STUDY ON THE INFLUENCE BENZYLADENINE (BA), ADDED IN VARIOUS CONCENTRATIONS TO THE CULTURE MEDIUM, OF REGENERATION EXPLAIN *Aylostera* (Speg.) *heliosa*

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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 explants were inoculated in a mineral medium - macro Murashige-Skoog (1962) with the addition of growth regulators, micronutrients Heller (1953), supplemented with benzyladenine - BA or 6-benzylaminopurine - BAP at different concentrations.

Evolution 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 *Aylostera heliosa* explants grown in culture medium supplemented with 2 mg /l BA (V_3) had the most and largest strain newly formed. Procrsul of callus was manifested only explants grown in culture medium devoid of growth regulators (V_0 - control), whereas no rooting was observed in any of varinatele experienced.

Keywords: cacti, vitrocultures, benzyladenine BA, young stems.

INTRODUCTION

Cytokinins that are plant hormones in absence of non-dividing cells, and tissue cultures which stimulate cell division and processes to the formation withstand the strains inoculated in which generate strains (Mauseth, 1976), also prevents senescence, auxin exerts antagonistic effect annihilating apical dominance, favoring cell dediferenţierea, etc., (Cachiță et al., 2004).

Escobar et al (1986) reported that the most effective plant growth regulator tested in vitro cultures of cactus, for multiplying the plant material is benzyladenine - BA or 6-benzylaminopurine - BAP, added to the culture medium to generate a number caulinare than neoformation.

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).

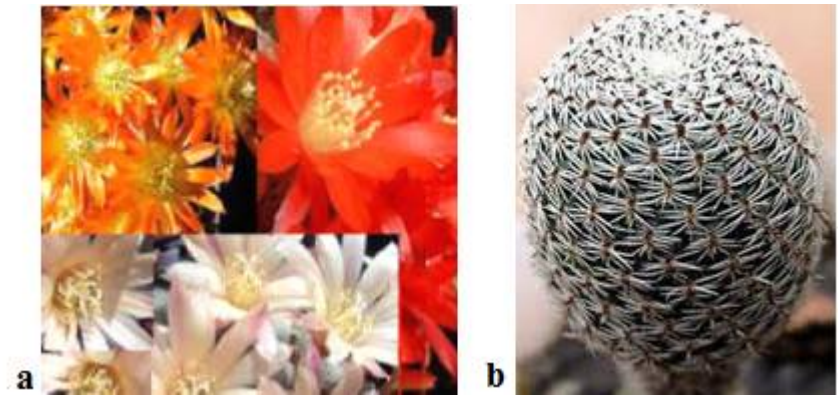

Fig. 1. Plant of *Aylostera heliosa*, where: a - colored flowers; b- strain silvery white spines aligned as comb.

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 of benzyladenine (BA). In order to obtain the proposed variants, we added new nutrient medium prepared devoid of growth regulators (V_0), version control, different concentrations of BA, namely: 1 mg/l BA (V_1); 1,5mg/l BA (V_2) and 2mg/l BA (V_3).

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).

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.

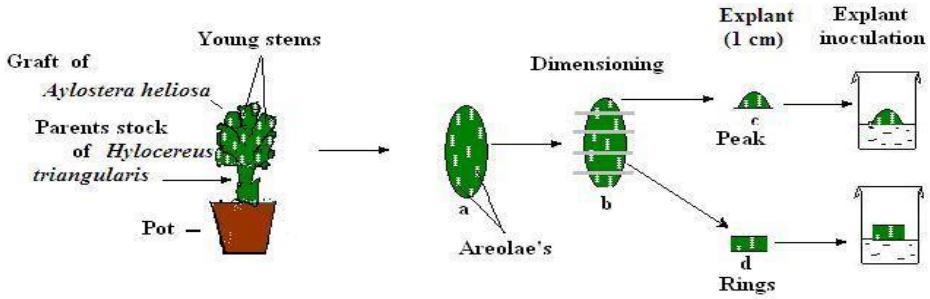

Fig.2. Schematic representation of sectioning method of the young stems to obtain *Aylostera* (Speg.) *heliosa* explants (where: a-young strain, b-sizing of young stems, c-explant represented from young stem d- explant represented as spherical rings).

In order to obtain the proposed alternatives, we added new developed nutrient medium devoid of growth regulators (V_0), version control, different concentrations of BA, 1mg/l BA (V_1), 1,5 mg/l BA (V_2) and 2mg/l BA (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

Readings taken at 90 days after initiation of *Aylostera heliosa* vitro cultures have shown that in vitro culture initiation is possible at this cactus, all culture media used but there were significant differences in the response fitoinoculilor nutrient medium composition (Vidican et at., 2009).

