STUDY ON THE REGENERATIVE CAPACITY AND ORGANOGENESISOF *Aylostera* (Speg.) *heliosa* EXPLANTS, IN THE PRESENCE OF 2,4 - DICHLOROPHENOXYACETIC ACID (2,4-D) IN CULTURE MEDIUM

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Abstract
In the case of cacti which are growing more difficult, as is Aylostera heliosa, a decorative species with flowers and harbor, finding a fast and efficient multiplication method is for researchers, a goal. Induction of a calusogenesis phenomena in cacti vitrocultures provides a valuable biological material from which new forms can be done legally outstanding ornamentals. 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 done on a mineral basic medium culture Murashige-Skoog (1962) with - macroelements, with growth regulators, Heller (1953) micronutrients, supplemented with 2.5 mg / l 2.4-dichlorophenoxyacetic acid (2,4 D).

The evolution of vitrocultures was followed for 90 days. Explants reaction was different depending on the nature of culture substrate. Finally, it appeared that explants evolution was differentiated according to the composition of culture medium, as expected, callus induction was favorable to the inoculum grown on medium supplemented with 2.5 mg / l 2,4 - dichlorophenoxyacetic acid (2,4 D).

Keywords: cacti, vitrocultures, callus, micropropagation, 2,4-D.

INTRODUCTION

Aylostera heliosa, is much appreciated cactus by connoisseurs (Fig. 1), both by being decorative with red or orange flowers and a port due to marginal white-silver spin, comb-aligned (Mihalte et al., 2008), but rather are very hard to multiply by grafting (Myeong et al., 2004). Fast and efficient multiplication is done by micropropragation *in vitro* of cacti (Karimi1 et al., 2010). In mineral medium cultures rich in growth regulators, cacti are considered to be highly susceptible to dedifferentiation process, invariably inducing a rich mass of *callus* formation (Jaafar et al., 2009), genetic instability and polyploidy of callus cells, makes it a valuable biological material (Copăcescu, 2001). In the literature there are a number of different results in terms of callus formation depending on the nature of the growth regulators present in the culture medium, as (Johnson and Emino, 1979b) found that by adding the culture medium an auxine like 2,4-D for *Mammillaria elongata* vitrocultures can produce a friable *callus*,

while (Papafotiou et al., 2001) obtained callus, the same species of cactus, in the presence citokinine - BA.

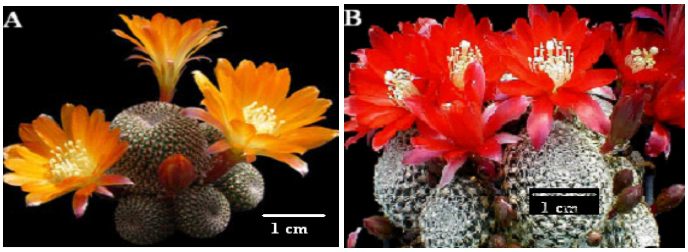

Fig.1. Image of *Aylostera* (Speg.) *heliosa* (where: A, B – flowers and stems).

In turn, (Starling and Dodds, 1983) reported callus induction from explants of *Mammillaria glassii* grown on MS medium with addition of 1.0 mg / l BA and (Pérez et al., 1998) have succeed *callus* induction on the cut surface of the *Mammillaria formosa galeotti* and *Mammillaria sphacelata* Mart. explants, and another 21 species of Mexican cacti on MS medium supplemented with a mixture of 1 mg/l BA + 1 mg/l NAA. But the most used and recommended stimulant for callus induction is the addition of 2.4-dichlorophenoxyacetic acid (2,4-D) in different concentrations, from 1 mg/l to 10 mg/l (Biswas t al., 2009).

Our aim was to initiate a *in vitro* culture of *Aylostera heliosa* and to study the regenerative capacity and organogenesis of the explants the presence of 2.5 mg/l 2.4-dichlorophenoxyacetic acid (2,4-D) in the culture medium.

MATERIALS AND METHODS

In this experiment in order to initiate the *Aylostera heliosa* vitroculture, 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 (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: macro-elements 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.

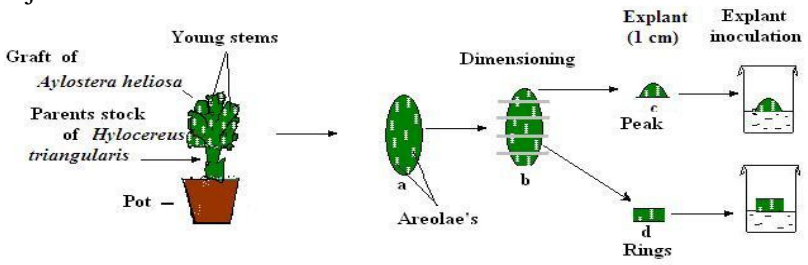

Fig. 2. Schematic representation of sectioning method of the young stems to obtain *Aylostera* (Speg.) *heliosa* explants (where a-young strain, b-sizing of young stems, c-explant represented from young stem d- explant represented as spherical rings).

