RESEARCH ON THE REGENERATIVE CAPACITY AND ORGANOGENOUS VITRO CULTURES OF *Echinocactus (Pfiff.) mihanovichii* THE PRESENCE IN THE CULTURE MEDIUM CYTOKININS benzyladenine (BA)

Vidican Iuliana Teodora*

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

Abstract

Hostility is offset sharp spikes in Echinocactus mihanovichii, cactus chlorophyll-deficient, not only by tenderness and beauty of the flowers that fascinates the viewer and totally unexpected appearance of skin coloring. This last feature is kept only by cloning. Thus the establishment of in vitro cultures of Echinocactus mihanovichii, we harvested coastal portions that have fragmented so that each explant hold 3-4 areola The explants were inoculated in a mineral medium - macro Murashige-Skoog (1962) with the addition of growth regulators, micronutrients Heller (1953), supplemented with benzyladenine - BA different concentrations.

Evolution explants was monitored for 90 days. Their response was different depending on the concentration of BA present in the culture medium. Finally, it was shown that explants Echinocactus mihanovichii grown in a culture medium supplemented with 2 mg/l BA (V_3) had the largest and most strains newly formed. The process of rootedness and callus was not shown on any of culture media studied.

Key words: benzyladenine (BA), vitro cultures, strains newly formed, callus, rootedness

INTRODUCTION

Echinocactus mihanovichii plant native to Paraguay (Copăcescu, 2001), is part of chlorophyll-deficient cacti. It is a cactus with red skin with exceptional decorative qualities, being highly appreciated appearance of 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).

Cytokinins that are plant hormones in absence of non-dividing cells, and tissue cultures which stimulate cell division and processes to the formation withstand the strains inoculated in which generate strains (Mauseth, 1976), also prevents senescence, auxin exerts antagonistic effect annihilating apical dominance, favoring cell dediferenţierea, etc., (Cachiță et al., 2004).

Escobar et al (1986) reported that the most effective plant growth regulator tested in vitro cultures of cactus, for multiplying the plant material is benzyladenine - BA added to the culture medium to generate a number caulinare than neoformation.

MATERIAL AND METHOD

The research was conducted in 2015 in the laboratory of plant biotechnology at the Faculty of Sciences at the University of Oradea, Department of Biology.

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 (Dabenkausse 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. 1a). 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.1b), and moved to portioning future inoculated explant so that each hold 3-4 areola, and sizes to fit into already established (Fig.1c).

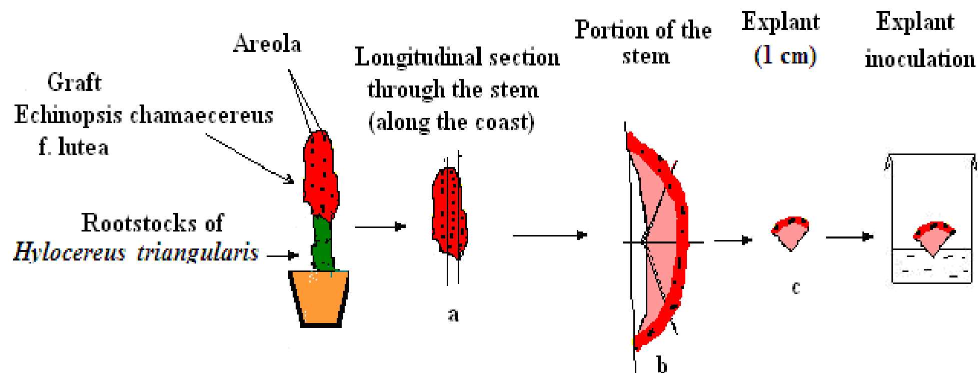

Fig. 1. Schematic representation of the manner in which the shoots, the ribs and the pieces of *Echinocactus (Pfiff) mihanovichii* were sliced to give inocula.

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 this experiment, our aim was to study reactions *Echinocactus mihanovichii* cactus grown on a culture medium supplemented with benzyladenine (BA). To obtain the proposed variants, we prepared and added to the nutrient medium lacking growth regulators (V_0), different concentrations of benzyladenine (BA), as follows: 1 mg/l BA (V_1); 1,5 mg/l BA (V_2) and 2mg/l BA (V_3).

