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COMPARATIVE STUDY ON THE CAPACITY OF REGENERATIVE ORGANOGENESIS IN *OPUNTIA FRAGILIS* VAR *FRAGILIS* AND *AYLOSTERA HELIOSA* GROW IN VITRO ON MEDIUM SUPPLEMENTED WITH 2,5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D)

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Abstract

In this experiment we chose two species of cactus, respectively: Opuntia fragilis var fragilis belonging to the genus Opuntia, a cactus with great economic importance for which there is a continuous growth for virus-free seedlings, obtained only by in vitro micropropagation (Johnson and Emino 1979, Escobar et al., 1986; Rubluo et al., 1996; Smith et al. 1991) and Aylostera heliosa, a decorative cactus with both port and flowers, but which is difficult to propagate by grafting but much easier by in vitro micropropagation. These cacti can be successfully acclimatized by developing a strong root system.

In order to establish the in vitro culture, we harvested buds from the stems of Opuntia fragilis var fragilis and Aylostera heliosa. We inoculated the explants on a culture medium consisting of macroelements and Fe EDTA Murashige-Skoog (1962), Heller microelements (1953), supplemented with 2,5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D).

The evolution of the explants was monitored for 90 days. The response of the explants of Opuntia fragilis var fragilis and Aylostera heliosa to the presence in the culture medium of an amount of 2,5 mg/l 2,4-D was different, so the presence in the culture medium of auxin as expected favorably influenced callus induction in both species while rhizogenesis was noted only in Opuntia fragilis var fragilis with an increase of 135,29% in the number of roots formed and 138,23% in their average length.

Keywords: in vitro cultures, 2,4-dichlorophenoxyacetic acid, roots, callus

INTRODUCTION

Ontogenetic development of plants is determined by endogenous and exogenous factors that can have an action more or less specific. Phytohormones, are endogenous stimuli, but may be added to the culture medium, the exogenous form of synthetic compounds that have the capacity to mimic the effects of natural growth regulators.

Auxins are phytohormones or growth regulators frequently used in tissue cultures, having a stimulating action on rhizogenesis, considering that the cumulative effect of endogenous auxins with the intake of exogenous auxins, leads to obtaining as many roots as possible (Juárez et al. , 2002). Among the synthetic auxins is 2,4-dichlorophenoxyacetic acid (2,4-D), which is the most used and recommended growth regulator that stimulates the generation of calluses in explants. At moderate concentrations, it boosts

cell division in the cambium, but becomes toxic at higher concentrations. In callus culture, auxin provides high friability, facilitating the separation of cells in cell suspensions and somatic embryogenesis. Cacti are considered to be extremely susceptible in the differentiation process when they are grown in mineral environments rich in growth regulators (Copăceacu, 2001) invariably inducing organogenesis processes.

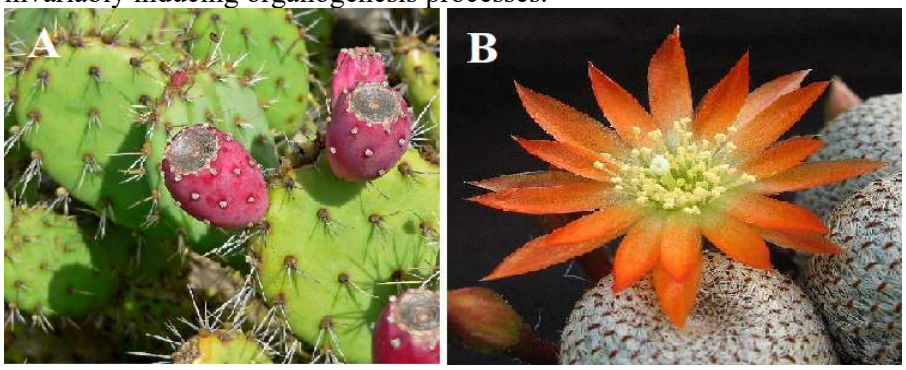

Fig.1. Image representing cacti, where: A - *Opuntia fragilis var fragilis*; B – *Aylostera heliosa*.

After Griffith, 2001a and Pinkava, 2002 genus *Opuntia* (fig.1A) cacti are the most studied species in the world, due to the economic importance of this cactus. *Opuntia* cactus is a valuable economically, is eaten as a vegetable, but also has edible fruit, also used as fodder (Kluge and Ting, 1978; Casas and Barbera, 2002). This plant is considered a good indicator of the presence of pollutants (Nobel, 1994), it is also considered as an important tool to combat desertification (El Gamrat, 2004). Like other species of cactus, *Opuntia fragilis var fragilis*, can multiply rapidly and efficiently by micropropragare in vitro (Karimi1 et al., 2010).

