STUDY ON ABILITY TO REGENERATIVE ORGANOGENESIS Opuntia (Tourney.) Mill. fragilis var. fragilis VITRO CURRENTLY GROWN IN SUBSTRATE A MIXTURE OF EQUAL AMOUNTS OF 3-INDOLEBUTYRIC (IBA) AND BENZYLADENINE (BA)

Vidican Iuliana Teodora*

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

Abstract

With great economic importance for cacti of the genus Opuntia there is a continuing increase in the demand for seedlings free of viruses, achievable only through micropropagation in vitro (Johnson and Emino 1979, Escobar et al., 1986; Rubluo et al., 1996; Smith et al. 1991).

Opuntia fragilis var fragilis to initiate vitro cultures of lime strains have prelevet hold, with sectioned the mature aureoles fragments of about 1 cm long and 0,5 cm thick but at least 2-3 areolas. Sterilized the explants were deposited on a culture medium consists of macro and Fe EDTA Murashige-Skoog (1962), trace elements Heller (1953), supplementation with a combination of equal amounts between auxin (3-indolilbutiric acid - 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) or 2 mg/l IBA + 2 mg/l BA (V_3).

Explants evolution was monitored for 90 days. Their response was different depending on the concentration of 3-indolilbutiric format (IBA) and benzyladenine (BA), added in equal parts. The proportion obtimă mixture of the two regulators of growth was found to be 2 mg/l IBA + 2 mg/l BA (V_3) this culture medium both the number and and the size of the shoots and roots of the newly formed were larger also explants and generating callus.

Keywords: vitro cultures, benzyladenină (BA), 3-indolilbutiric (IBA), newly formed stems, roots, callus.

INTRODUCTION

Phytohormones are endogenous stimuli, but may be added to the culture medium, the exogenous form of synthetic compounds that have the capacity to mimic the effects of natural growth regulators.

The combination of growth regulators in vitro cultures, we get the so-called hormonal balance, having the function of control of organogenesis, within limits, by changing the concentration or ratio of growth regulators present in the culture medium. The presence in the culture medium of high concentrations, but equal, auxin and cytokinins will drive both the processes of morphogenesis as well as the generation and growth of callus, while there in the culture medium together with an cytokinins, of increased concentrations of

auxin stimulate processes of rootedness, while an increase in the content cytokinins stimulate the formation of buds (Cachiţă, 2004).

Hormonal balance in the culture is influenced to a great extent endogenous phytohormones report can not be totally controlled. Rubluo et al., (1996), believes that in vitro cultures of cactus interaction between cytokinins and auxins added to the culture medium in the form of exogenous growth regulators directly influence rootedness. After Taiz et al., (1998), in addition to the hormonal balance of the culture, the processes of morphogenesis is influenced by the amount of light they are exposed vitro cultures.

After Griffith, 2001 Pinkava, 2002 *Opuntia* cactus genus are the most studied species in the world, due to the economic importance of this cactus. *Opuntia* cactus is a valuable economically, is eaten as a vegetable, but also has edible fruits, also used as fodder (Kluge and Ting, 1978; Casas and Barbera, 2002). This plant is considered a good indicator of the presence of pollutant (Nobel, 1994), it is also considered as an important tool to combat desertification (Valdez-Flores, 1994; El Gamrat, 2004).

This experiment was aimed at studying how they react *Opuntia fragilis var fragilis* to supplement the culture medium V_0 with a combination of equal amounts between 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) or 2 mg/l IBA + 2 mg/l BA (V_3). V_0 variant consists of the new modified basic medium without growth regulators and is 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 secţiont 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).

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.

