STUDY ON THE INFLUENCE OF 3 INDOLILBUTIRIC (AIB), ADDED IN DIFFERENT CONCENTRATIONS IN THE CULTURE MEDIUM, THE REGENERATION CAPACITY OF EXPLANTS *Aylostera* (Speg.) *heliosa*

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Abstract

Aylostera heliosa, decorative cactus both the port and the flower is a difficult species propagated by grafting, but found a viable method of multiplication in vitro micropropagation. For this cactus can be successfully acclimatized need to develop a strong root system. In this experiment we investigated how phytohormones AIB (3 indolilbutiric acid) added to the culture medium favored rootedness.

In order to establish a Aylostera heliosa vitroculture, we sampled young stems from mother-plants grown in greenhouses, which were subsequently designed in segments that have at least 2-3 areolae. Inoculation was made alkaline mineral culture medium Murashige-Skoog (1962) with - macro and micronutrients Heller (1953), without growth regulators (V_0), version control, and supplemented with 3 indolilbutiric acid in different concentrations as follows: 1mg/l IBA (V_1), 1.5 mg/l IBA (V_2) and 2mg/l IBA (V_3).

The evolution of vitrocultures was followed for 90 days. Explants reaction was different depending on the nature of culture substrate. Finally, as expected, the phenomenon of rootedness was favorable in terms of both the number and size of newly formed roots in culture medium supplemented with 2 mg/l of 3-indolilbutiric (V_3).

Keywords: cacti, vitrocultures, 3 indolilbutiric acid, rootedness.

INTRODUCTION

Plant hormones or growth regulators are organic compounds in concentrations much lower than those nutrients or vitamins, stimulates or inhibits growth and morphogenesis, respectively regulate different phy-siological processes in the tissues and organs of the plant (Davies, 2004).

3 indolilbutiric acid (AIB) is a class of synthetic auxin, but apparently can be found in nature, but only in some plant species (after Moore, Cachiță et al., 2004). Auxins are commonly used in tissue culture with stimulants rootedness process and with cytokinins, and they play a major role in the proliferation and growth of plant cells. Fito inoculated tissues synthesized, as well, auxin - or IBA - in the apical meristems, and by adding to the culture medium of the phytohormone produced by synthesis, are favored rootedness. At vitroculturile of *Opuntia ellisiana* on culture media with added acid 3 indolilbutiric (AIB), the percentage of rooting explants was 100%, considering that the cumulative effect of endogenous

auxin in the intake of exogenous auxins leads getting a large number of roots (Juárez et al., 2002).

Aylostera heliosa, cactus, decorative (figure 1) both in port - due thorns silvery-white edge aligned comb (Perez et al., 2002) and by red or orange flowers is a very difficult species multiplied by grafting (Myeong et al., 2004). *Aylostera heliosa* like other cacti can multiply rapidly and effectively by micropropragare in vitro (Karimil et al., 2010).

Fig. 1. *Aylostera heliosa* stems and flowers

Cacti are considered to be highly susceptible to the process of differen-tiation when grown in mineral-rich medium growth regulators (Copăceacu, 2001) invariably induces organogenesis processes.

In this experiment our goal was to study the reactions of *Aylostera heliosa* plant inocula the existence in the culture medium 3 indolilbutiric acid (AIB).

MATERIALS AND METHODS

In this experiment in order to initiate the *Aylostera heliosa* in vitro culture, the plant material consisted from young stems harvested from mother plants. The material was sterilized by placing for one minute, in alcohol 96º, followed by a submersion operation, in a sodium hypochlorite solution 0.8% in proportion of 1:2 with water (one part sodium hypochlorite, 2 parts sterile water), which were added three drops of Tween 20, shaking continuously (Cachiță et al., 2004). After 20 minutes, the removal of disinfectant agent was achieved by washing the plant material in sterile water, in five consecutive rinses, of five minutes each, after which the plant material was deposited on aseptic filter paper rings, introduced in sterile Petri dishes. Sizing future inocula was performed under aseptic conditions in horizontal laminar flow hood, with sterile air. Young stems were cut into spherical slices, which had the following dimensions: 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. Explants modeling (figure 2) were done so that each has at least 2-3 areolae (Karimil et al., 2010).