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

From observations made within 90 days of the initiation of the experiment was seen a steady increase in size of the explants, this phenomenon is directly proportional to the amount of cytokinins (BA) from the culture medium. This remark explants variant V_3 (medium supplemented with 2 mg/l BA) as having the highest average basal diameter of the main stem – 1,6 cm - which represents an increase of 45,45% compared to the same parameter value recorded at V_0 control group. The explants belonging to the variants V_1 (medium supplemented with 1 mg/l BA), and V_2 (medium supplemented with 1,5 mg/l BA) has an average value of this parameter of 1,3 cm, 1,4 cm, respectively (Fig.2A), marking an increase of 18,11% and 27,27% the second case (Fig.3A).

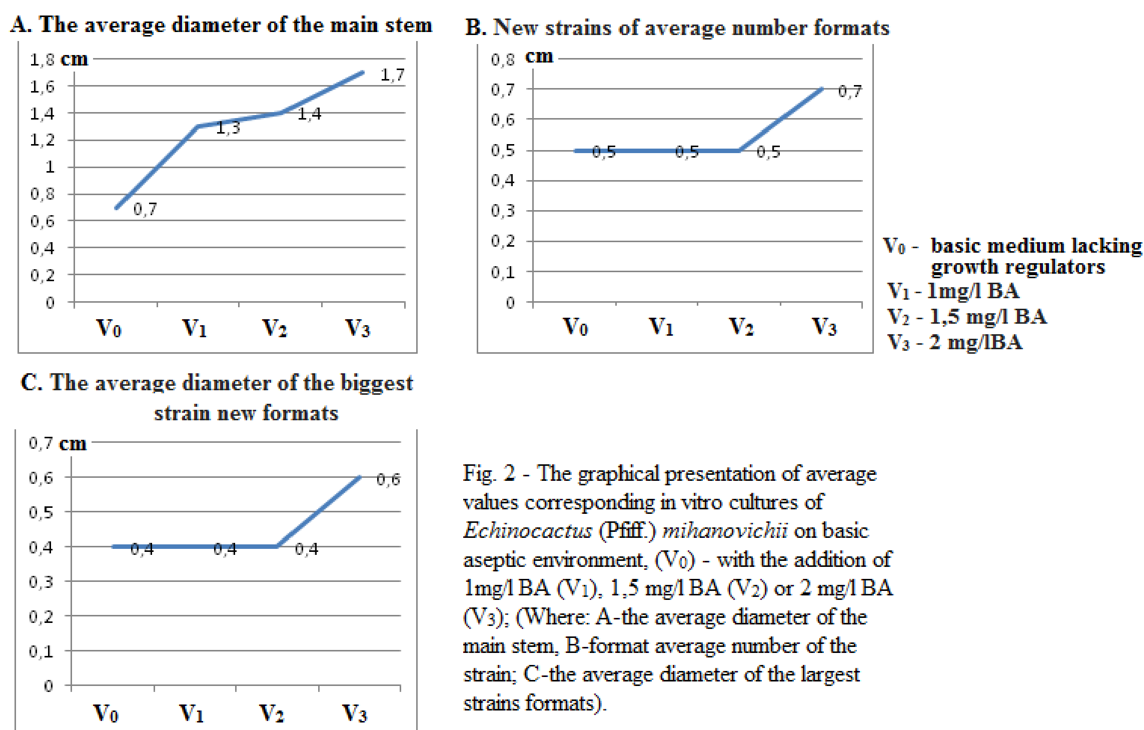

Fig. 2 - The graphical presentation of average values corresponding in vitro cultures of *Echinocactus* (Pfiff.) *mihanovichii* on basic aseptic environment, (V_0) - with the addition of 1mg/l BA (V_1), 1,5 mg/l BA (V_2) or 2 mg/l BA (V_3); (Where: A-the average diameter of the main stem, B-format average number of the strain; C-the average diameter of the largest strains formats).

By adding to the culture medium of benzyladenine (BA) was favored the generation and increase in diameter of the newly formed stems. The average number of newly formed stems of the control equalized V_0 (0,5 shoots/variant) in the case of the variant V_2 explants (medium supplemented with 1,5 mg/l BA), while in the variant V_3 (medium supplemented with 2 mg/l BA) this parameter was higher by 0,2 buds/variant (Fig.2B), which

represents an increase of 60% (Fig.3B).