To obtain experimental variants we supplemented the mineral culture medium without growth regulators V_0, and with 2.5 mg/l 2.4-dichlorophenoxyacetic acid (2,4 D).

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*. Bio-metric assessments were taken at intervals of *30 days*. Observations consisted from bio-measured: 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

Explants of *Aylostera heliosa* reactions were observed for *90 days* and in the 60^{th} respectively 90^{th} day observation were made: on basal stem diameter, number of caulinar neoformations, caulinar basal new formation diameter, the number and length of roots, also the number of callus formation and callus diameter, the recorded values were reported the control

group V_0 - vitroplantlets grown on basic mineral medium culture MB - MS, without growth regulators - were considered - 100%.

Observations made after *60 days* after initiation of the experiment show that at that time belonging *Aylostera heliosa* explants of variant V_1 (mineral medium culture MS supplemented with 2.5 mg/l 2,4-D) with an diameter of stems 1.3 cm (Fig. 3A), were matched the witness V_0 (mineral medium culture MS without growth regulators) (Fig. 4A). Regarding the explants caulogenesis belonging to variant V_1 (mineral medium culture MS supplemented with 2.5 mg/l 2,4-D) the number of caulinar neoformations was higher with 0.1 buds/variant (Fig. 3B) compared to medium values of the same parameter registered the control group V_0 (0.7 buds/variant). The absolute values expressed in percentages lead to an increase of the average number of caulinar neoformations 14.28% (Fig. 4B) to variant V_1 (mineral medium culture MS supplemented with 2.5 mg/l 2,4-D) reported to control group V_0.

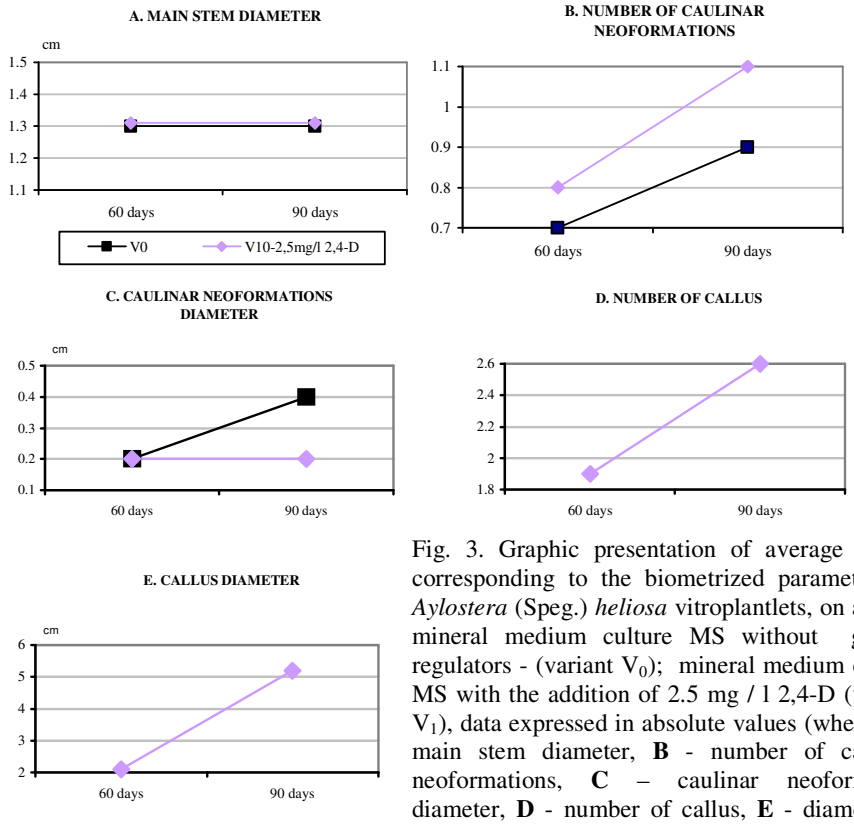

Fig. 3. Graphic presentation of average values corresponding to the biometrized parameters of *Aylostera* (Speg.) *heliosa* vitroplantlets, on aseptic mineral medium culture MS without growth regulators - (variant V_0); mineral medium culture MS with the addition of 2.5 mg / l 2,4-D (variant V_1), data expressed in absolute values (where: **A** - main stem diameter, **B** - number of caulinar neoformations, **C** – caulinar neoformation diameter, **D** - number of callus, **E** - diameter of callus).