The mineral medium culture used in this experiment consisted of: macroelements and Fe-EDTA, (Murashige and Skoog, 1962), microelements (Medeiros et al., 2006), mineral mixture to which were added vitamins: HCl pyridoxine, HCl thiamine and nicotinic acid (each 1 mg/l), 100 mg/l m-inositol, 20 g/l sucrose and 7 g/l agar-agar, pH of the medium was adjusted to a value of 5.8.

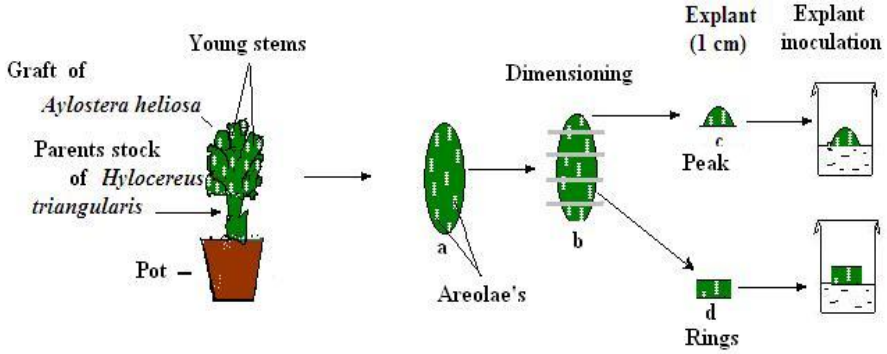

Fig. 2. Schematic representation of sectioning method of the young stems to obtain *Aylostera* (Speg.) *heliosa* explants (where: a-young strain, b-sizing of young stems, c- explant represented from young stem d- explant represented as spherical rings).

In order to obtain the proposed alternatives, we added new developed nutrient medium devoid of growth regulators (V_0), version control, different concentrations of AIB, ie 1mg/l IBA (V_1), 1.5 mg/l IBA (V_2) and 2mg/l IBA (V_3).

Sterilization of vials with medium was performed by autoclaving at a temperature of 121 °C for 30 minutes. The recipients with medium culture had a capacity of 15 ml, and each were placed 5 ml of the medium. After cooling the media proceeded to inoculate explants, operation conducted in aseptic camera on a laminar flow hood, horizontal, with sterile air.

After inoculation, explants were vials were filled with polyethylene folia. Conditions in the growth chamber were as follows: illuminated with white light emitted by fluorescent tubes, photoperiod was under 16 hours light/24 h 1700 lux light intensity, temperature between 20-24°C.

Vitroplantlets reaction after inoculation was monitored for 12 weeks. Biometric assessments were taken at intervals of 30 days. Observations consisted from biomeasured: vitroplantlets length regenerated from explants, number of rotes, callus formation, determining the number of neostems and branches developed on the initial inocula.

RESULTS AND DISCUSSION

Exlantelor reaction was monitored for 90 days at 60 and 90 days after initiation of *Aylostera heliosa* vitroculturilor after appraisals were determined: percentage of survival fitoinoculilor, based on botanical characteristics of species - stalks spheroids - diameter and its length is not measured, the number strains newly formed, the largest diameter caulinare new formation, root number and length of the largest root, also the number and callus formations, dimetra largest callus, the values recorded by the control group V_0 - plant inocula raised the basal medium devoid of growth regulators - were considered as 100%, were reported in all the readings taken from other experimental variants.

