COMPARATIVE STUDY ON THE REGENERETIVE AND ORGANOGENIC CAPACITY *Aylostera (speg.) heliosa* IN THE PRESENCE IN CULTURE MEDIUM 2,5 mg/l benzyladenine (BA) and 2,5 mg/l 2,4 dichlophenoxyacetic acid (2,4-D)

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Abstract

Aylostera heliosa t is a cactus that, like other cacti, can multiply quickly and efficiently by in vitro micropropagation (Karimi1 et al., 2010) which ensures a virus-free planting material. It is considered a species with very high decorative potential both by flowers, port, and by the presence of thorns with white-silver edges aligned in the form of (Fig.1b) comb (Mihalte et al., 2008), but which multiplies very hard by grafting (Myeong et al., 2004).

Future inocula of Aylostera heliosa were harvested under sterile conditions from young stems sectioned into spherical slices about 1 cm long, 0,5 cm thick and 0,5-1,5 cm in diameter, depending on the area from which they were take, and with at least 2-3 areolae.

The explants were inoculated in a mineral environment - macro, Murashige-Skoog (1962), with the addition of growth regulators, microelements Heller (1953) - V_0 (control), and on media supplemented with 2,5 mg/l benzyladenine (V_1) and 2,5 mg/l 2,4 D (V_2).

The evolution of the explants was monitored for 90 days. Their response was different depending on the phytohormones added to the culture medium, auxin (2,4 D) or cytokinin (BA). Aylostera heliosa explants, grown on culture medium supplemented with 2,5 mg/l BA (V_1), were shown to have the largest and largest newly formed strains. The process of callusogenesis was manifested with my best results in explants grown on culture medium supplemented with 2,5 mg/l 2,4 D (V_2) but also on medium without growth regulators (V_0 - control), while rhizogenesis did not occur in any of the varicose veins experienced.

Keywords: cacti, vitrocultures, benzyladenine BA, dichlorophenoxyacetic 2,4 D, callus

INTRODUCTION

It is known that auxins together with cytokinins, are substances with direct action on cell growth, they stimulate cell division, (Cachiţă et al., 2004).

Benzyladenine (BA) is a cytokinin with a role in boosting the processes of cell division and caulogenesis, bud formation in the inoculum, from which new strains will be generated (Mauseth, 1976), exerting an antagonistic effect on auxins, inhibiting rhizogenesis. (Cachiţă et al., 2004), In in vitro cactus cultures, it is considered that in order to multiply the plant material, the most effective growth regulator to generate new strains is BA (Escobar et al, 1986).

Dichlorophenoxyacetic acid (2,4-D) or is an auxin with great efficacy in callus formation and rhizogenesis, according to Sandra Aparecida et al., 1996, its introduction into the culture medium is sufficient to induce callus, later it can be detached, cut and then transferred to fresh culture medium to obtain seedlings.

Aylostera heliosa or *Rebutia heliosa* is a species native to Bolivia, one of the cacti with the most spectacular and abundant blooms, which makes them particularly decorative. (Fig. 1a and b)

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

The aim of this research was to study how the presence in the culture medium of an equal amount of cytokinin (2,5 mg/l benzyladenine) and auxin (2,4 dichlorophenoxyacetic acid) influences the regenerative and organogenic capacity of vitroplants *Aylostera heliosa.*

MATERIAL AND METHOD

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).

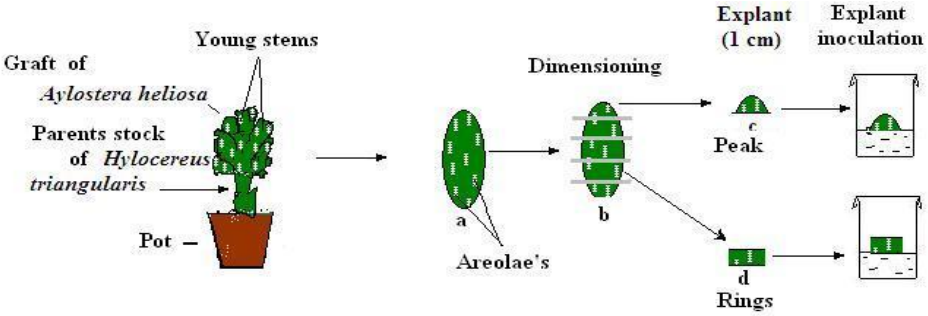

Fig.2. Schematic representation of sectioning method of the young stems to obtain *Aylostera* (Speg.) *heliosa* explants (where:
-young strain, b-sizing of young stems, c- explant represented from young stem d- explant represented as spherical rings).