Aylostera heliosa (fig.1B), cactus, decorative both in the port - thorns due to the comb aligned to the white-silver edge (Perez et al., 2002), and by red or orange flowers is a very difficult species multiplied by grafting (Myeong et al., 2004). *Aylostera heliosa* like other cacti can multiply quickly and efficiently by in vitro micropropagation (Karimi1 et al., 2010).

In this experiment our goal was to study the reactions of phytoinocles of *Opuntia fragilis* var *fragilis* and *Aylostera heliosa* to the existence in the culture medium of 2,4 - dichlorophenoxyacetic acid (2,4-D) added in the same concentration, of 2,5 mg/l. We thus obtained the following variants: V_0 or control group (medium without growth regulators), V_1 in which we cultivated inoculi of *Opuntia fragilis* var *fragilis* on a medium supplemented with 2,5 mg/l 2,4 - dichlorophenoxyacetic and V_2 in which we cultured *Aylostera heliosa* inocula on a medium supplemented with 2,5 mg/l 2,4 - dichlorophenoxyacetic.

MATERIAL AND METHOD

To initiate *in vitro* cultures of *Opuntia fragilis* var *fragilis* and *Aylostera heliosa* i keep prelevet strains with mature areolas but with less thorns trainers, shorts and white. The material so obtained was secţiont transverse operation which resulted dished washers that were divided so that eventually fragments were inoculated following dimensions: about 1 cm long and 0,5 cm thick, yet have minimum 2-3 areola. After these operations we obtain the explants from mid dial and lateral (Fig. 2).

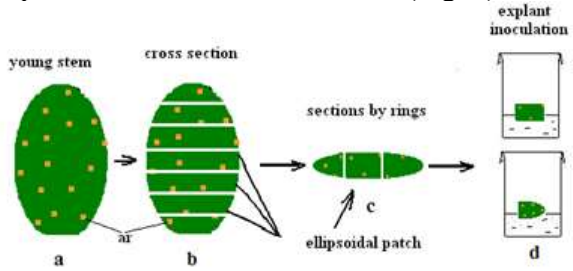

Fig. 2. Schemetic representation of *Opuntia fragilis* var *fragilis* and *Aylostera heliosa* young stems (a, b), and how slicing it into rings ellipsoid (c) and lateral explants inoculated on media centers and aseptic (d), where: ar - areola.

Knowing that *in vitro* cultures of naturally occurring cacti - the areola - some long hairs and bristles, host parties for a variety of organisms (Garcia-Saucedo et al., 2005), sanitized of plant material was achieved by submersare for one minute at 96 º alcohol, followed by the coating process it with a solution of 0.8% sodium hypochlorite mixed with water in a ratio of 1:2, which were added three drops of Tween 20 as surfactant (Cachiță et al., 2004). Sanitized lasted 20 minutes, during which the plant material was continuously stirred. After decanting disinfectant plant material was washed with sterile distilled water to remove chlorine, achieving five consecutive rinses, of five minutes each.

After sterilization, the plant material was deposited in Petri capsules on filter paper discs (previously sterilized in the oven) in a laminar flow hood, horizontal air sterile operation, followed by sizing operation and future inocula removal of necrotic parts thereof.

Culture medium used for growth explants consisted of: macro Murashige-Skoog EDTA and Fe (1962), Heller microelements (1953), mineral mixture to which was added vitamins: pyridoxine HCl, thiamine HCl and nicotinic acid (containing 1 mg/l each), m-Inositol - 100 mg/l, sucrose - 20 g/l and agar 7 g/l pH of the medium was adjusted to a value of 5,8, its first autoclaving. The basal medium (MB) presented, we added 2,4-dichlorophenoxyacetic acid (2,4-D) concentration of 2,5 mg/l 2,4-D,

achieving the following: V_0 or control group (medium without growth regulators), V_1 in which we cultivated inoculi of *Opuntia fragilis* var *fragilis* on a medium supplemented with 2,5 mg/l 2,4 - dichlorophenoxyacetic and V_2 in which we cultured *Aylostera heliosa* inocula on a medium supplemented with 2,5 mg/l 2,4 - dichlorophenoxyacetic.