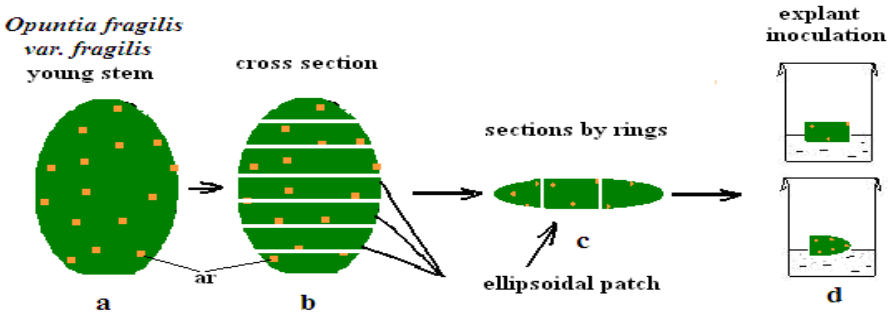

Fig. 1. Schemetic representation of *Opuntia fragilis* var *fragilis* young stems (a, b), and how slicing it into rings ellipsoid (c) and lateral explants inoculated on media centers and aseptic (d), where: ar - areola.

The basal medium (MB) presented, added with a combination of equal amounts between auxin (3-indolilbutiric - IBA) and cytokinins (benzyladenine - BA) in different concentrations respectively:

V_0 - the control, medium lacking growth regulators,
V_1 - medium supplemented with 1 mg/l IBA + 1 mg/l BA
V_2 - medium supplemented with 1,5 mg/l IBA + 1,5 mg/l BA
V_3 - medium supplemented with 2 mg/l IBA + 2 mg/l 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, explants 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 at 90 days after inoculation of explants culture medium compared to monitored data from 60 days vitro cultures, it appears that the average length of the main stem exceeded waist strain control group in explants variants V_2 (medium supplemented with 1,5 mg/l IBA + 1,5 mg/l BA) and V_3 (medium supplemented with 2 mg/l IBA + 2 mg/l BA) registering an increase of 6,25% and 18,75% in the second case (fig. 2A). In this experimental variant V_1(medium supplemented with 1 mg/l IBA + 1 mg/l BA) parameter values was under control batch marking a minus of 12,5% (fig. 2A).

At the same time the average number of new strains formed in all experimental variants exceeded the values recorded by this parameter to witness V_0 (medium lacking growth regulators), thus marking an increase of 14,28% at V_1 (medium supplemented with 1 mg/l IBA + 1 mg/l BA), the 71,42% to 100% of the variant V_2 (medium supplemented with 1,5 mg/l IBA + 1,5 mg/l BA) and V_3 (medium supplemented with 2 mg/l IBA + 2 mg/l BA), (fig. 2B). These results are consistent with those published by Clayton et al. (1990), who reported that in cultures "in vitro" some species of cactus, to increase production of axillary buds require a high level of exogenous auxin and cytokinins.

The maximum values of the average length of most newly formed strains were recorded at V_3 (medium supplemented with 2 mg/l IBA + 2 mg/l BA) version is marking an increase of 73,52%, followed by V_2 (medium supplemented with 1,5 mg/l IBA + 1,5 mg/l BA) with an increase of 47,05% compared with the control to V_0, while Why the explants variant V_1(medium supplemented with 1 mg/l IBA + 1 mg/l BA) recorded an increase of only 2,94% (fig. 2C).

The average number of roots that date is positioned over the control value V_0 variants V_3 (medium supplemented with 2 mg/l IBA + 2 mg/l BA) with 88.23% and 76.47% V_2 (medium supplemented with 1,5 mg/l IBA + 1,5 mg/l BA), V_1 (medium supplemented with 1 mg/l IBA + 1 mg/l BA) version of this parameter, leveling control (fig. 2D).

