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RESEARCH ON THE DEVELOPMENT OF INOCULUM *Aylostera (SPEG.) heliosa* CULTIVATED IN VITRO IN THE PRESENCE OF A MIXTURE OF EQUAL AMOUNTS OF 3-indolylbutyric acid (AIB) AND benziladenine (BA) IN THE SUBSTRATE

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Abstract

Aylostera heliosa is a cactus with a great decorative potential, both the flowers and the harbor, but difficult multiplied by grafting, for which it seeks a way of spreading fast and secure and virus free seedlings. Future inoculated *Aylostera heliosa* were harvested under sterile conditions from young stems cut into slices about 1 cm length spherical, 0,5 cm thick and a diameter of 0,5-1,5 cm, depending on the area that were take, and at least 2-3 areolae.

The culture medium used was composed of macro-elements, Murashige-Skoog (1962) with the addition of growth regulators, micronutrients Heller (1953), with the addition of growth regulators consists of a mixture of equal amounts of an 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) and 2 mg/l IBA+2 mg/l BA (V_3).

The exhibits were monitored for 90 days. By using a culture medium supplemented with a mixture of growth regulators consisting of equal amounts of 3-indolylbutyric acid (AIB) and benziladenine (BA), we obtained from the *Aylostera heliosa* explants different reactions depending on the concentration in which they were added to the nutrient substrate. Finally, it has been shown that the obese proportion of the mixture of the two growth regulators is 2 mg/l AIB + 2 mg/l BA (V_3). The stimulatory effect of this combination was manifested on caulogenesis, rhizogenesis and calusophenesis.

Keywords: cacti, vitro cultures, 3-indolilbutiric IBA, benzyladenine BA, young stems.

INTRODUCTION

In vitro cultures, the combination of growth regulators called organogenesis hormonal balance adjustment can be achieved within certain limits by changing the concentration or ratio of regulatory present in the growth medium. After the Cachiță et al. (2004), the existence of a culture medium of high concentrations of 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 presence of high concentrations, but equal, the two compounds will be driven with the morphogenesis process, so the generation of callus and its growth.

Hormonal balance in the culture medium can not be fully controlled, it is influenced to a large extent on the endogenous phytohormone ratio. The Rubluo et al. (1996), believes that in vitro cultures of cactus, rooting is the result of interaction between cytokinins and auxins added to the culture medium in the form of exogenous growth regulators, but which, in Taiz et al. (1998), are affected by the amount of light they are exposed vitro cultures.

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

In this experiment our goal was to study reactions Aylostera heliosa existence inoculated in the culture medium supplementation changed us - V_0 - medium lacking growth regulators - with a combination of equal amounts between an auxin (3-indolilbutiric - IBA) and cytokinins (benzyladenine - BA), added in 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) and 2 mg/l IBA + 2 mg/l BA (V_3).

Aylostera heliosa is a cactus ornamantal requires decorate both the different colored flowers (Fig.1) and the port, because thorns silvery-white edge aligned (Fig.1b) comb (Mihalte et al., 2008). Aylostera heliosa is a species of cactus that is very difficult multiplied by grafting (Myeong et al., 2004). But like other cacti can multiply rapidly and efficiently by micropropragare in vitro (Karimi1 et al., 2010).

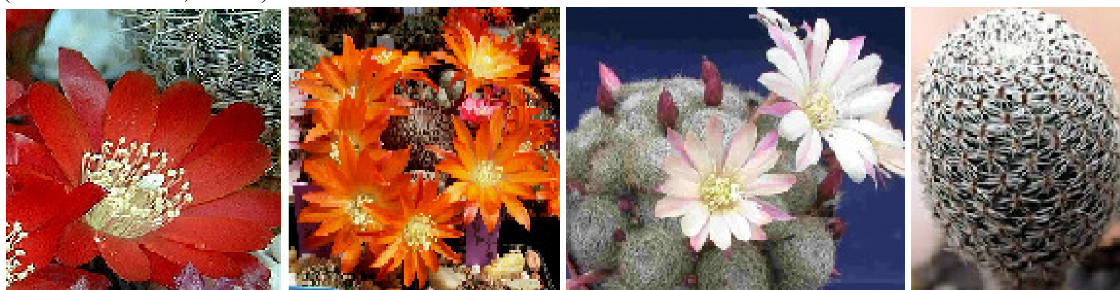

Fig. 1. Plant of *Aylostera heliosa*

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 (Cachiţă 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 (Fig. 2) were done so that each has at least 2-3 areolae (Karimi1 et al., 2010).