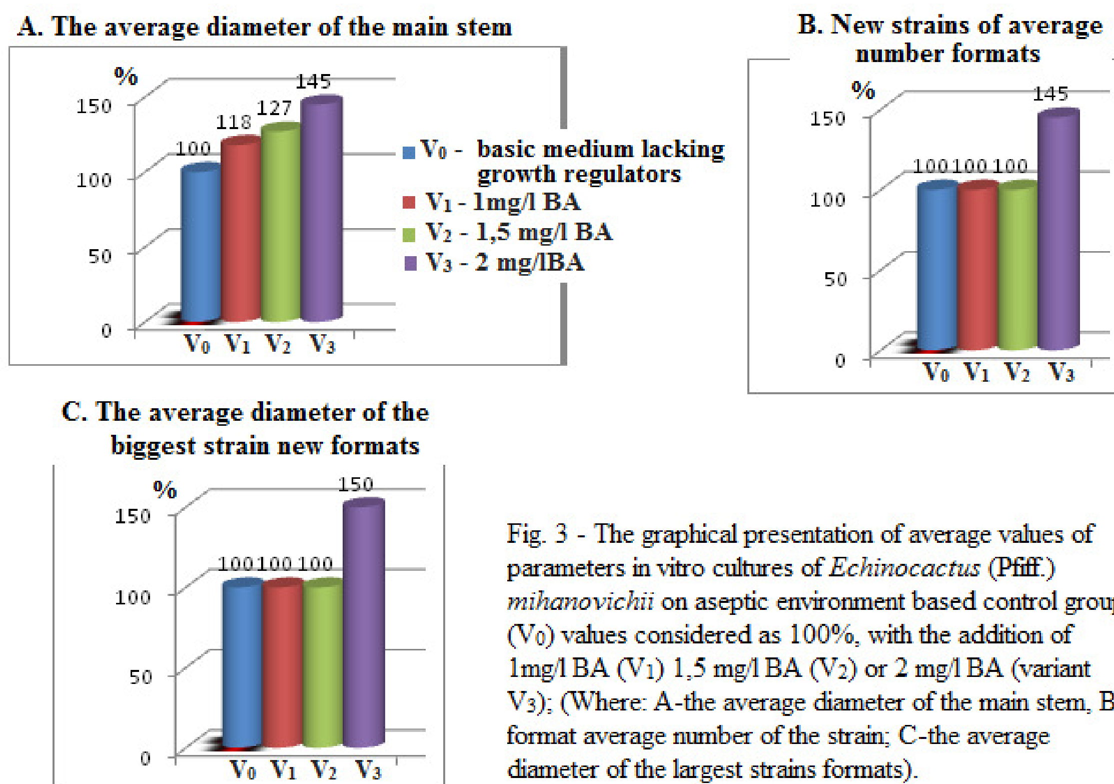

Fig. 3 - The graphical presentation of average values of parameters in vitro cultures of *Echinocactus* (Pfiff.) *mihanovichii* on aseptic environment based control group (V_0) values considered as 100%, with the addition of 1mg/l BA (V_1) 1,5 mg/l BA (V_2) or 2 mg/l BA (variant V_3); (Where: A-the average diameter of the main stem, B-format average number of the strain; C-the average diameter of the largest strains formats).

The average diameter of the strain newly formed (Fig.2C) was 0,4 cm V_0 to the control (medium lacking growth regulators) and V_2 (medium supplemented with 1,5 mg/l BA), while the values a parameter has reached an average of 0,6 cm from explants inoculated and grown in medium supplemented with 2 mg/l BA (V_3), which is retained in the control group an increase of 50% (Fig.3C). These results are consistent with those obtained by Pérez et al. (2002), which in vitro cultures of *Pachycereus pringlei* and *Stenocereus thurberi* obtained only on media supplemented caulinare branches with 1,5 mg/l BA, respectively, with 2 mg/l BA.