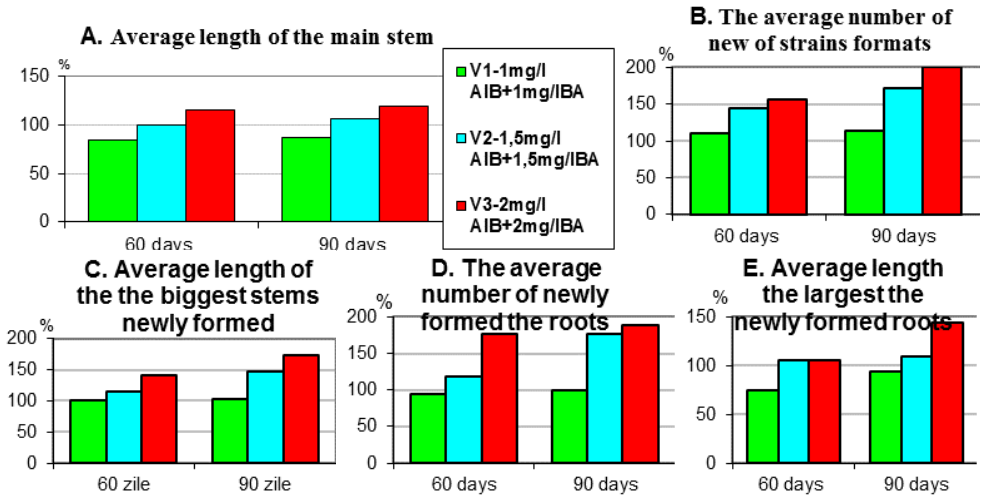

Fig. 2. Graphical representtation of average values corresponding to the track parameters vitro cultures of *Opuntia (Tournef.) Mill. fragilis var. fragilis*, modified based on the new aseptic environment, with additives consisting of a mixture of equal amounts of 1 mg/l IBA and 1 mg/l BA (V_1), 1,5 mg/l IBA and 1,5 mg/l BA (V_2) or 2mg/l IBA and 2 mg/l BA (V_3), data expressed as a percentage, obtained by reporting the values pursued the results achieved in the respective parameters biometrizaţi the control group (V_0), without rules growth values considered as 100%; (where: A-average length main stem; B-average number of stems the newly formed; C-average length of most stems the newly formed; D-average number of the roots; E-average length the largest the roots the newly formed.

Comparing the average length of the largest root in the experimental variants studied, it is above the average of the control group V_2 (medium supplemented with 1,5 mg/l IBA + 1,5 mg/l BA) an increase of 8, 82% and 44.11% at V_3 (medium supplemented with 2 mg/l IBA + 2 mg/l BA) version. The lowest is noted again that the V_1 (medium supplemented with 1 mg/l IBA + 1 mg/l BA) version, based on V0, scored a less the of 5.89% (fig. 2E).

At this time the genesis of callus was shown only in the variant V_3 (medium supplemented with 2 mg/l IBA + 2 mg/l BA).

Based on the results achieved in this experiment, we can say that the presence in the culture of a mixture of equal amounts of 3-indolilbutiric (IBA) and benzyladenine (BA) helped expression morphogenetic explants *Opuntia fragilis var. fragilis*, resulting in the generation of their level of both the new strains and the roots, also, by supplying the substrate with a combination of 2 mg/l IBA + 2 mg/l BA (V_3) was sufficient to stimulate callus formation.