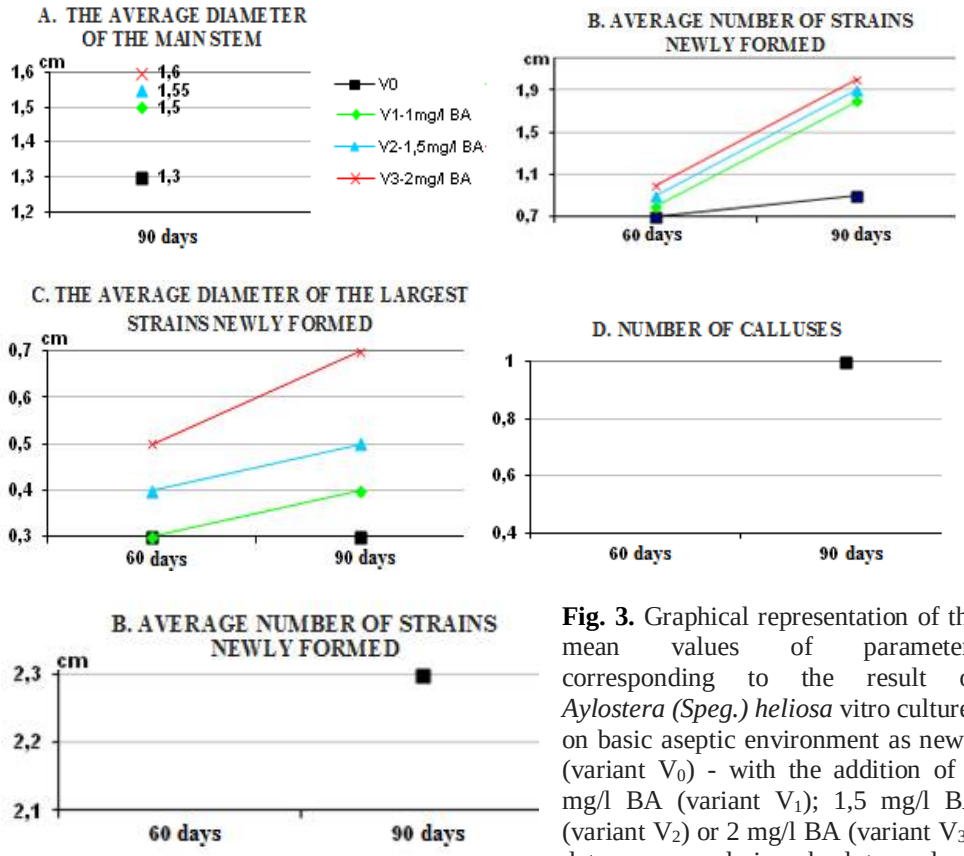

Fig. 3. Graphical representation of the mean values of parameters corresponding to the result of *Aylostera (Speg.) heliosa* vitro cultures on basic aseptic environment as new - (variant V_0) - with the addition of 1 mg/l BA (variant V_1); 1,5 mg/l BA (variant V_2) or 2 mg/l BA (variant V_3), data expressed in absolute values; (where: A- the average diameter of the main stem; B-strains, the average number of newly formed, the average diameter; C-of the newly formed higher strains; D-the average number of calli; E-average diameter of calluses).

During this time the baseline of the average diameter of the stems so that an increased value of the parameter of 1,5 cm (Fig. 3A) belonging to the variants V_1 explants (medium supplemented with 1 mg/l BA), and V_2 (medium supplemented with 1,5 mg/l BA) also showed a 15,38% (Fig. 4A), while the highest value – 1,6 cm - was recorded at explants inoculated and grown in medium supplemented with 2 mg/l BA (V_3), which represents an increase of 23,07%.

The literature stated that of all cytokinins, most cactus species respond favorably to the cultivation medium supplemented with benzyladenine (BA) at different concentrations 0,1-10 mg/l (Wellens, 2003).