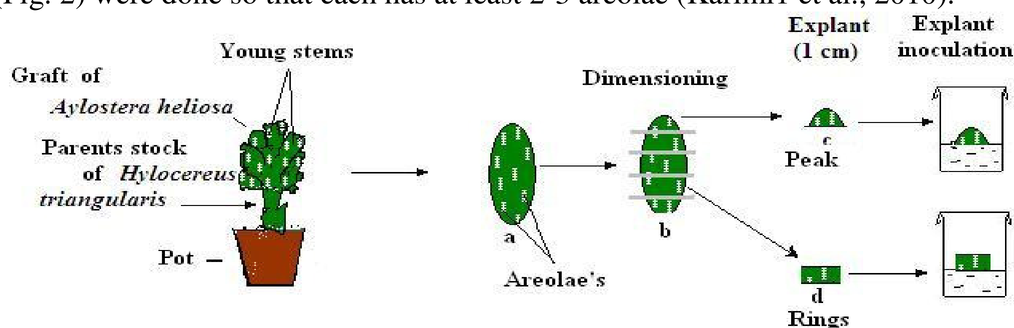

Fig.2. Schematic representation of sectioning method of the young stems to obtain *Aylostera* (Speg.) *heliosa* explants (where: a-young strain, b-sizing of young stems, c- explant represented from young stem d- explant represented as spherical rings).

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.

The basal medium (MB), V_0 - version control, added a mixture of equal amounts of 1 mg/l IBA and 1 mg/l BA (variant V_1), 1,5 mg/l IBA and 1,5 mg/l BA (variant V_2) or 2 mg/l IBA and 2 mg/l BA (variant 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 24°C 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, inocula 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

After 90 days of in vitro culture, it was found that the mean basal diameter of the strain had a value of 1,3 cm (Figures 3A) in all variants studied in this experiment.

At this time, it was observed that the four variants of culture media studied in the present experiment at V_1 variant explants (medium supplemented with a mixture of 1 mg/l AIB and 1 mg/l BA) did not generate strains we.

In the literature, a similar situation is also reported by Sawsan et al., (2005), who concluded that the addition of the Murashige-Skoog culture medium (1962) to the cactus vitro cultures with a combination of auxin and a cytokinin, depending on their concentration, the combination may become a restrictive factor for the formation of shoots.

The average number of newly formed strains exceeded in all cases the values of this parameter recorded in control V_0 (medium lacking growth regulators) with 0,1 newly formed/ variant strains (Figures 3B) at V_2 (medium supplemented with a mixture of 1,5 mg/l AIB+BA), thus marking an increase of 11,11% and 0,4 newly formed/variant strains at V_3 (medium supplemented with a mixture of 2 mg/l AIB+BA), which in this case led to an increase of 44,44% (Figures 4B).

The mean basal diameter of the newly formed strains was also above the V_0 control (0,3 cm), 0,1 cm to V_2 (medium supplemented with 1,5 mg/l AIB+BA) and 0,2 cm (Figures 3C) to V_3 (medium supplemented with a mixture

of 2 mg/l AIB+BA), which represents a 33,33% increase of 66,66% (Figures 4C).

