STUDY ON REGENERETIVE CAPACITY AND ORGANOGENIC EXPLANTS OF *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis* GROWN IN VITRO IN THE PRESENCE OF CYTOKININS IN THE CULTURA MEDIUM BENYZLADENIN (BA)

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Abstract

Opuntia genus includes a large number of species of great economic importance, for which it is seeking a rapid propagation method after showing materiel freely propagating viruses.To establish vitroculturii of Opuntia fragilis var, fragilis shoots 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 benziladenina - or 6-BA benzilaminopurina - BAP in different concentrations.

The evolution of 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 of Opuntia fragilis var fragilis, performed best on culture medium supplemented with supplemented with 2 mg/l BA (V_3), in which both the number and size of newly formed shoots and roots were highest.

Keywords: cacti, vitrocultures, benzyladenin (BA), newly formed stems, rootedness.

INTRODUCTION

Cytokinins are plant hormones without which non-dividing cells, and in tissue culture stimulates cell division processes and caulogenesis, prevent senescence, auxin exerts an antagonistic effect annihilating apical dominance, promotes cell dediferențierea etc. (Cachiță et al., 2004). Generally cytokinins are considered essential in vitro cultures cactus, they stimulate caulogenesis respectively neoformation of buds in the inoculum from which to generate strains (Mauseth, 1977). Escobar et al., (1986) reported that in order to multiply plant material benziladenina - or 6-BA benzilaminopurina - BAP is the most effective growth regulator tested vitroculturile cactus.

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).

The purpose of this experiment is to study how *Opuntia fragilis* var. *fragilis* explants responded to introduction into the culture medium of varying amounts of benziladenina - or 6-benzilaminopurina BA - PAO, respectively: 1 mg/l BA (V_1) of 1.5 mg/l BA (V_2) and 2 mg/l BA (V_3), it is known that the nutrient substrate added cytokinins generates a large number of shoots. Variant - V_0 - made up of a basic medium as the new growth regulators without being considered as representing 100%.

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 secționt 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).

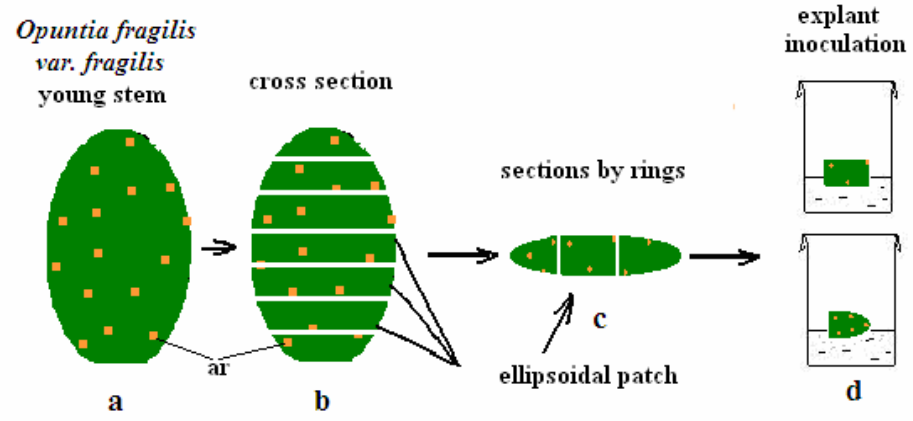

Fig. 1. Schemetic representation of *Opuntia fragilis* var *fragilis* young stems (a, b), and how slicing it into rings ellipsoid (c) and lateral explants inoculated on media centers and aseptic (d), where: ar - areola.

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 (Cachiță 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 6-benzilaminopurina (BA), obtaining the following experiments: V_0 - version control, medium without growth regulators; V_1 - medium supplemented with 1 mg/l BA (6-benzilaminopurina BA); V_2 - medium supplemented with 1.5 mg/l BA (6-benzilaminopurina BA);V_3 - medium supplemented with 2 mg/l BA (6-benzilaminopurina BA).