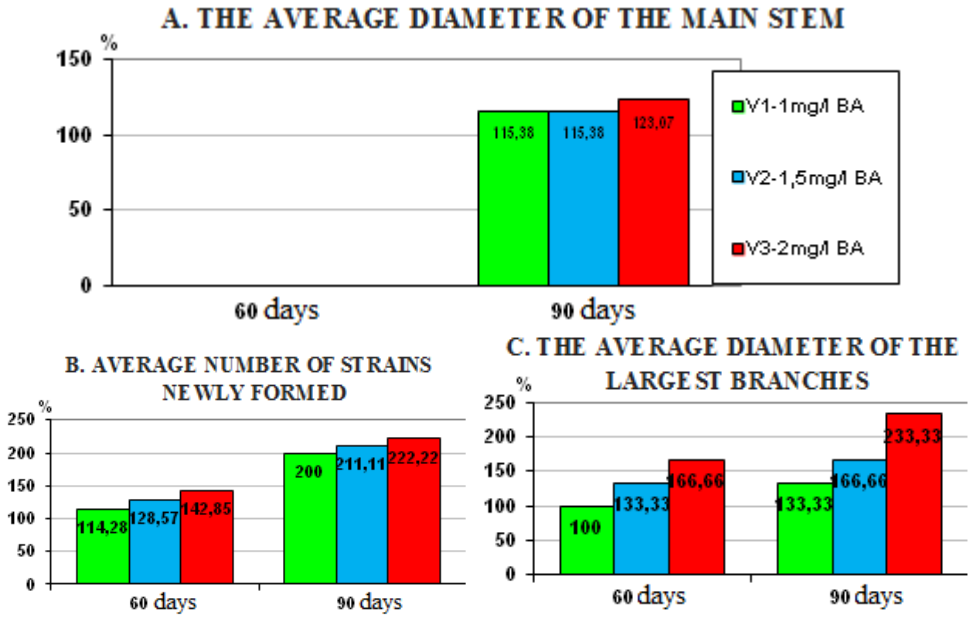

Fig. 4. Graphical presentation of mean values corresponding to the track parameters of *Aylostera (Speg.) heliosa* vitro cultures on basic aseptic environment as new, with the addition of 1 mg/l BA (variant V_1), 1,5 mg/l BA (variant V_2) or 2 mg /l BA (variant V_3), data expressed as a percentage, obtained by reporting the results achieved target values to those parameters monitored in the control group (V_0), lacking growth regulators, values considered as 100% ; (where: A-average diameter of the main stem; B-average number of new strains formed; C-average dimeter largest stalks newly formed).

In the current experiment, supplementation of culture medium with BA positively affected the number of newly formed strains, which doubled (1,8 stems newly formed/variant) to explants belonging to variant V_1 (medium supplemented with 1 mg/l BA) large values of this parameter and experimental groups were recorded for the growth regulator concentration was higher. Thus, compared to the values V_0 in the control group (medium lacking growth regulators), this parameter has been greater than 1 newly formed stem/variant (Fig. 3B) to V_2 (medium supplemented with 1,5 mg/l BA), and 1,1 stems newly formed/variant V_3 (medium supplemented with 2 mg/l BA), which represents an increase of 111,11% to 122,22%, respectively (Fig. 4B).