Main stem diameter generated at caulinar neoformations for the inoculated explants grown on mineral medium culture supplemented with 2.5mg/l 2.4-dichloropheno-xyacetic acid (2,4-D) was 0.2 cm (Fig. 3C), equating the control group (Fig. 4C).

As it was expected the presence in the culture medium of 2.4-dichloro-phenoxyacetic acid (2,4-D) positively influenced *callus* induction, the explants grown on this nutrient substrate registered a number of 1.9 callus/variant (Fig. 3D), which represents 0.9 calluses/variant V_0, more than the control group (mineral medium culture MS without growth regulators), ie an increase of 90% (Fig. 4D). And after (Steinhart, 1962) in *Trichocereus spachianus* vitrocultures, *callus* induction was performed in culture medium in the presence of growth regulator 2,4-D, at least 50% of the explants were presented *callus,* its growth phenomenon it is very fast. The *callus diameter* (measured at the widest area) was 0.4 cm (Fig. 3E) higher on the experimental variant with mineral medium culture MS supplemented with 2.5 mg/l 2,4-D (V_1) compared with the same parameter values recorded to control group V_0 (mineral medium culture MS without growth regulators), these differences represent an increase of 23.52% (Fig. 4E).

At *90 days* after initiating the experiment, similar to previous readings, the *main stem diameter* of *Aylostera heliosa* vitroplants remained in both cases - V_0 (mineral medium culture MS without growth regulators) and V_1 (mineral medium culture MS supplemented with 2.5 mg/l 2,4-D) - unchanged, it was 1.3 cm (Fig. 3A).

The *number of caulinar neoformations* was 0.2 buds/variant (Fig. 3B) higher values shown by vitroplants from variant V_1 (mineral medium culture MS supplemented with 2.5 mg/l 2,4-D) compared with the same recorded parameter to control group V_0 (0.9 buds/variant), which represents an increase of 22.22% (Fig. 4B). Regarding to *caulinar neformations diameter* generated at the inoculated explants grown on mineral medium culture MS without growth regulators (V_0), the value of this parameter was 0.4 cm (Fig. 3C) marked an increase of 50% (Fig. 4C) compared with the recorded values from the explants cultured on values supplemented with the 2.5 mg/l 2,4-D (V_1).

Rhizogenesis phenomenon remained elusive until this date to any of the experimental variations studied.

After *90 days* of culture *in vitro* can be noted that to *Aylostera heliosa* vitroplants the *number of calluses*/variant is 1.6 (Fig. 3D) is more higher for the vitroplants from variant V_1 (mineral medium culture MS supplemented with 2.5 mg/l 2,4-D) reported to the same parameter values recorded to control group (mineral medium culture without growth regulators) V_0, and the percentage values representing an increase of 160% (Fig. 4D). The results obtained by us in this experiment are similar to those reported by

(Mata et al., 2001) at *Gymnocalicium turbinicarpus*, which received varying degrees of friable *callus* induction from the explants of such vitrocultures.

