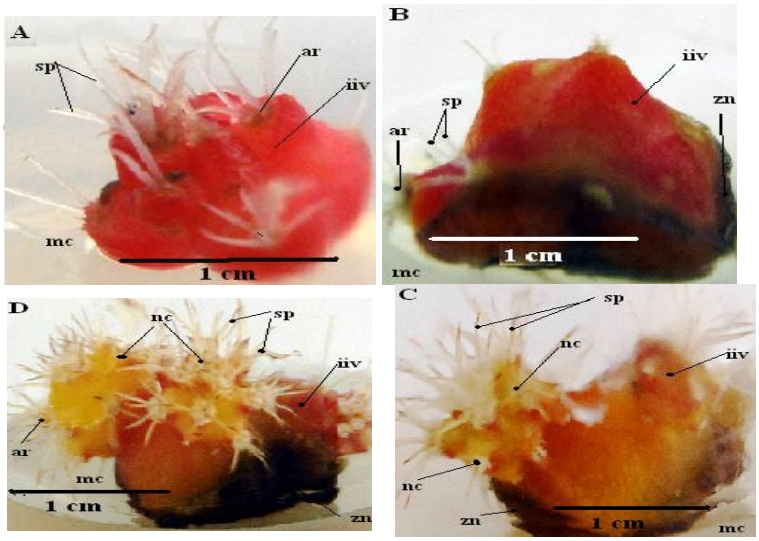

Fig. 5. *Echinocactus* (Pfiff.) *mihanovichii* inoculum, 90 days after inoculation in vitro explant. Where: A-modified base-the new environment lacking growth regulators (V_0); B-base medium with the addition of a mixture consisting of equal amounts of 1 mg/l IBA and 1 mg/l BA (V_1); C-base medium with the addition of a mixture consisting of equal amounts of 1,5 mg/l IBA and 1,5 mg/l BA (V_2); D-basic medium with the addition of a mixture consisting of equal amounts of 2 mg/l IBA and 2 mg/l BA (V_3); (Iiiv -starting viable inoculum; mc-culture medium; nc-new strains formed; ar-areola; sp-thorns; zn-necrotic area).

Comparing the data recorded at 90 days after initiation of *Echinocactus mihanovichii* vitroculturii culture media supplemented with a mixture of equal parts of 3-indolilbutiric (AIB) and benzyladenine (BA), but added in different concentrations, we found that studied experimental variants were reacted according to the different nutrient substrate copoziţia (Vidican et al., 2010). In a stitch no reaction (the formation of roots, shoots and callus) was inoculated and grown explants showed medium supplemented with a mixture of 1 mg/l IBA + 1 mg/l BA (V_1). Results similar to those of the control V_0 (medium lacking growth regulators) - the generation of new strains have recorded the explants variant V_2 (medium supplemented with a mixture of 1,5 mg/l IBA and 1,5 mg/l BA). According to the results obtained, we can say that the most beneficial combination for this experiment was formed from a mixture that consists of 2 mg/l IBA and 2 mg/l BA (V_3) to the explants grown on the substrate they've generated 20% more young stems that reached basal medium diameter 25% higher compared with the same parameter values recorded in the control V_0. Note that in none of the experimental variants have not been rooting phenomena or callus.

CONCLUSION

1. At 90 days after initiation of in vitro culture of *Echinocactus mihanovichii* found that the growth of explants is different depending on the composition of the culture medium, remarking accelerated growth on medium supplemented with a mixture of equal parts of two mg/l IBA and 2

mg/l BA (V_3).

2. The explants grown in medium supplemented with a mixture of 1 mg/l IBA and 1 mg/l BA (V_1) of the composition remained immune nutrient substrate, the reaction of which is the same as explants inoculated culture medium without growth regulators (V_0).

3. The beneficial effect of the medium supplemented with a mixture of 2 mg/l IBA and 2 mg/l BA (V_3) has been the process of generating new buds grow on this medium such inoculii generating a number of new strains 0,65 formats/variant, relative to the values recorded in the control group, represented an increase of 20%.

4. By this time the phenomenon of rooting and callus did not result in any of the variants tested.

5. Analyzing characteristics of explants after 90 days of culture in vitro, it is noted that explants which have retained the original color, red, V_0 reared control group (medium lacking growth regulators), the other experimental variants suplimantate the mixture of equal parts of 3-indolilbutiric (AIB) and benzyladenine (BA), regardless of the added concentration, both explants and generate buds are colored in different shades of orange to yellow.

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