STUDY ON THE REGENERATIVE CAPACITY AND ORGANOGENESIS OF 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 micropropagation 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 cytokinine - BA.

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 et 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 96°, 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 (Karimi 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.

To obtain experimental variants we supplemented the mineral culture medium without growth regulators V₀, 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 60th respectively 90th 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 V₀ - 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 Aylóstera heliosa explants of variant V₁ (mineral medium culture MS supplemented with 2.5 mg/l 2,4-D) with a diameter of stems 1.3 cm (Fig. 3A), were matched the witness V₀ (mineral medium culture MS without growth regulators) (Fig. 4A). Regarding the explants caulogenesis belonging to variant V₁ (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 V₀ (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 V₁ (mineral medium culture MS supplemented with 2.5 mg/l 2,4-D) reported to control group V₀.

Fig. 3. Graphic presentation of average values corresponding to the biometrized parameters of Aylóstera (Speg.) heliosa vitroplantlets, on aseptic mineral medium culture MS without growth regulators - (variant V₀); mineral medium culture MS with the addition of 2.5 mg/l 2,4-D (variant V₁), 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-dichlorophenoxyacetic 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 neoformations 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.

![Graphs showing biometric parameters of Aylostera heliosa vitroplantlets](image)

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<sub>0</sub>); mineral medium culture MS with the addition of 2.5 mg / l 2,4-D (variant V<sub>1</sub>), 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<sub>1</sub>), which exceeded the witness V<sub>0</sub> with 2.9 cm (Fig. 4E), 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
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 (V₀), B - mineral medium culture MS supplemented with 2.5 mg / l 2,4-D (V₁) (iiiv- viable initial inocula; nc - caulinar neoformation; ar- areoles, sp-spines, cl-callus, mc-medium culture).](image)

Starting from the obtained results, respectively getting vitrocultivated explants on culture medium supplemented with 2.5 mg / l 2,4-D (V₁), 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 (V₀) and mineral medium culture supplemented with 2.5 mg / l 2,4-dichlorophenoxyacetic acid (2,4-D) (V₁) 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) (V₁) compared with those grown on medium without growth regulator (V₀) 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 (V₁) showed higher values than the same parameter values recorded on the control group V₀ (mineral medium culture without growth regulators ) with 22.22%, while
the caulinar neoformations diameter of neoformed buds was 50% lower than in V₀.  
Rhizogenesis in this period was not observed in any of the following experiments.

REFERENCES


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