STUDY ON THE REGENERATIVE AND ORGANOGENIC CAPACITYOF *Echinopsis (zucc.) chamaecereus f. lutea*IN VITRO CULTUREON AN ADDITION MEDIUM OF A MIXTURE FORMED IN EQUAL QUANTITIES OF 3-INDOLYLBUTIRIC ACID (AIB) AND OF BENZYLADENINE (BA)

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Abstract.

Cactus with yellow epidermis, Echinopsis chamaecereus f. lutea, is part of the group of chlorophyll-deficient cacti, which occur spontaneously in cultures due to mutations, which are unable to synthesize chlorophyll survive only if they are grafted.

In order to establish an in vitro culture of Echinopsis chamaecereus f. Lutea, we took explants represented by minibus (seedlings) from mother plants grown in the greenhouse. Inoculation of the explants was done on a culture medium consisting of macroelements and EDTA Murashige-Skoog Fe (1962), Heller microelements (1953), supplementation with medium supplemented with a mixture of equal amounts of 3-indolylbutyric acid (AIB) and of benzyladenine (BA).

The evolution of the explants was monitored for 90 days. Response of explants of Echinopsis chamaecereus f. lutea to the presence in the culture medium supplemented with a mixture of equal amounts of 2mg/l 3-indolylbutyric acid (AIB) and 2mg/l benzyladenine (BA), variant V_3, demonstrated the beneficial effect on the generation of new strains, finding an increase of 133,33%, and in terms of their dimensions, this parameter marked an increase of 150%, compared to the values of control group V_0 (average lacking growth regulators). It is worth noting that, during this experiment, the rhizogenesis and calusogenesis process did not take place.

Keywords: vitroculture, 3 indolylbutyric acid (AIB), benzyladenine (BA), newly formed stems.

INTRODUCTION

Echinopsis chamaecereus f. lutea, is a chlorophyll - deficient cactus, with yellow epidermis (Copăcescu, 2001), lacking the possibility of synthesizing chlorophyll due to the small number of chloroplasts, about 1/3 of the total plastids (Shemorakov, 2003).

The depigmentation process is determined by the spontaneous emergence in cultures of some mutations (Shemorakov, 2001) influenced to a large extent by temperature and light. According to Skoulkin (2000) plants kept at a lower temperature than the one obtained in the shade, rarely, or even at all, develop such mutations. Russian researchers have shown a special interest in the chlorophyll-deficient species of cacti, and thus they have been classified according to the color of the epidermis (Shemorakov,

2003), according to which *Echinopsis chamaecereus f. lutea* is part of the monocolor group.

According to Shemorakov (2001) the reversible mutation of the plastids during meiosis causes that by reproductive generation in *Echinopsis chamaecereus f. lutea* has minimal chances for it to retain its color (Kornilova, 2008). Thus it was concluded that plants can retain this particular property only reproduced by cloning.

In Vitro cultures, the combination of growth regulators called organogenesis hormonal balance adjustment can be achieved within certain limits by changing the concentrationratio of regulatory present in the growth medium. After theCachiță et al.(2004), the existence of aculturemedium of high concentration soft 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 present high concentrations, but equal, the two compounds will bedrivenwiththe morphogenesis process, the generation of callusandits growth.

Hormonalbalancein the culture mediumcan not be fully controlled, it is influenced to a large extent on the endogenous phytohormone ratio. TheRubluoet al. (1996), believes that invitro culturesofcactus, rooting is the result of interaction between cytokinins and auxins added to the culture medium in the form of exogenous growth regulators, but which, inTaizet al.(1998), are affected bythe amount of lightthey are exposedvitro cultures.

This experiment was aimed at studying the way they react cactus explantsinculture medium supplementation changes -V_0 -medium lacking growth regulators-with a combination of equal amounts between an auxin (3-indolilbutiric -IBA) andcytokinins(benzyladenine-BA), added in different concentrations, respectively, 1 mg/lIBA+1mg/lBA(V_1); 1,5mg/lIBA+1,5mg/lBA(V_2) and2mg/lIBA+2 mg/lBA(V_3).

The purpose of the experiment covered by this article is to analyze the reaction variability of the vitrocultures of *Echinopsis chamaecereus f. lutea*, in the presence in the culture medium of a mixture consisting of equal amounts of 3-indolylbutyric acid (AIB) and benzyladenine (BA).

MATERIALS AND METHODS

The biological material used in our experiments consisted of regenerated seedlings on *Echinopsis chamaecereus f. lutea* (fig. 1). The explants were about 1 cm long, 0,5 cm thick and a diameter of 0.5-1.5 cm, depending on the area from which they were harvested (fig. 2).