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

Comparing the results of the readings taken within 90 days from the start presented show that the average length is found mainly strain explants inoculated and grown on medium supplemented with 1 mg/l BA (V_1) with a 1.6 cm equalized witness V_0. In the variants V_2 (medium supplemented with 1.5 mg/l BA), and V_3 (medium supplemented with 2 mg/l BA) mean value of this parameter was 1.9 cm (Fig. 2A), which is an existing, increase of 18.75% (Fig. 3A) in relation to witness V_0 (medium lacking growth regulators).

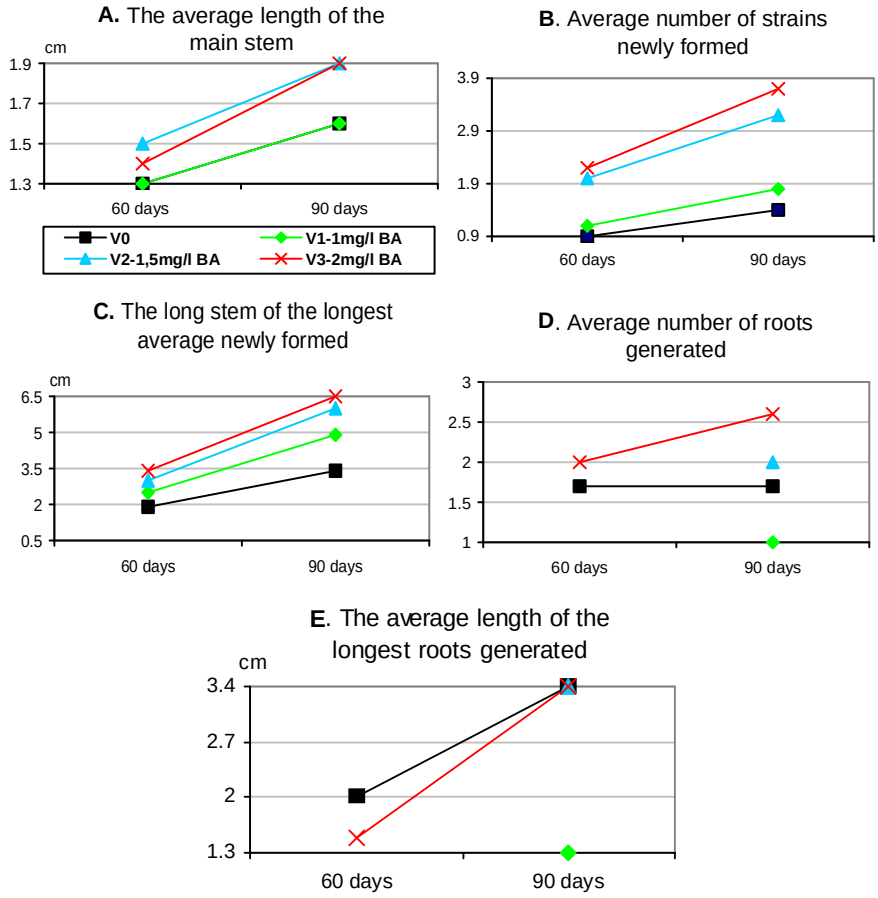

Fig. 2. Graphical presentation of mean values corresponding to the data analyzed in the in vitro cultures of *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis*, the basic aseptic environment as new - (V_0 version) - with the addition of 1 mg/l BA (V_1), 1.5 mg/l BA (variant V_2) or 2 mg/l BA (version V_3), data expressed in absolute values, (where: A-average length of the main stem, B-average number of newly formed strains, C-largest average length of newly formed strains, D-average number of roots generated, E-length average root of the largest foreground).

The addition of BA in the culture medium exerted a stimulating effect on caulogenesis in vitro cultures of *Opuntia fragilis* var. *fragilis*, the average number of newly formed strains has exceeded the similar data recorded at the control V_0 (medium lacking growth regulators) in all experimental groups on the substrate nutrient was supplemented with different concentrations benziladenină.