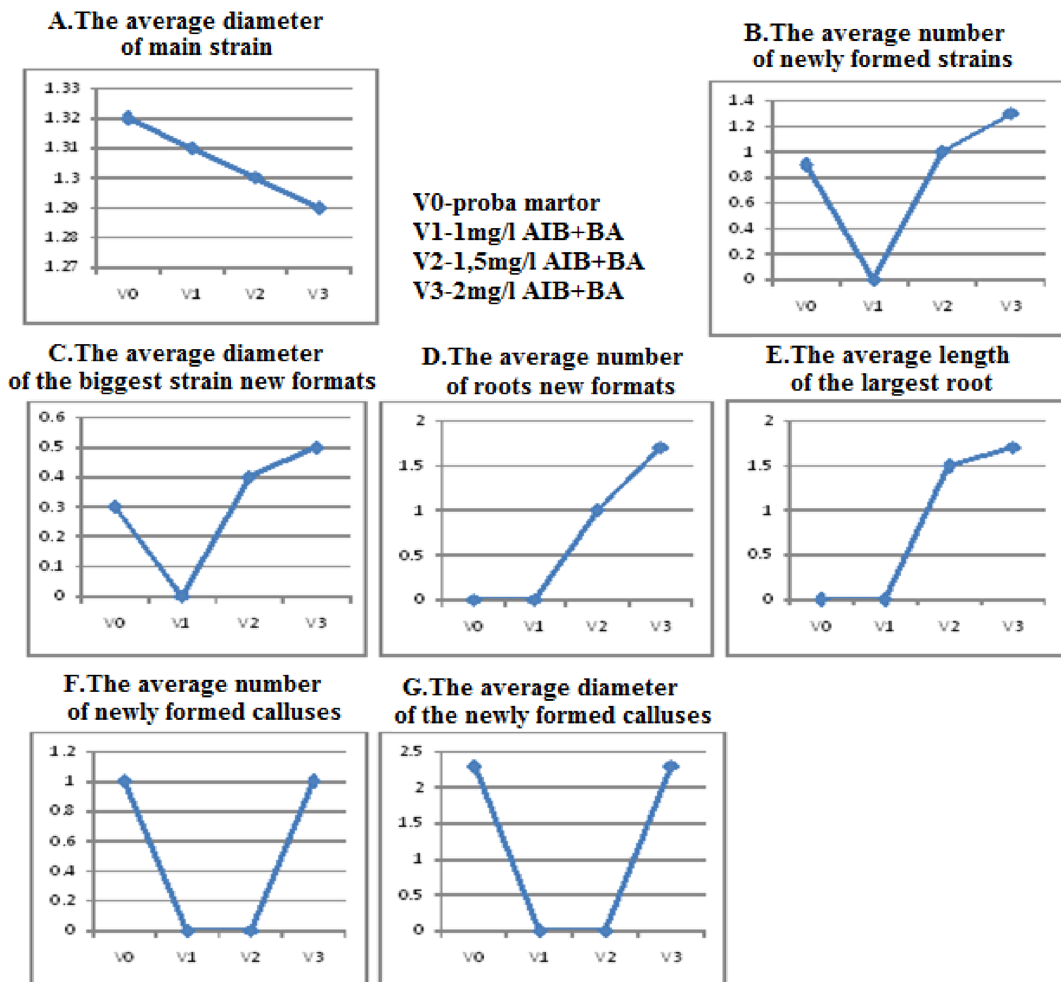

Fig.3. Graphical presentation of the mean values corresponding to the parameters in the in vitro cultures of *Aylostera (Speg.) heliosa* on aseptic base modified by us (variant V_0) - with an addition of a combination of equal amounts of 1 mg/l AIB and 1 mg/l BA (variant V_1), 1,5 mg/l AIB and 1,5 mg/l BA (variant V_2) or 2 mg/l AIB and 2 mg/l BA (variant V_3) ; (where: A-average diameter of the main strain; B-medium number of newly formed stems; C-average diameter of the largest newly formed stems; D-average number of newly formed roots; E-average length of the largest root new formats; F-average number of calluses; G-average callus diameter).