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.

To obtain the proposed results, we added in the culture medium without regulators of (V_0) and considered as a control, 2,5 mg/l benzyladenine (V_1) and 2,5 mg/l 2,4 D (V_2).

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

He readings made 90 days after the initiation of in vitro cultures of *Aylostera heliosa* showed that, during this time, the average basal diameter of the stem recorded the highest value -1,6 cm - cm (Fig.3) in the explants.

belonging to variants V_1 (average supplemented with 2,5 mg/l BA) values by 0,3 cm above the control V_0, which represents an increase of 23,7%, while the same parameter for explants grown on V_2 (medium supplemented with 2,5 mg/l 2,4-D) marked an increase of 0,1 compared to the control, a percentage increase of 7,69% (Fig.5). Results that, statistically, are appreciated as being distinctly significant (Table 1).

Supplementation of the culture medium with 2,5 mg/l BA (V_1) positively influenced the formation of new strains (Fig.4), which recorded the highest values, respectively 2 buds/variant, compared to 0,9 buds/variant in the case of control V_0 (medium without growth regulators) and 1,1 buds/variant (Fig.3) in explants grown on culture medium supplemented with 2,5 mg/l 2,4-D (V_2). These values represent in both cases an increase, by 122,22% (Fig.5) in V_1 explants (supplemented with 2,5 mg/l BA) and by 22,22% in V_2 (supplemented with 2,5 mg/l 2,4-D). Results which, statistically, are appreciated as being distinctly significant (Table 1)

Table 1

Results of biometric evaluations of vitro seedlings of *Aylostera* (Speg.) *heliosa* performed 90 days after inoculation of explants on basic aseptic culture media with the addition of 2,5 mg/l BA (V_1) and 2,5 mg/l 2,4 D (V_2)

Parametrul / Varianta	Lungimea medie a tulpinitei principale ± abaterea standard	Varianța	Semnificația	Numărul mediu al neoformațiunilor caulinare ± abaterea standard	Varianța	Semnificația	Lungimea medie a celei mai mari neoformațiuni caulinare ± abaterea standard	Varianța	Semnificația	Numărul mediu de rădăcinițe ± abaterea standard	Varianța	Semnificația	Lungimea medie a celei mai mari rădăcinițe ± abaterea standard	Varianța	Semnificația	Numărul mediu de calusuri ± abaterea standard	Varianța	Semnificația	Diametrul mediu al calusurilor ± abaterea standard	Varianța	Semnificația
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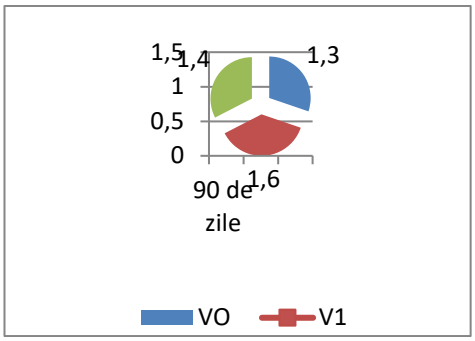
V0	1,30±0,36	0,1326	**	0,90±0,30	0,0884	**	0,30±0,07	0,0053	**	0	0		0	0		1,00±0,65	0,4211	NS	2,30±1,21	1,4526	
V1	1,60±0,41	0,1700	**	2,00±0,46	0,2105	**	0,70±0,22	0,0474	**	0	0		0	0		0	0		0	0	
V2	1,30±0,32	0,1000	**	1,10±0,33	0,1079	**	0,20±0,09	0,0084	*	0	0		0	0		2,60±0,41	0,1684	***	5,20±0,58	0,3368	***