It is noted that the higher amount of BA increases the average number of newly formed strains/variant is higher, so the highest value of this parameter is detached explants variant V_3 (medium supplemented with 2 mg/l BA) the newly formed strains 3.7/variant (Fig. 2B) marked an increase of 164.28% (Fig. 3B).

On the version V_1 (medium supplemented with 1 mg/l BA) and V_2 (medium supplemented with 1.5 mg/l BA) have been a number of newly formed strains 1.8/approach, and new 3.2 formed strains/variant which is related to the values V_0 of the control group, added to 128.57% 28.57% and in the second case.

The average length of the largest strains newly formed ranged in value over witness V_0 (medium lacking growth regulators) in all experimental variants in the culture medium was supplemented with BA.

Again it is noted explants variant V_3 (medium supplemented with 2 mg/l BA) that with an average of 6.5 cm (Fig. 2C), marked an increase of 91.17%, while a average length of the largest new strain formed 6.0 cm explants grown in culture medium supplemented with 1.5 mg/l BA (V_2) recorded a plus of 76.47% (Fig. 3C).

The lowest values of this parameter were recorded in the experimental variant which culture medium was supplemented with the smallest amount of BA and 1 mg/l (V_1), where we recorded an average length of most branches caulinars 4.9 cm, which compared to the control group V_0 is added 44.11%. These results are treated statistically as very significant.

At this time it was noted that, in the rootedness, the phenomenon was observed in all the experimental variants under study. The average number of regenerated roots from the explants of *Opuntia fragilis* var. *fragilis* was 1 roots/variant V_1 (medium supplemented with 1 mg/l BA), the two roots/variant V_2 (medium supplemented with 1.5 mg/l BA) and 2.6 roots/variant V_3 (medium supplemented with 2 mg/l BA) (Fig. 2D) the absolute values relative to those recorded in the control group V_0 (medium lacking growth regulators) showed a deficit of 41.42% in the first case, and an increase 17.64% and 52.94% at V_2 and V_3 (Fig. 3D). These differences in statistical terms, are estimated to be significant.