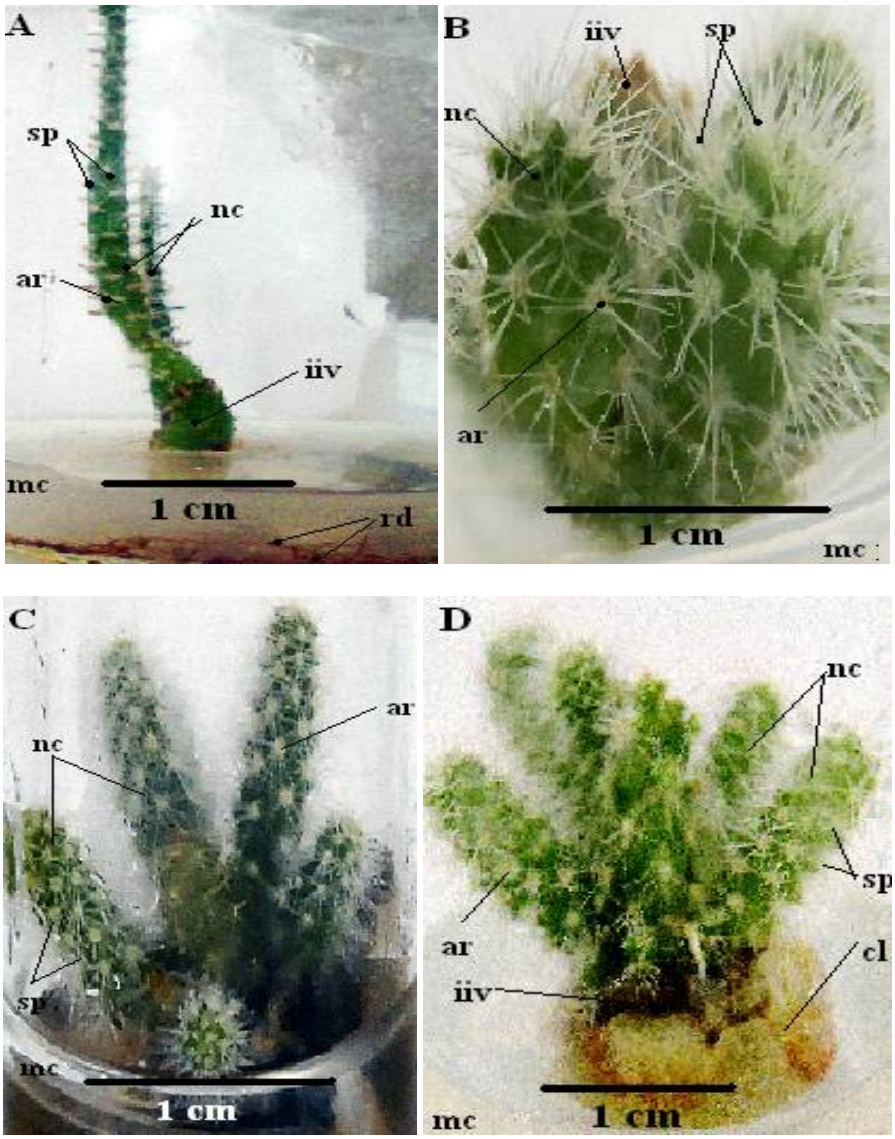

Fig. 3. The inoculum *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis*,, 90 days after the inoculation of the explant "in vitro", where: A-the basic aseptic environment of new modified without growth regulators (V_0); B-basic medium with an addition consisting of a mixture of equal amounts of 1 mg/l IBA and 1 mg/l BA (V_1); C-basic medium with an addition consisting of a mixture of equal amounts of 1,5 mg/l IBA and 1,5 mg/l BA (V_2); D-basic medium with an addition consisting of a mixture of equal amounts of 2 mg/l IBA and 2 mg/l BA (V_3); (where: iiv-initially inoculum viable; mc-culture medium, nc-strains newly created; rd-roots; ar-areoles; sp-thorns; cl-callus).

It notes that follow parameter values increase with the concentration of the mixture of the two growth regulators added to the culture medium (Vidican, 2014).

Comparing the images in figure 3 notes the beneficial effect of the two growth regulators that significantly increased the number of shoots per explant, they managed to elongate and form rosettes also flattened shape sprouts specific plants of the genus *Opuntia* formed on environment culture which contained the lowest amount of growth regulators respectively 1 mg/l IBA + 1 mg/l BA (V_1). In the explants cultured on medium with the addition consists of 2 mg/l IBA + 2 mg/l BA (V_3) to generate and callus, which is currently based explant as a sleeve different color from one area to another, in shades of white, cream to tan (fig. 3D).

CONCLUSION

1. After 90 days of initiating *Opuntia fragilis var. fragilis* in vitro culture noted that explants reacted positively to the incentive effect due to the presence in the culture medium the mixture of 3-indolilbutiric (IBA) and benzyladenine (BA), added in equal shares, share best mixture of the two regulators growth turned out to be 2 mg/l IBA + 2 mg/l BA (V_3).
2. Formation of buds 100% higher in explants grown in culture medium supplemented with 2 mg/l IBA + 2 mg/l BA (V_3), all this topped with 73,52% witnessed in height.
3. The explants grown in culture medium supplemented with 2 mg/l IBA + 2 mg/l BA (V_3) generated by 88,23% more root, which is longer than the 44,11% as compared with the same parameters registered the blank V_0 (medium lacking growth regulators).
4. Genesis callus manifested only inoculii reared culture medium improved by 2 mg/l IBA + 2 mg/l BA (V_3).

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