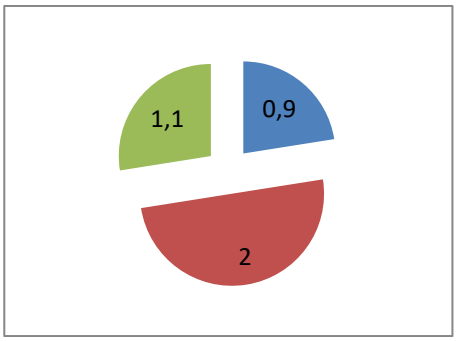
Legenda : „***" - foarte semnificativ; „**" - distinct semnificativ; „*" - semnificativ; „NS" - nesemnificativ.

As expected, the average basal diameter of the newly formed buds is larger in explants grown on culture medium supplemented with 2,5 mg/l BA (V_1) (Fig.4) and 0,7 cm, respectively (Fig.3), compared to the control group V_0 (Vidican et al, 2014) in which this parameter recorded a value of 0,3 cm and 0,4 cm in explants grown on V_2 (average supplemented with 2,5 mg/l 2, 4-D).

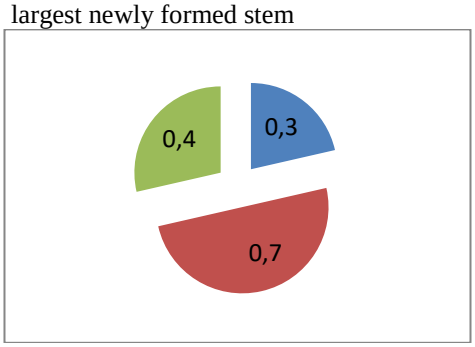
A.The average diameter of the stem



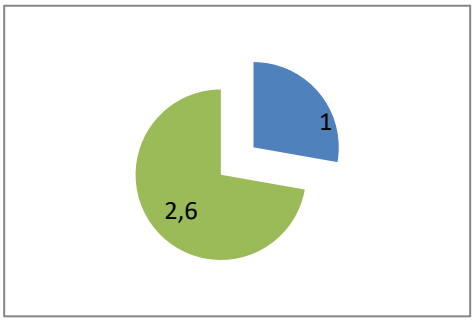
B. Avarage number of newly main formed strains



C. The average diameter of the largest newly formed stem



D. The average number of calluses



E. The average diameter of the calluses

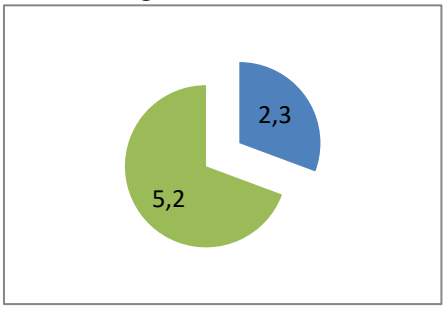


Fig.3. Graphic presentation of the average values corresponding to the parameters read at the level of in vitro cultures of *Aylostera* (Speg.) *heliosa*, on basic aseptic medium modified by us - (variant V_0) - with the addition of 2,5 mg/l BA (variant V_1) and of 2,5 mg/l 2,4 D (variant V_2), data expressed in absolute values; (where: A– average diameter of the main stem; B – average number of newly formed strains; C– average diameter of the largest newly formed stem; D- average number of calluses; E-average diameter of calluses).

The results recorded by us are in line with those obtained by Wellens (2003) which shows that of all the cytokines, most cactus species respond favorably to cultivation on a medium supplemented with benzyladenine (BA) in different concentrations 0,1 - 10 mg / l (Fig. 4). Compared to the values recorded in the control group V0 (average without growth regulators) this parameter expressed in percentage values was higher by 133,33% in variant V_1 and by 33,33% in V_2. (Fig.5)

The phenomenon of rhizogenesis has not manifested itself until this date in any of the experimental variants studied (Fig. 4).