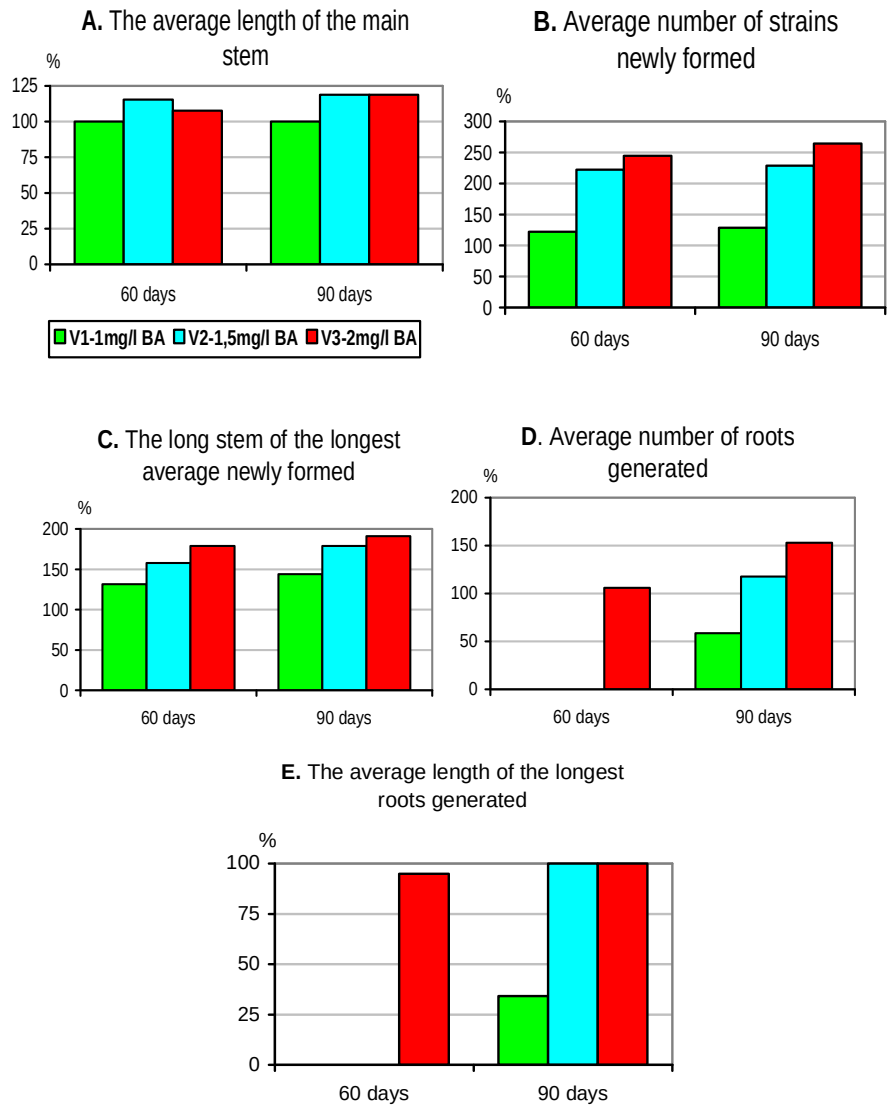

Fig. 3. Graphical presentation of mean values corresponding to the parameters investigated in vitro cultures of *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis*, modified based on new aseptic environment with the addition of 1 mg/l BA (V_1), 1.5 mg/l BA (variant V_2) or 2 mg/l BA (variant V_3), data expressed as a percentage, obtained for reporting the results analyzed values recorded in the control group studied parameters (V_0), without growth regulators, values considered as 100% (where: A-the average length of the main stem, B-average number of strains newly formed , C-the average length of the newly-formed higher strains, D-mean number of roots caused, E-greater the average length of the roots generated).