The synergistic influence of the mixture in equal amounts between 3-indolylbutyric acid (AIB) and benziladenine (BA) stimulated the rhinogenesis, thus at explants of V_2 variants (medium supplemented with 1,5 mg/l AIB+BA) or V_3 (medium supplemented with a mixture of 2 mg/l AIB+BA), the average number of newly formed roots was 1 root/variant and 1,1 root/variant respectively (Figures 3D). The beneficial effects of the composition of the culture medium were also evidenced by the growth capacity of the newly formed roots, which had an average length of 1,5 cm in variant V_2 (medium supplemented with a mixture of 1,5 mg/l AIB+ BA) and 1,7 cm to V_3 (medium supplemented with a mixture of 2 mg/l AIB+BA) (Figures 3E); neither at this time the witness has rooted (Vidican, 2014).

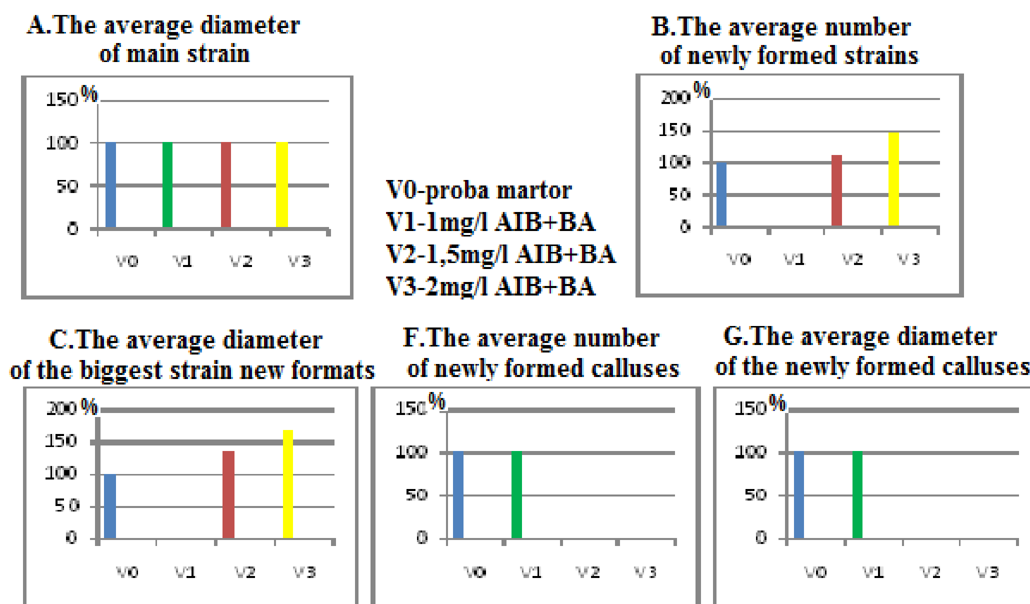

Fig.4. Graphical presentation of the mean values corresponding to the parameters in the in vitro cultures of *Aylostera (Speg.) heliosa* on aseptic base modified by us (variant V_0) - with an addition of a combination of equal amounts of 1 mg/l AIB and 1 mg/l BA (variant V_1), 1,5 mg/l AIB and 1,5 mg/l BA (variant V_2) or 2 mg/l AIB and 2 mg/l BA (variant V_3), data expressed as a percentage, obtained from the reading of the readings to the results recorded at the respective parameters monitored in the control group (V_0), lacking growth regulators, values considered as 100%; where: A-mean diameter of main strain; B-average number of newly formed stems; C-mean diameter of the largest newly formed stem; D-average number of calluses; E-average caliber diameter).

After 90 days of vitro culture, it was noted the triggering of the callus genesis at the explants of control variant V_0 (medium lacking growth regulators). The calus grew so fast that it reached the same values in terms of the average number of calluses/variation - 1 calus/variant (Figures 3F) - and the average calius diameter – 2,3 cm (Figures 3G) - with those recorded in variant V_3 (medium supplemented with a mixture of 2 mg/l AIB+BA) at which callus induction was signaled from 30 days of vitro culture. According to Pasqual et al., (1992) and Martínez-Vázquez et al., (1989), in the Mammillaria genus cultivated on MS medium supplemented with a mixture of an auxin and a citiquinin, there are many precedents for producing calus.