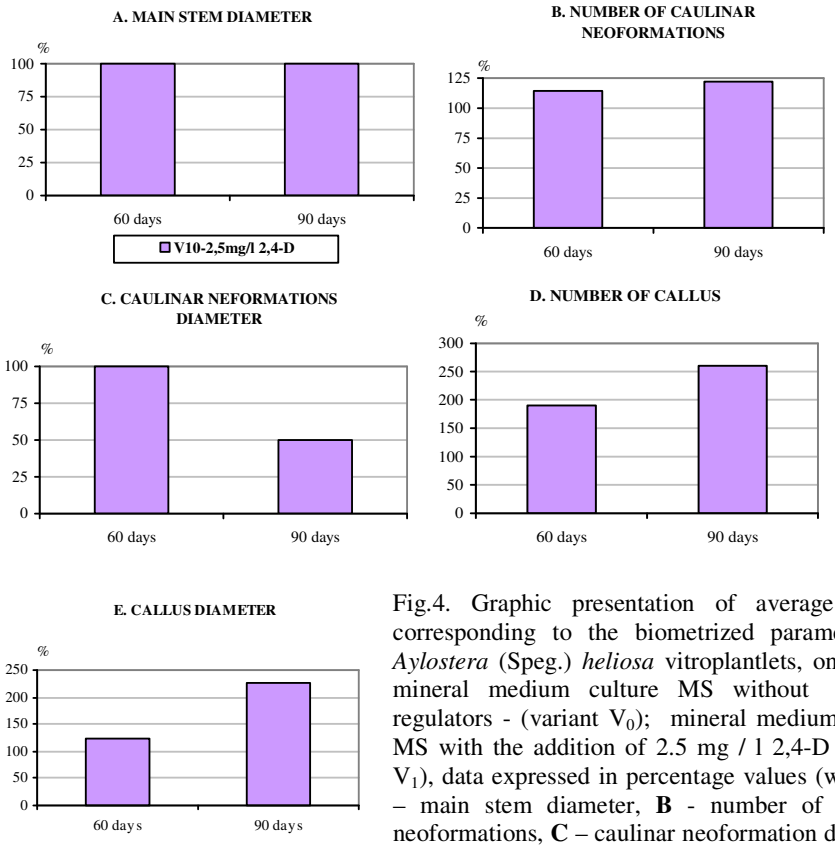

Fig.4. Graphic presentation of average values corresponding to the biometrized parameters of *Aylostera* (Speg.) *heliosa* vitroplantlets, on aseptic mineral medium culture MS without growth regulators - (variant V_0); mineral medium culture MS with the addition of 2.5 mg / l 2,4-D (variant V_1), data expressed in percentage values (where: **A** – main stem diameter, **B** - number of caulinar neoformations, **C** – caulinar neoformation diameter, **D** - number of callus, **E** - diameter of callus).

The *diameter of the callus*, showed highest values recorded throughout the explants were inoculated and grown on mineral medium culture MS supplemented with 2.5 mg / l 2,4-D (V_1), which exceeded the witness V_0 with 2.9 cm (Fig. 3E), which represents an increase of 126.1% (Fig. 4E). Maintaining a fast growing *callus* was obtained at the level of *Cereus peruvianus* vitrocultures (Sandra Aparecida et al., 1996) and *Notocactus magnificus* (Medeiros et al., 2006) grown on MS medium supplemented with 2,4-dichlorophenoxyacetic acid.

This study showed that *callus* is generated from cactus explants cultured on medium without growth regulators and it was located on the surface of the explant and medium culture, it shows signs of early senescence, as indicated by a light brown or cream color (Fig . 5A). The explants inoculated on culture medium supplemented with 2.5 mg / l 2,4-D

(V_1) the *callus* was friable, with white color (Fig. 5B), due to the abundance covered the entire area of culture medium. As shown in Figure 5A and B, the generated stems at both experimental variants retained their particular characters of *Aylostera heliosa* species in terms of size and color of thorns, but mostly aligned in comb form.

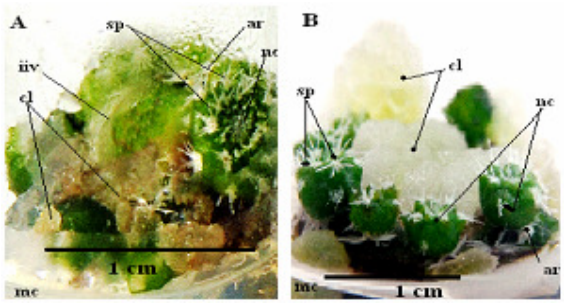

Fig. 5. *Aylostera* (Speg.) *heliosa* vitroplantlets, *90 days* after explants inoculation *"in vitro"*, where: **A** - modified mineral medium culture MS without growth regulators (V_0), **B** - mineral medium culture MS supplemented with 2.5 mg / l 2,4-D (V_1) (iiv- viable initial inocula; nc - caulinar neoformation; ar- areolae, sp-spines, cl-callus, mc- medium culture).

Starting from the obtained results, respectively getting vitrocultivated explants on culture medium supplemented with 2.5 mg / l 2,4-D (V_1), a rich mass of *callus* and numerous shoots, through these formations transferred on medium culture with different hormonal balance can be selected somaclones of particular ornamental value (Corneanu et al., 1990). The literature shows that although the *callus* it maintains its regenerative capacity for a longer period, subculturing may lead to a higher frequency of mutants, in particular by subculturing it on culture medium supplemented with high concentrations of 2, 4-D (Choi et al., 2002).

CONCLUSIONS

Initiation of *Aylostera heliosa* vitroculture proved to be possible on both experimental variants: mineral medium culture without growth regulators (V_0) and mineral medium culture supplemented with 2.5 mg / l 2.4-dichlorophenoxyacetic acid (2,4-D) (V_1) the explants evolution was differentiated according to its composition.