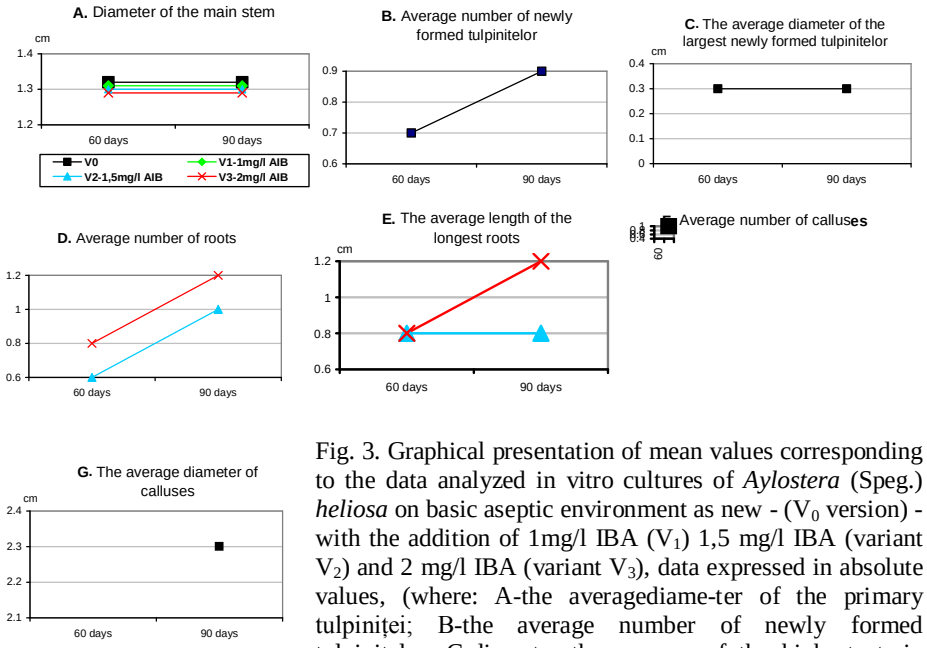

Fig. 3. Graphical presentation of mean values corresponding to the data analyzed in vitro cultures of *Aylostera* (Speg.) *heliosa* on basic aseptic environment as new - (V_0 version) - with the addition of 1mg/l IBA (V_1) 1,5 mg/l IBA (variant V_2) and 2 mg/l IBA (variant V_3), data expressed in absolute values, (where: A-the averagediame-ter of the primary tulpiniței; B-the average number of newly formed tulpinitelor; C-diameter the average of the highest strain newly formed; D-number of roots; E-greater the average length of the roots; F-the average number of calli; G-the average diameter of calluses).

After 5 days after inoculation was observed that the number of containers, *Aylostera heliosa* inoculated explants, which showed infection was between 12-15% - which is a good sterilization biological material - both in control V_0 (free environment growth regulators) as well as other experimental versions that we culture medium supplemented with auxin, or 3-indolilbutiric acid (IBA) concentration of 1 mg/l (V_1), 1.5 mg/l (V_2) or 2 mg/l (V_3). In vitro culture after 90 days, the percentage of survival of

explants was only 57-62% of the total inoculated initially cause that led to the loss of plant material was the emergence and proliferation of necrosis, a phenomenon by George et al., (1984) occurs due to the release of the plant inocula - in culture medium - of phenolic compounds, toxic substances that act antagonistically inhibiting the growth neogenesis, and causing the color change explants, due to the synthesis of quinones, very active in this matter of view (Pierik, 1990, Deberg et al., 1991).

At 90 days after initiation of the experiment, the absolute average basal diameter *Aylostera heliosa* strains remained constant - and 1.3 cm (figure 3A) - both in the control group belonging plant inocula V_0 (medium lacking growth regulators) as well as those grown in medium supplemented with IBA (figure 4).

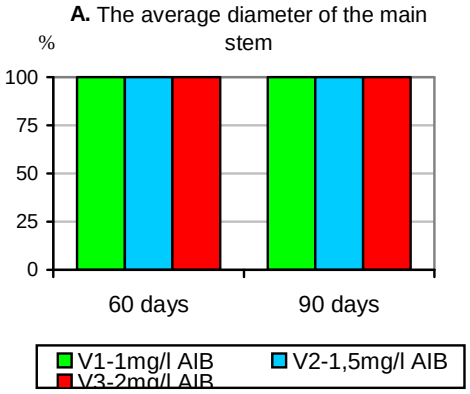

Fig. 4. Graphical presentation of mean values corresponding to the data analyzed in the in vitro cultures of *Aylostera* (Speg.) *heliosa*, modified based on new aseptic environment with the addition of 1mg/l IBA (V_1), 1.5 mg/l IBA (V_2 version) or 2 mg/l IBA (variant V_3), given in percentage, obtained by reporting the results of target values from the data recorded in the control group studied (V_0), without growth regulators, values considered 100% (where: A-the average diameter of the main stem).

Analyzing biometric data recorded in the 90th day of in vitro culture shows that at this time only in explants inoculated and reared control group V_0 (medium lacking growth regulators) were generated caulinare new formation (figure 5A).

Regarding rootedness, similar situation presented before reading this phenomenon manifested only in the variants belonging plant inocula V_2 (medium supplemented with 1.5 mg/l IBA) and V_3 (medium supplemented with 2 mg/l IBA), the average number The root/variant is of 1 and 1.2 (figure 3D). Roots newly formed according characteristics species are thick and swivel.