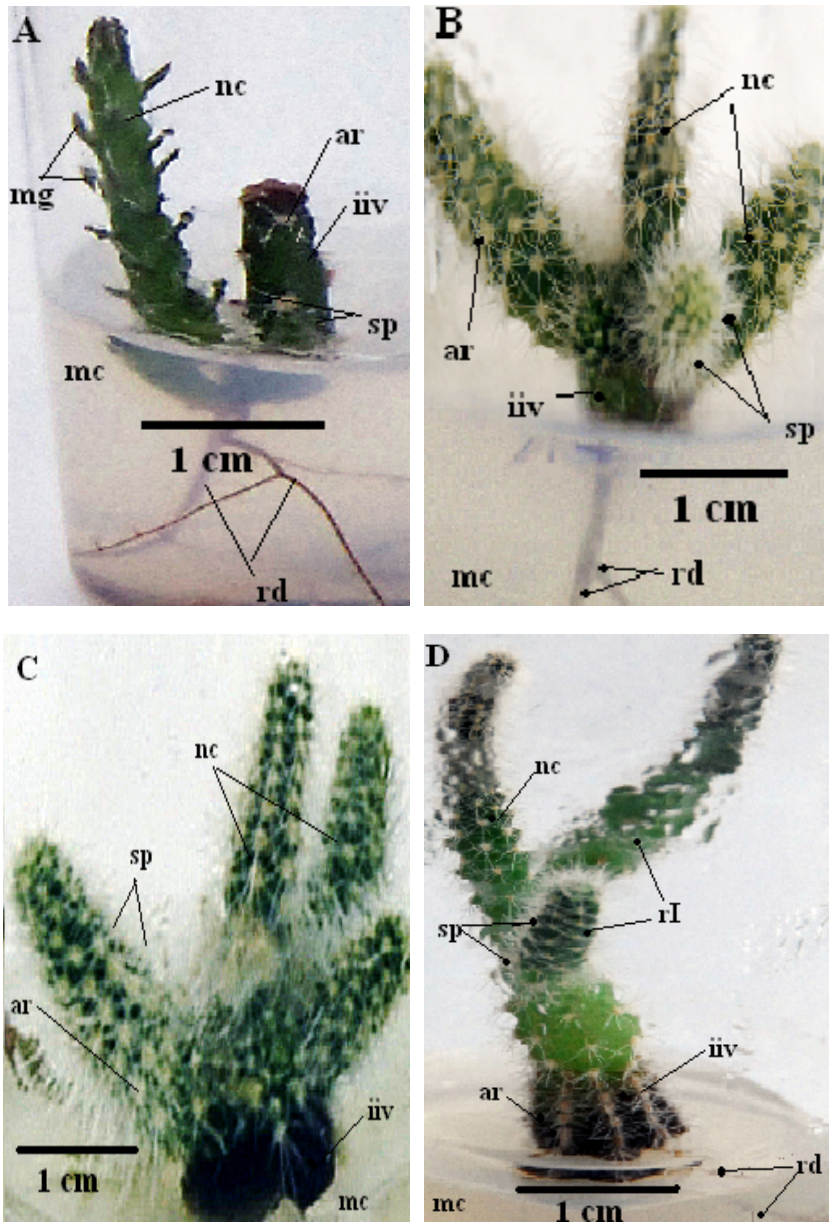

Fig. 4. *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis* inoculum 90 days after the start of the experiment, in which: A-the basic aseptic environment as new and without growth regulators (V_0), B-basal medium with addition of 1 mg/l BA (V_1) , C-basal medium with addition of 1.5 mg/l BA (V_2), D-basal medium with the addition of 2 mg/l BA (V_3), (iiv-the original inoculum viable, mc-medium culture, nc-newly formed stem, rd-root row, ar-areola, sp-thorns, rI-order branches, mg-buds).

Based on the results obtained it can be said that, by increasing the concentration of cytokinins in the culture medium - if this experiment benziladeninei (BA) added in a concentration of 1 mg/l (V_1), 1.5 mg/l (V_2) or 2 mg/l (V_3) - to obtain a large number of strains newly created/version, and also shoots per explant length is considerably higher, results similar to those reported Vidican et al. of 2010.

For the current experiment the culture medium was supplemented with up to 2 mg/l BA, but the results obtained allow us to think that we are in agreement with reports made by Khalafalla et al., 2007, which concluded that the most effective the culture medium in which the number and length of sprouts in the *Cactacaea* in vitro cultures is the suplimentet with benziladenină (BA), these parameters peaking at concentration 5.0 mg/l BA.

We also noted that the presence of BA in the culture medium was a positive stimulus that caused swelling of the areola to 100% of their differing bud a variable number, issue and found Juarez et al., (2002).

New seedlings generated from explants cultured on medium supplemented with cytokinins - BA - have areolas are well developed and long spines, white, puffy keeps the same (Fig. 4B, C and D).

It should be noted that in explants grown in medium supplemented with 2 mg/l BA (V_3) in the new strains have been generated by the first-order branches (Fig. 4D).

The average length of the longest root was 3.4 cm in the case of explants inoculated and grown in culture medium supplemented with 1.5 mg/l BA (V_2) and 2 mg/l BA (V_3), so that they V_0 failed to match the blank, while in the version V_1 (medium supplemented with 1 mg/l BA), this parameter has reached a value of only 1.3 cm (Fig. 2E) which is one less than 61, 77% (Fig. 3E).

Callus was found not to be present, no date, at any of the following experiments studied.

CONCLUSION

The data monitored and evaluated for 90 days I found particularly favorable effect which manifests benziladenina (BA) on the formation of new strains in vitroculturile of *Opuntia fragilis* var. *fragilis*.

From this point of view, your favorite the culture medium was found to be the supplemented with 2 mg/l BA (V_3), the explants inoculated and grown on the substrate were observed in most of the newly formed stemvariant, respectively 164.28% above the values recorded in the control group, while the average length of the largest shoot is 91.17% above average.

Regarding rootedness - noted all version V_3 (medium supplemented with 2 mg/l BA) which recorded an increase of 52.94% of the average number of roots/variant, while the average size of the longest roots tied witness V_0.

Callus not manifested - until the end of the experiment - in any of the variants studied.

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