The vegetal material, seedlings of *Echinopsis chamaecereus f. lutea*, was asepsis by immersion, for one minute, in 96º ethyl alcohol, followed by its coating with 0.8% sodium hypochlorite solution, mixed with water in

relation to 1: 2; To the disinfectant solution, three drops of Tween 20 are added (as a surfactant) (Cachiţă et al., 2004).

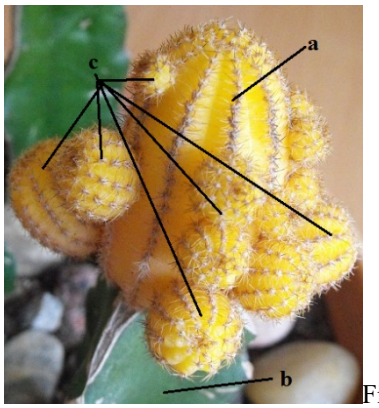

Fig.1. Young plant of *Echinopsis chamaecereus f. lutea*, grown in a greenhouse (where: a-grapes, b-rootstocks, c-buds newly formed)

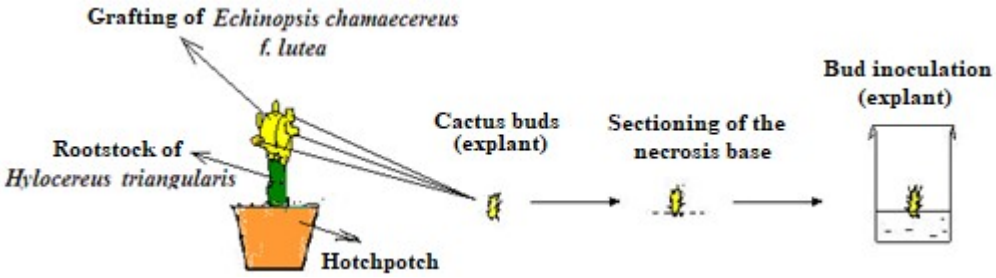

Fig. 2. Schematicrepresentation of the exploitation mode of the fragments of *Echinopsischamaecereus f. lutea*, whichwillbeinoculated on aseptic media.

During the 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, five minutes each. Then, the plant material was deposited in aseptic conditions, in the hood with horizontal laminar flow, of sterile air, in operation, on the filter paper rounds sterilized in the oven, introduced in aseptic Petri dishes. Subsequently, the necrotic parts of the future inocula were removed.

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 ofthe medium wasadjusted to5,8, the first toautoclaving. Thebasal medium (MB) added amixture of equal amounts of mg/lIBAand 1 mg/lBA(variantV_1), 1,5 mg/lIBAand

1,5mg/lBA(variantV_2) or 2mg/lIBAand2 mg/lBA(variantV_3), obtaining the followingexperimental: V_0 - version control, medium without growthregulators; V_1 -1 mg/lIBAand 1 mg/lBA; V_2 - 1,5 mg/lIBAand 1,5 mg/lBA and V_3 -2 mg/lIBAand 2 mg/lBA.

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/ 4h, lighting achieving cultures with the white light emitted by fluorescent lamps, the intensity of 1700 lux.

Explantsandexplantsreactionprogresswas monitoredfor 90days.Inthis time periodwere conduct periodic observations andreadingsevery30 days.Values thus obtainedin the control group(V_0, the explants grow in basic medium, without growth regulators)were consideredhe referenceas 100% to these all the other recorded values are related.

RESULTS AND DISCUSSION

After 90 days of viticulture, the basal average diameter of the main strain of *Echinopsis chamaecereus f. lutea* exceeded for all experimental variants the values recorded in the control sample V_0 (the medium lacking growth regulators), by 0,1 cm at V_1 (supplemented medium), with a mixture of 1 mg/l AIB and 1 mg/l BA) which represents a further 22,22%, and 0,2 cm (Fig. 3A) in the case of explants belonging to V_1 variants (medium supplemented with a a mixture of 1 mg/l AIB and 1 mg/l BA) and V_3 (medium supplemented with a mixture of 2 mg/l AIB and 2 mg/l BA), thus increasing 33,33% (Fig. 4A).

During this time period, new strains were generated in explants belonging to variant V_3 (medium supplemented with a mixture of 2 mg/l AIB and 2 mg/l BA) which by an average of 0,1 numerically exceeded the new strains/variant (Fig. 3B), compared to control V_0, thus marking an increase of 33,33% (Fig. 4B), and with a mean basal diameter of 0,6 cm, they registered an increase of 50%. At the level of explants inoculated and raised on experimental variants V_1 (medium supplemented with a mixture of 1 mg/l AIB and 1 mg/l BA) or V_2 (medium supplemented with a mixture of 1,5 mg/l AIB and 1, 5 mg/l BA) no new strains were generated, probably due to the reciprocal inhibition effect on the two growth regulators. Our

results are in agreement with those published by Mata et al., (2001), who in the *Turbinicarpus cactus*, observed that by supplementing the culture medium with a combination of auxin and cytokinin, in different concentrations, it has become a restrictive factor for the formation of shoots.