The addition of 2 mg/l IBA (V_3) in the culture medium has had a stimulating effect on the growth of the explants roots length grown in vitro on the substrate, the average length of the longest root - in this case - was 1,2 cm, while those of the variant V_2 (medium supplemented with 1.5 mg/l IBA), this parameter has not changed since last read, maintained at 0.8 cm (figure 3E), the differences are considered in terms of statistically significant as distinct. On the basis of these results, we consider the addition

to the culture medium to 2 mg/l IBA (V_3) is an effective measure for obtaining and increasing the explants *Aylostera heliosa* roots. Callus induction did not show any at this time to of experimental variations studied in the culture medium was supplemented with IBA.

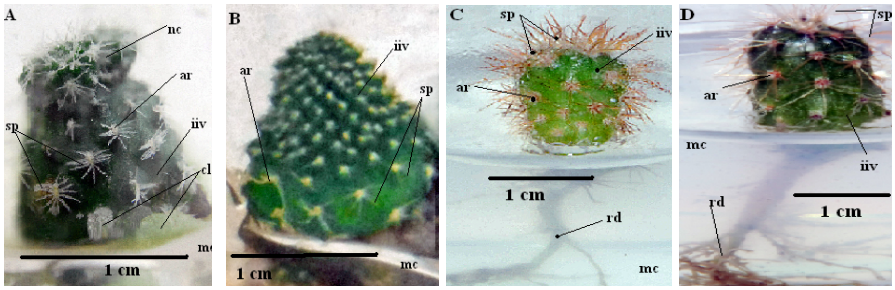

Fig. 5. *Aylostera* (Speg.) *heliosa* inoculum, 90 days after the inoculation of the explant "in vitro", where: A-the basal medium lacking growth regulators (V_0); B-the basal medium with addition of 1 mg/l IBA (V_1); C- the basal medium with the addition of 1.5 mg/l IBA (V_2), D-the basic medium with the addition of 2 mg/l IBA (V_3); (iiv-the original inoculum viable, mc-culture medium, nc-strain newly formed; rd-root, ar-areola, sp-thorns cl-callus).

Note that although the feature is *Aylostera heliosa* alignment comb thorns edges are white - silver, with thickened basal area dark brown color, if reared plant inocula culture medium supplemented with 1.5 mg/l AIB (V_2) or 2 mg/l IBA (V_3), central spines were scattered long reddish deviating from species characteristic appearance (figure 5C and D). If plant inocula grown on a culture medium without growth regulators (V_0), the spins maintained characteristics with regard to shape, size and color (figure 5A), while the explants cultured on nutrient substrate supplemented with 1 mg/l IBA (V_1) developed areolas are normal but special characters *Aylostera* thorns meet only at its base, the middle and upper portion, up to that time not fully developed. These results allow us to assume the presence in the culture medium of a certain amount of growth regulator enhances great morphological plasticity *Aylostera heliosa* catus species, which will be noticed in all the experiments of this thesis that the biological material that plant. It is worth mentioning that, in relation to the characteristics of the spinal fitoinoculii variants belonging to V_2 (medium supplemented with 1.5 mg/l IBA) and V_3 (medium supplemented with 2 mg/l IBA), which are specific to the characteristics of the genus *Aylostera*, which spins center or the edges are very different from one species to another, both in terms of shape (aciformi right, bend, etc..), their length (0.1 to 2.0 mm), positioning the areola (fan, align the comb) or their color (from white to transparent black, passing through a wide range of shades of other colors).

DISCUSSIONS

Analyzing the data from the assessments *Aylostera heliosa* explants after 90 days vitrocultură, we found it possible to initiate in vitro cultures at this cactus, on culture media supplemented with 3 indolilbutiric acid (AIB) in different concentrations (or 1 mg/l 1.5 mg/l, or 2 mg/l), and that there were significant differences in the response to the composition of the nutrient medium fitoinoculilor

Explants cultured on medium lacking growth regulators (V_0) or medium supplemented with 1 mg/l IBA (V_1) were noted by generating strains, but not a root.

The presence in the culture medium of 1.5 mg/l IBA (V_2) or 2 mg/l IBA (V_3) stimulated rootedness. The best results from explants variant V_3 (medium supplemented with 2 mg/l IBA) who had an increase of 200% compared to the control, both in terms of the number of roots and length of these is generated.

Callus induction was not noticed - until the end of the experiment - in any of the variants studied, in which the culture medium was supplemented with IBA.

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