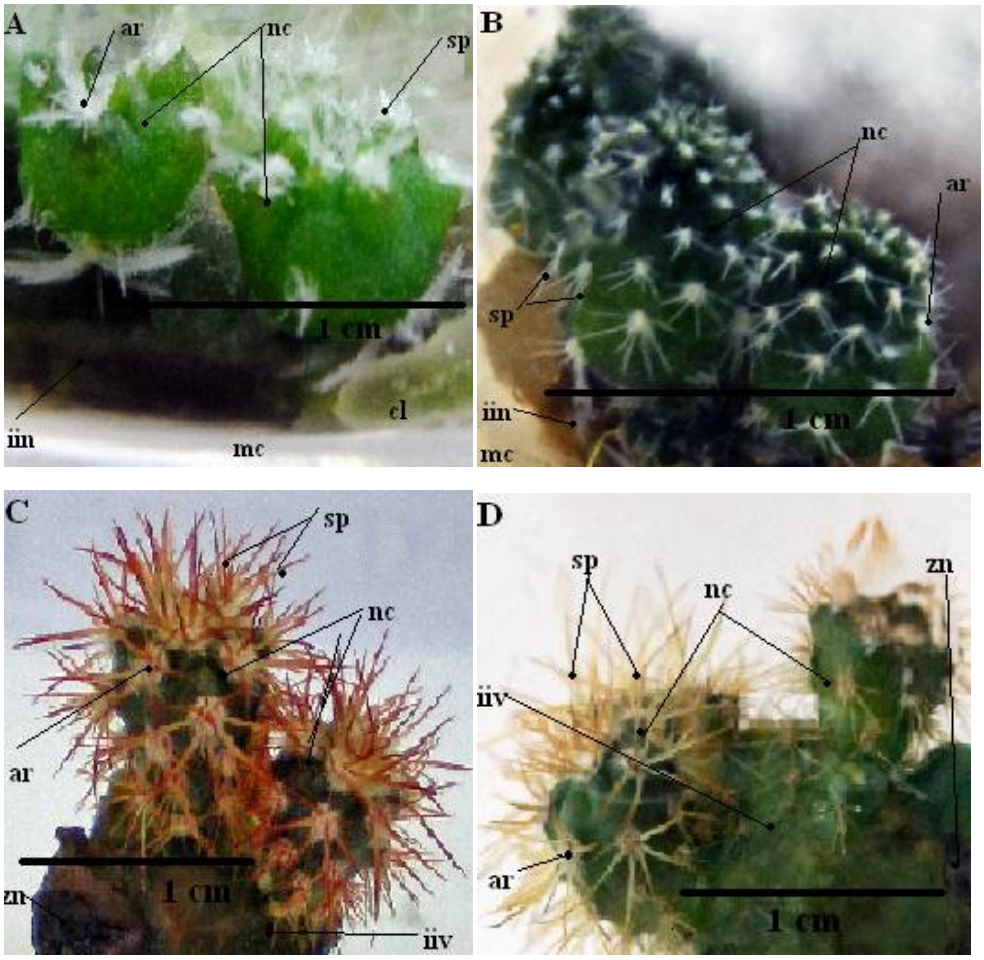

Fig. 5. *Aylostera (Speg.) heliosa* inoculum, 90 days after explant inoculation "in vitro", where: A- the basic aseptic environment as we lacking growth regulators (V_0); B-base medium with the addition of 1 mg/l BA (V_1); C-base medium with the addition of 1,5 mg /l BA (V_2); D-basic medium with the addition of 2 mg/l BA (V_3); (iiv-initial inoculum viable; iin-initial necrotic inoculum; mc-culture medium; nc-young stems; ar-areola; sp-thorns; cl-callus; zn-necrotic area).

The culture medium supplemented with 1 mg/l BA (V_1) to favor and increase in diameter of the newly formed stems to an average of 0,4 cm (Fig. 3C), as compared to the control V_0 (0,3 cm), representing an increase of 33.33%. The beneficial effect of benzyl adenine on average basal diameter of newly formed strains was noted but the explants grown on medium supplemented with 1,5 mg/l BA (V_2) and 2 mg/l BA (V_3) which recorded a value of this parameter of 0,5 cm, 0,7 cm, respectively, relative to control values without an increase of 66,66% to 133,33%, respectively (Fig. 4C).

Only callus induction was observed in the inoculated explants and grown in culture medium without growth regulators (V_0), where there was an average of 1 calluses/variant (Fig. 3D), with an average size of 2,3 cm (Fig. 3E).

Analyzing images from figure 5 it can be noted that the explants inoculated and grown on medium lacking growth regulators (V_0) or one supplemented with 1 mg/l BA (V_1), spins and characters kept species (Fig. 5 A and B); the same can not be said about those grown on substrates that had the composition to 1,5 mg/l BA (V_2) or 2 mg/l BA (V_3) situation where thorns have changed shape and color - are longer and fan compared to those of the mother plant (donor inocula) - becoming the first case tan and brown, in the second case; areolas are large variations both well developed, cream-colored glohide (Fig. 5 C and D).

Monitoring the reaction *Aylostera heliosa* explants for a period of 90 days have been found to have a beneficial effect on the culture medium supplemented with 2 mg/l BA (V_3) of regeneration, in the which the average number has exceeded newly formed strains to 122,22% control V_0 and the diameter thereof basal medium, which was 133,33% higher than the same parameter recorded in the control group.

Rooting not manifested in this time in any of the experimental variants
studied.

Of the four culture media investigated in this experiment it was found that only explants inoculated and grown on medium lacking growth regulators (V_0) generated callus.

DISCUSSIONS

1. Analyzing the data from the assessments *Aylostera heliosa* explants after 90 days vitro cultures, we found that it is possible to initiate in vitro cultures at this cactus, on culture media supplemented with benzyladenine (BA) in different concentrations (1 mg/l; 1,5 mg/l and 2 mg/l), and that there were significant differences in the response to the composition of the nutrient medium inoculants;
2. Explants cultured on medium supplemented with 2 mg/l BA (V_3) were noted by generating new strains, and obtaining the best results with an increase of 222,22% to 233,33% of their number and in what for their size, compared to the control.
3. Rooting not manifested in this time in any of the experimental variants studied.
4. Callus induction was observed only in explants inoculated and grown on medium lacking growth regulators (V_0).

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