The response of *Aylostera heliosa* explants on mineral medium culture with 2.5 mg / l 2.4-dichlorophenoxyacetic acid (2,4-D) (V_1) compared with those grown on medium without growth regulator (V_0) as expected, showed a positive callus induction, so after *90 days* had an increase of 160% of the *number of calluses* / variant and a plus value of 126.1% *diameter of the callus* value.

Caulinar neoformation number generated at inoculated explants grown on culture medium supplemented with 2.5 mg / l 2,4-D (V_1) showed higher values than the same parameter values recorded on the control group V_0 (mineral medium culture without growth regulators) with 22.22%, while

the *caulinar neoformations diameter* of neoformed buds was 50% lower than in V_0.

Rhizogenesis in this period was not observed in any of the following experiments.

REFERENCES

1. Biswas M.K., U.K. Roy, R. Islam, M. Hossain, 2009, Callus culture from leaf blade, nodal, and runner segments of three strawberry(*Fragaria* sp.) clones. Turk. J. Biol., 33: 1-6.
2. Cachiță C.D., C. Deliu, R.L. Tican, A. Ardelean, 2004, Tratat de biotehnologie vegetală. vol.I, Editura Dacia, Cluj-Napoca, pp. 29-154.
3. Choi J.M., S. Zhang, P.G. Lemaux, 2002, Reduced Somaclonal Variation in Barley Is Associated with Culturing Highly Differentiated, Meristematic Tissues Phil Bregitzer, Crop Science, 42: 1303-1308.
4. Copăcescu V.S., 2001, Cactuşii, monografie; Ed. Ceres, Bucuresti, pp. 135-150.
5. Corneanu M., G.C. Corneanu, V.S. Copăcescu, 1990, Plant regeneration with somaclomal variability from *Mammilllaria sp.* callus. Abstract Book, Vllth Intern.Congress of Plant Tissue and Cell Culture, Amsterdam, pp. 95.
6. Heller R., (1953): Rescherches sur la nutrition minérale des tissus végétaux cultives *in vitro*. Ann.Sci. Nat. Bot. Veg. Ser.,vol. II : 1-5.
7. Jaafar S.N., M. S. Norihan, K. Shafii, 2009, *In vitro* culture of *Pereskia bleo*, Acta Hort. (ISHS), 829: 99-104.
8. Johnson J., E. Emino, 1979b, *In vitro* propagation of *Mammillaria elongate.* HortScience, 14(5): 605 – 606.
9. Karimi1 N., M.R. Mofid, M. Ebrahimi, R. Naderi, 2010, Effect of areole and culture medium on callus induction and regeneration *Cereus peruvianus* Mill. (*Cactaceae*), Trakia 10. Journal of Sciences, 8(2): 31-35.
10. Mata M., M, Monroy, K. Goldmmer, V. Chavez, 2001, Micropropagation of *Turbinicarpus laui* glass et Foster, an endemic and endangered species. In vitro Cellular Development Biology Plant, 37: 100-104.
11. Medeiros L., R. Ribeiro, L.A. Gallo, E. Oliveira, M.P. Dematte, 2006, *In vitro* propagation of *Notocactus magnificus*, Plant Cell, Tissue and Organ Culture, Springer, 84(1): 165-169.
12. Mihalte L., R. Sestras, G. Fesyt, 2008, Assessing genetic variability at different genotypes of cacti plants by means of rapd analysis**,** Bulletin UASVM, Horticulture, 65(1): 110 -115.
13. Murashige T., F. Skoog, 1962, A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15: 473 –497.
14. Myeong I.J., C. Ghang-Hui, L. Jung-Myung, 2004, Production and Breeding of cacti for grafting in Korea, Chronica horticulturae, Korea, 44(3): 7-10.
15. Papafotiou M., G. Balots, P. Louka, J. Chronopoulos, 2001, *In vitro* plant regeneration of *Mammillaria elongata* normal and cristate forms. Plant Cell, Tissue and Organ Culture, 65: 163 – 167.
16. Pérez E., M. Perez, E. Villalobos, E. Meza, L. Morones, H. Lizalde, 1998, Micropropagation of 21 species of mexican cacti by axillary proliferation. In vitro Cellular Development Biology Plant, 34: 131– 135.
17. Sandra Aparecida O., M.F.P. Silva Machado, M.A. Claudete Aparecida, (1996), Micropropagation of *Cereus peruvianus mill.* (*Cactaceae*) by areole activation. In Vitro Cellular & Developmental Biology – Plant, Springer Berlin/ Heidelberg, 32(3): 47-50.
18. Starling R., J. Dodds, (1983): Tissue culture propagation of cacti and other succulents. Bradleya, 1: 84 – 90.
19. Steinhart C. E., 1962, Tissue cultures of a cactus. Science, 137: 545 – 546.