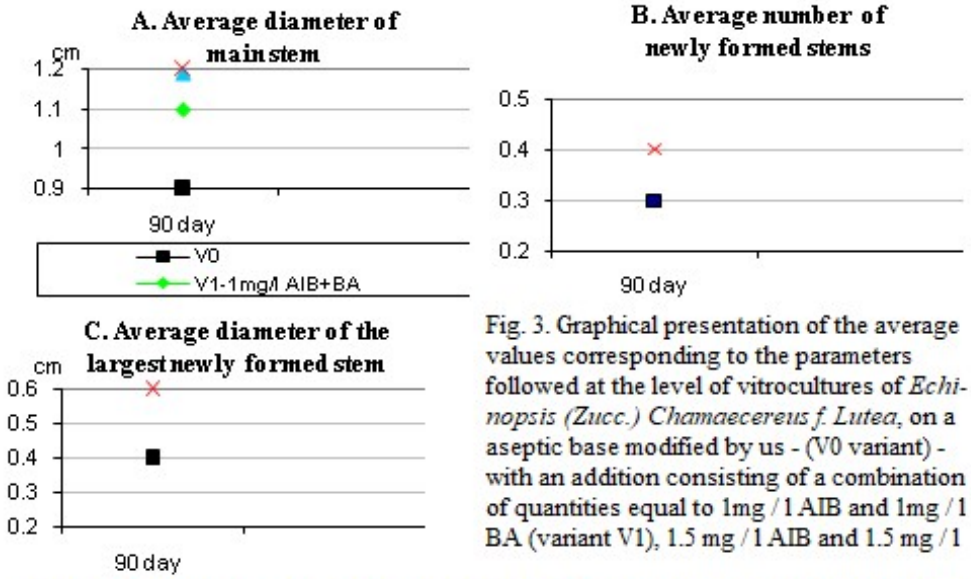

Fig. 3. Graphical presentation of the average values corresponding to the parameters followed at the level of vitrocultures of *Echinopsis (Zucc.) Chamaecereus f. Lutea*, on a aseptic base modified by us - (V0 variant) - with an addition consisting of a combination of quantities equal to 1mg / l AIB and 1mg / l BA (variant V1), 1.5 mg / l AIB and 1.5 mg / l BA (variant V2) or 2mg / l AIB and 2mg / l BA (variant V3), given expressed in absolute values; (where: A - average diameter of main stem; B - average number of newly formed stems; C - average diameter of the largest newly formed stem)

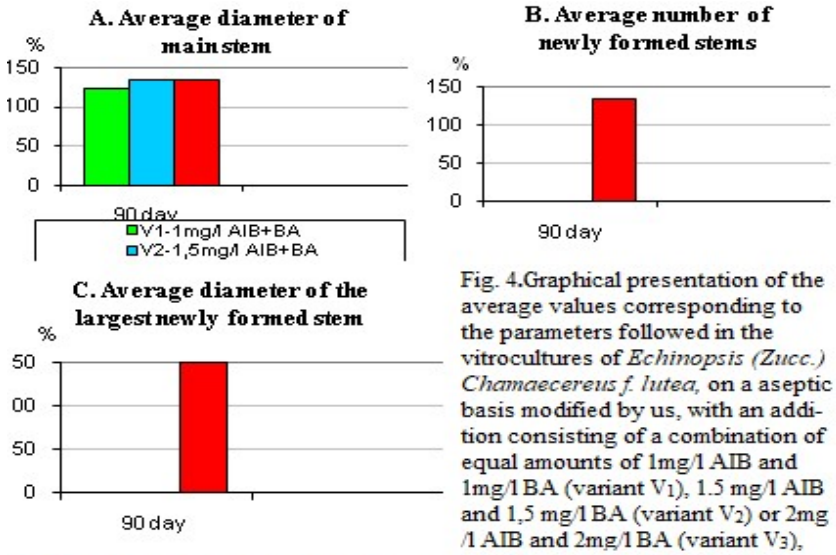

Fig. 4.Graphical presentation of the average values corresponding to the parameters followed in the vitrocultures of *Echinopsis (Zucc.) Chamaecereus f. lutea,* on a aseptic basis modified by us, with an addition consisting of a combination of equal amounts of 1mg/l AIB and 1mg/l BA (variant V_1), 1.5 mg/l AIB and 1,5 mg/l BA (variant V_2) or 2mg /l AIB and 2mg/l BA (variant V_3), /l AIB and 2mg/l BA (variant V_3), data expressed as a percentage, obtained from reporting the biometric values to the results recorded at the respective biometric parameters in the control group (V_0), without growth regulators, values considered to be 100%; (where: A - average diameter of main stem; B - average number of newly formed stems; C - average diameter of the largest newly formed stem).