Callus induction was noted both in inoculated and grown explants on culture medium lacking growth regulators (V_0) where an average number of 1,0 calluses/variant was recorded (Fig.3), with an average size of 2,3 cm (Fig.) As well as those grown on culture medium supplemented with 2,5 mg/l 2,4-D (V_2) which with a number of 2,6 calluses / variant that reached an average size of 5,2 cm (Fig.) Exceeded the control by 160% in the first case and by 126,08% (Fig.5) in the second case (Vidican et al, 2011)

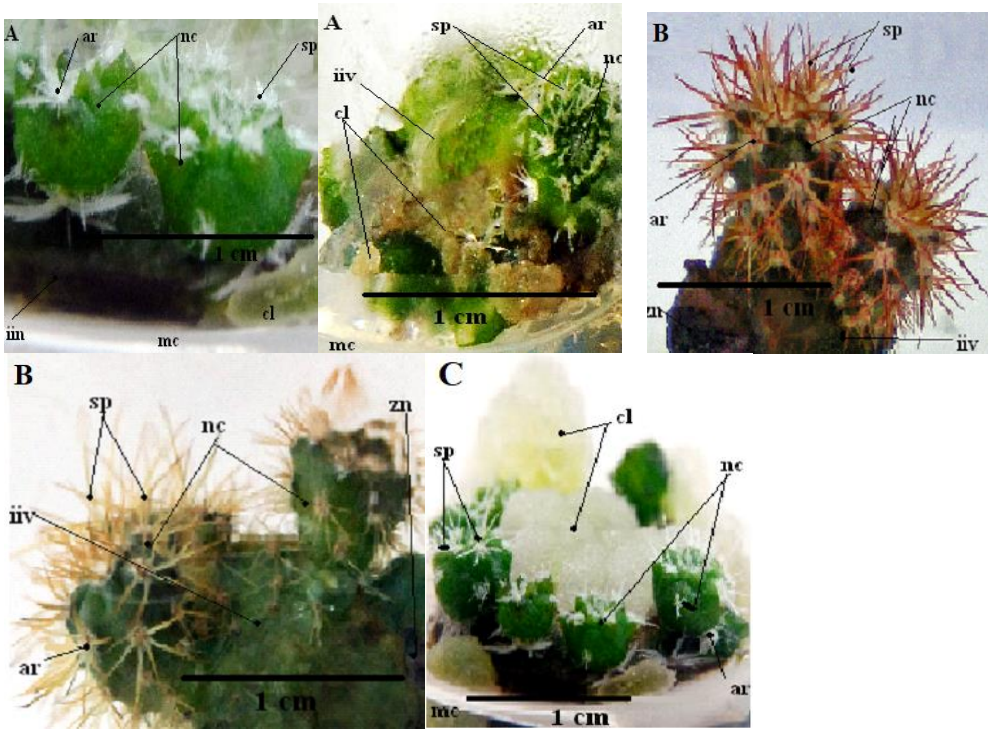
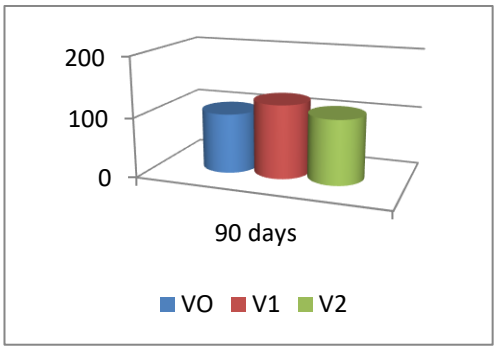


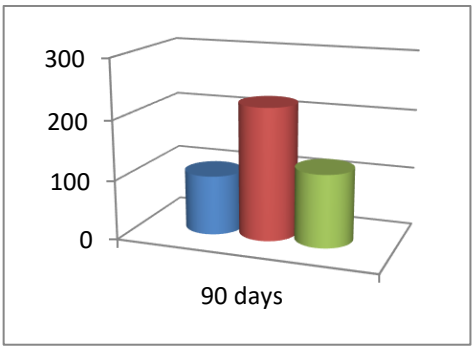
Fig.4. Inoculi of Aylostera (Speg.) Heliosa, 90 days after inoculation of the "in vitro" explant, where: A- basic aseptic medium without growth regulators (V0); B- Basic medium with the addition of 2.5 mg / l BA (V1); C- Base medium with the addition of 2.5 mg / l 2.4D (V2); where: iiv-viable initial inoculum; iin-initial necrotic inoculum; mc-culture medium; nc-young stems; ar-areola; sp-spines; cl-calus; zn-necrotic area.