From the images shown in figure 4 it may be noted that after 90 days vitro cultures explants *Echinocactus mihanovichii* grown in culture medium supplemented with 1 mg/l BA (V_1) have formed new buds, although the level of the control group V_0 (medium lacking growth regulators) this phenomenon to occur; explants these variants present in the basal portions of the original color - red - has changed, becoming orange or yellow. New strains explants formed the control group, are smaller and pinkish reddish compared to the averages suplimantate formed with benzyladenine (BA),

which is remarkable both by number, size and the color. And explants and newly formed buds on them, and changed color from turning red and via more shades of orange and yellow; noting that stems from the newly formed both areolas and thorns are well developed, like plants from the wild. Characteristic of all explants regardless of substrate nutrient that increased contact area is necrosis in environment-culture. Characteristic of all explants regardless of substrate nutrient that rose is necrosis in the contact zone environment-culture features of cacti chlorophyll deficient that we have observed and in other experiments (Vidican and Cachiță, 2009; Vidican 2012; Vidican 2014).

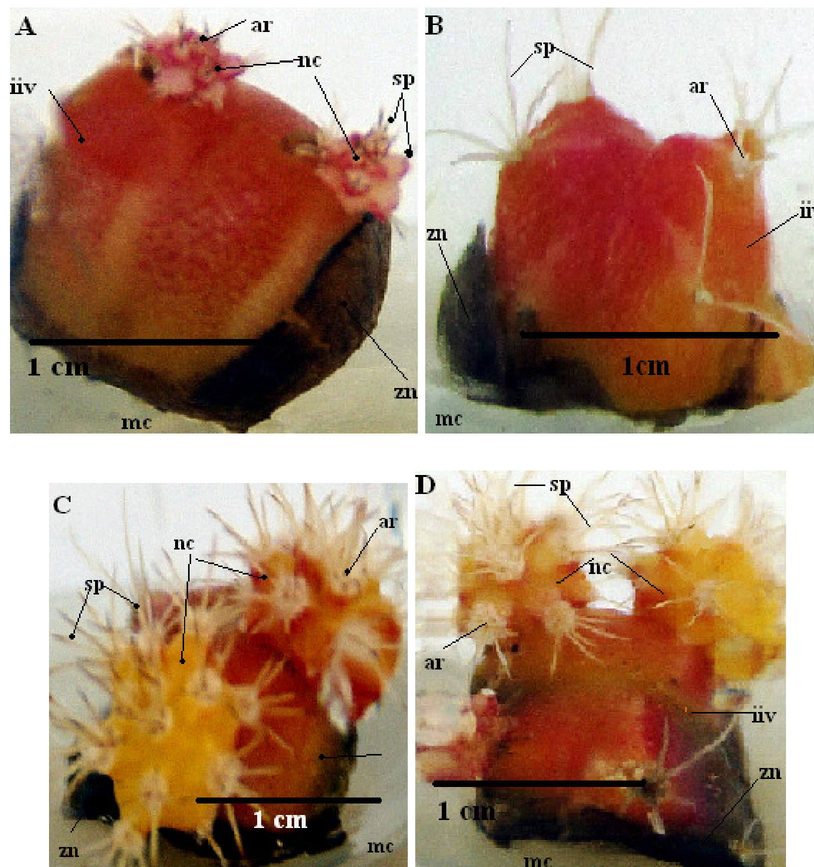

Fig.4. *Echinocactus (Pfiff.) mihanovichii* inoculum, 90 days after inoculation explant "in vitro", where A-modified basic aseptic environment on us and without growth regulators (V_0); B-base medium with the addition of 1 mg/l BA (V_1); C-basic medium with the addition of 1,5 mg/l BA (V_2); D-basic medium with the addition of 2 mg/l BA (V_3); (iiv-viable initial inoculum; mc-culture medium; nc-newly formed stems; ar-areola; sp-thorn; zn-area necrosis).

CONCLUSIONS

1. After 90 days *Echinocactus mihanovichii* vitro cultures found that the presence in the culture medium to 2 mg/l BA (V_3) stimulated caulogenesis.
2. Explants grown on this type of substrate showed an increase of 60% in terms of average number of strains newly formed, which has an average diameter basal by 50% higher compared to the parameter values remember recorded in the control group V_0 (free environment growth regulators).
3. At the inoculants of *Echinocactus mihanovichii* regardless of the composition of the culture medium was not manifested phenomena of rootedness or callus.

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