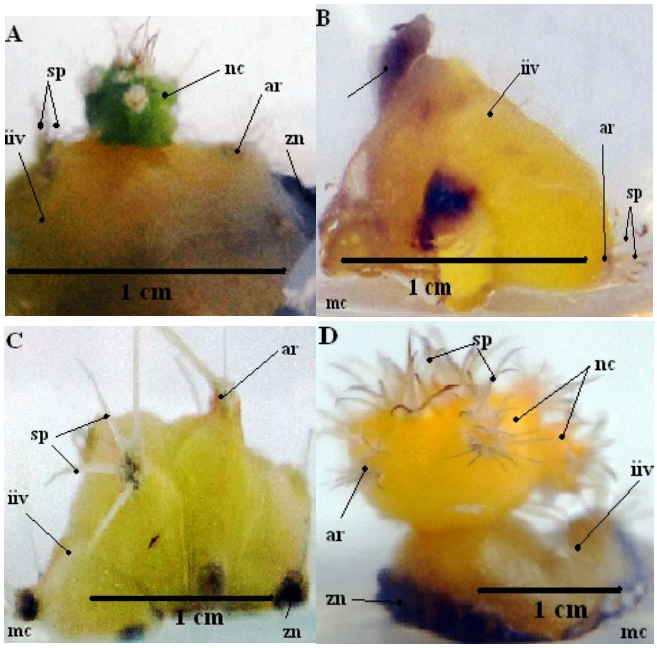

Fig. 5. Inoculi of Echinopsis (Zucc.) Chamaecereus f. Lutea, 90 days after inoculation of the explant "in vitro", where: A-base medium modified by us lacking growth regulators (V0); B-on basic medium with an addition consisting of a combination of equal amounts of 1mg/l AIB and 1mg /l BA (V_1); C-on basic medium with an addition consisting of a combination of equal amounts of 1,5mg/l AIB and 1,5mg/l BA (V_2); D-on basic medium with an addition consisting of a combination of equal amounts of 2 mg/l AIB and 2 mg/l BA (V_3); (iiv-inoc initially viable; mc-culture medium; nc-newly formed stem; ar-areola;sp-thorn; zn-area necrosis).

From figure 5B it can be seen that similar to the results obtained by us in other experiments (Vidicanet al.,2017), in the explants of *Echinopsis chamaecereus f. lutea* (yellow cactus) vitrocultivated on the control lot (culture medium without growth regulators - V_0), the new stems they are green (Fig. 5A), while those inoculated and grown on culture media supplemented with growth regulators, in this case with 2 mg/l AIB and 2 mg/l BA (V_3), they have retained yellow (Fig. 5D). In both the new strains and the explants, the areoles and spines have retained their species characteristics (white areola, also white spines, 1-1,5 cm long), being well developed (Fig. 5C). It is worth noting the presence of necrosis, regardless of the composition of the culture medium, located either in the contact area with the culture medium or on the surface of the explant (Fig. 5B and C).

For 90 days the presence in the culture medium of the mixture consisting of equal quantities of 3-indolylbutyric acid (AIB) and benzyladenine (BA), regardless of the amount in which it was added, did not stimulate either rhizogenesis (Vidicanet al.,2009) or calusogenesis.

Comparing the data obtained we found that the vitrocultures of Echinopsis *chamaecereus f. lutea* gave unsatisfactory results regarding their response to the composition of the nutrient substrate - in this case a mixture of an auxin (AIB) and a cytokinin (BA). - combination that failed to enhance the organogenic capacity of the explants.

CONCLUSIONS

1. After 90 days of in vitro culture initiation in *Echinopsis chamaecereus f. lutea* we found that supplementing the culture medium with a mixture of equal amounts (V3) of 2mg/l of 3-indolylbutyric acid (AIB) and 2mg/l benzyladenine (BA) had beneficial effects only on the generation of new strains, where it was found a 133,33% increase compared to the values of the control group V_0 (medium lacking growth regulators), in terms of their diameter, this parameter scored a 150% increase over the witness.
2. In explants of *Echinopsis chamaecereus f. lutea* (yellow cactus) grown on the control group (culture medium without growth regulators - V_0), the new strains are green while those grown on culture media supplemented with 2 mg/l AIB and 2 mg/l BA (V_3), remained yellow.
3. Regardless of the composition of the culture medium, the presence of necrosis located either in the contact area with it or on the surface of the explant is noted

4. The phenomenon of rhizogenesis and calusogenesis did not manifest in any of the variants the experiments taken in the study.

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