The callus generated from explants cultivated on a medium lacking growth regulators is located on the surface of the explant but also on the culture medium, it shows signs of early senescence, indicated by its cream or even light brown (Fig.4).

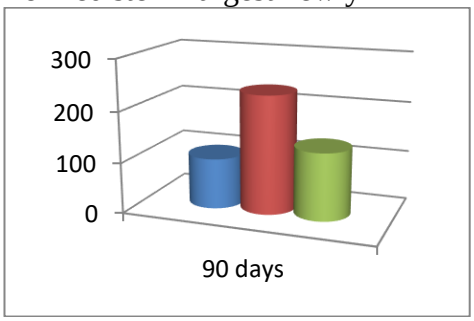
A.The average diameter of the stem formed strains



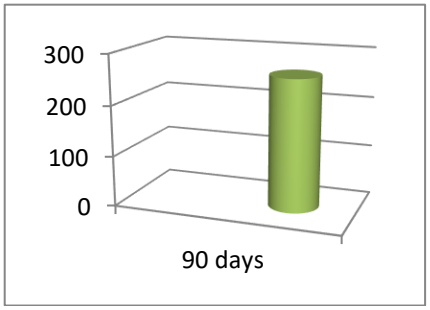
B. Avarage number of newly main



C. The average diameter of the formed stem largest newly



D. The average number of calluses



E. The average diameter of the calluses

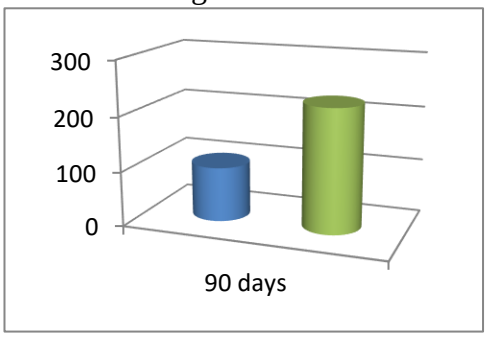


Fig.5. Graphical presentation of the average values corresponding to the parameters analyzed at the level of vitrocultures of *Aylostera* (Speg.) *heliosa*, on basic aseptic medium modified by us - (variant V_0) - with the addition of 2,5 mg/l BA (variant V_1) and 2,5 mg/l 2,4 D (variant V_2), data expressed as a percentage, obtained after reporting the analyzed values to the results recorded at the respective parameters read in the control group (V0), without growth regulators,

In the case of explants inoculated on culture medium supplemented with 2,5 mg/l 2,4-D (V_2), the callus was crumbly, snow-white (Fig. 4), and due to its abundance it covered the entire surface of the culture medium. As can be seen in Figure 4, the buds generated at the level of both experimental variants kept their particular species characteristics, respectively, the size and color of the spines, but especially the specific shape of the comb.

CONCLUSIONS

1. Analyzing the data analyzed on *Aylostera heliosa* explants after 90 days of in vitro culture, we found that it is possible to initiate in vitro

cultures in this cactus, on culture media supplemented with benzyladenine (BA) and 2,4 D

2. Monitoring the beneficial effect on the formation of buds and their average size by the addition in the culture medium of 2,5 mg/l auxin (2,4-D) and cytokinin (BA) we can say that the highest values we have recorded in variant V_1 (average supplemented with 2,5 mg/l BA) with 2 buds/variant respectively an average size of 0,7 cm compared to 1,1 buds/variant in V_2 (average supplemented with 2,5 mg/l 2,4 d) and an average size of 0,4 cm.

3. Rhizogenesis did not occur in any of the experimental variants studied.

4. Regarding the number of calluses formed at V_2 (mean supplemented with 2,5 mg/l 2,4 D) there was an increase of 1,6 calluses/variant compared to the control group V_0, an increase of 126,08% , and an average increase in size by 2,9 cm, respectively an increase of 126,08%

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