It is noteworthy that the spikes formed at the level of new buds from inoculated explants in growth free (V_0) and in V_1 inoculum medium (supplemented with a mixture of 1 mg/l AIB+BA) are characteristic of the species *Aylostera heliosa* (Figures 5A and B), whereas both phytoinocultures and newly formed stems are generated on media supplemented with 1,5 mg/l AIB+BA (V_2) or with 2 mg/l AIB+BA (V_3) under the influence of the culture medium, took over the *Aylostera* genus specific, longer, reddish and brownish-reddish (Figures 5C and D) . At the level of inoculated and elevated explants on variant V_3 (medium supplemented with a mixture of 2 mg/l AIB+BA), the induction of the callus phenomenus is noted, the calus is pale green, located on their surface.

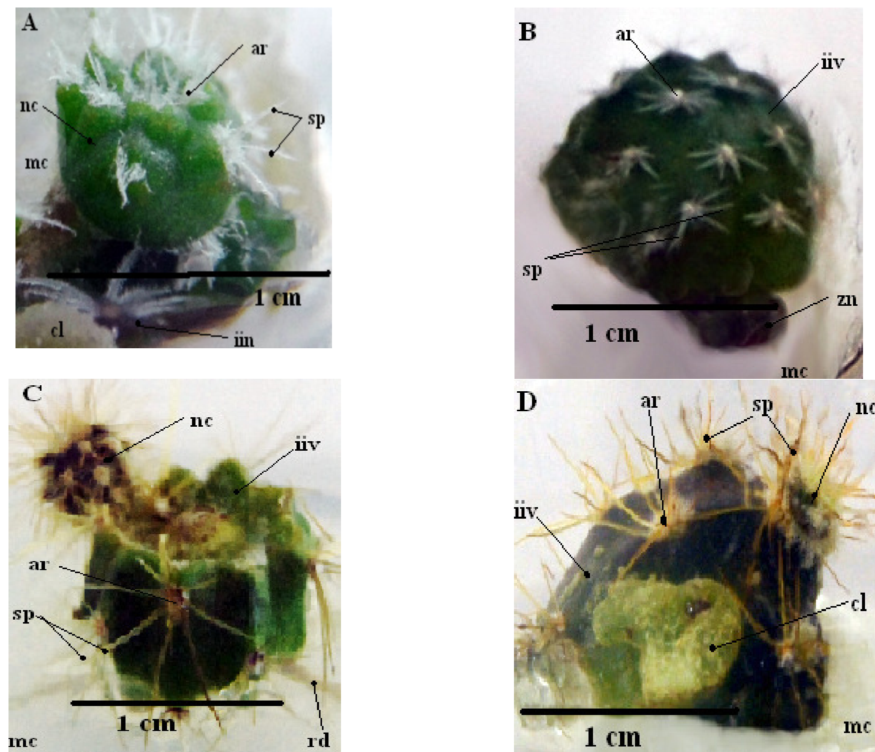

Fig. 5. *Aylostera (Speg.) heliosa* inocula at 90 days after in vitro explant inoculation where: A-on aseptic base modified by non-growth regulators (V_0); B-on base medium with an addition of a combination of equal amounts of 1mg/l AIB+BA (V_1); C-on base medium with an addition of a combination of equal amounts of 1,5 mg/l AIB+BA (V_2); D-on a basic medium with an addition of a combination of equal amounts of 2 mg/l AIB+BA (V_3); (iiv–viable in vivo inoculum, mc-culture media, nc-cauline neoformation, rd-roots, ar-arthales, sp-spines, cl-clusters, zn-necrosis zones).

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CONCLUSION

1. After 90 days at the *Aylostera heliosa* explants grown on a medium supplemented with a mixture of 2 mg/l AIB + BA (V_3), both caulogenesis and rhizogenesis were recorded.

2. Presence in the culture medium of 2 mg/l AIB+BA (V_3), in our case proved to be the obscured proportion of the mixture of the two growth regulators generating 44,44% more strains than the control V_0, which exceeded the witness by 66,66%.

3. On the same V_3 culture medium (2 mg/l AIB+BA) the newly formed roots had a length of 1,7 cm, phenomenon unobserved in the other experimental variants.

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