The culture medium was placed in a glass vial with a capacity of 15 ml (each container was placed 5 ml of medium). Medium vials were sterilized for 30 minutes, by autoclaving 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 inocula were transferred to room for growth, under the following conditions: temperature ranged from 24ºC in peroada light and 20° during the phase of darkness and light was the regime fotoperiodic 16 hours lumină/24h, lighting cultures achieving is the white light emitted by fluorescent lamps, the intensity of 1700 lux.

Reaction and evolution of explants was monitored for 90 days. In this time period were conducted periodic observations and readings every 30 days. Values recorded biometric control group (V_0, fitoinoculi 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

In the explants of *Opuntia fragilis* var *fragilis* grown on a medium supplemented with 2,5 mg/l 2,4-D (V_1), the average number of roots formed was 0,6 roots/variant above the value of the same parameter recorded in the control group V_0 increase) (Fig.3), which represents an increase of 135,29% (Fig.4). The presence of auxin 2,4-D at a concentration of 2,5 mg/l (V_1) in the culture medium also positively influenced the increase in root length, so the average length of the largest root formed - in absolute values - exceeded the value recorded by this parameter in the control group by 1,3 cm (Fig.3), thus marking an increase of 18,23% (Fig.4) (Vidican et al, 2016); these differences are considered, from a statistical point of view, to be very significant generated at the level of the explants (Table 1). While in the case of *Aylostera heliosa* explants the presence in the culture medium of 2,5 mg/l 2,4-D (V_2) did not stimulate the formation of roots, the phenomenon of rhizogenesis did not manifest itself until this date (Vidican et al, 2011).

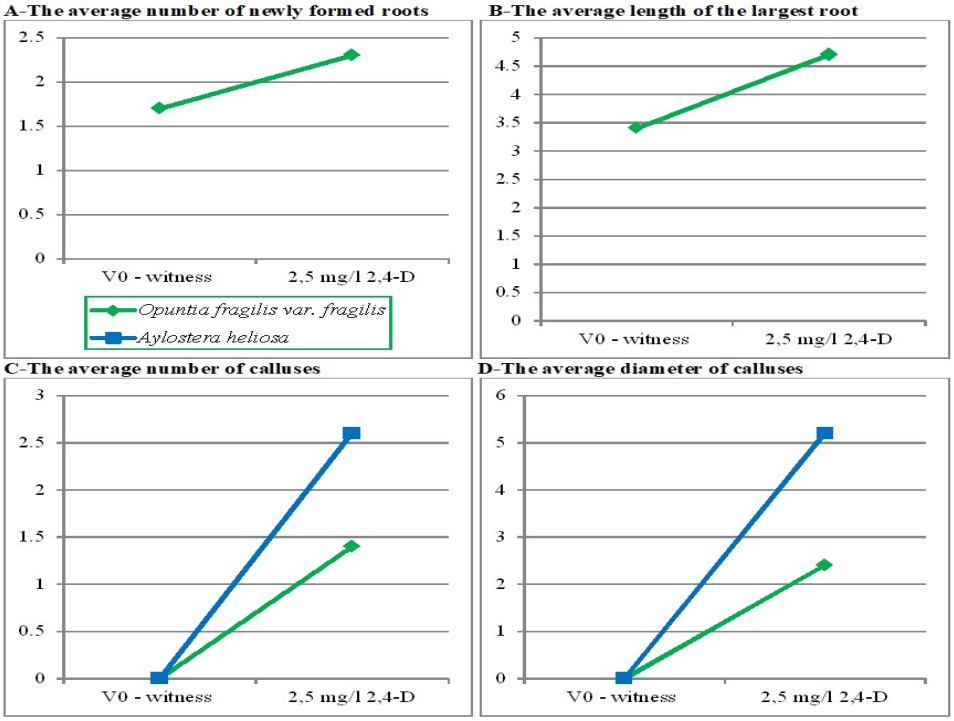

Fig.3. Graphic presentation of the average values corresponding to the parameters recorded at the level of vitrocultures of *Opuntia fragilis* var *fragilis* and *Aylostera heliosa* on aseptic medium modified by us - (variant V_0) - with the addition of 2,5 mg/l 2,4-D, data expressed in absolute values; (where: A-the average number of newly formed roots; B – the average length of the largest root; C-the average number of calluses; D-the average diameter of calluses).

The action of 2,4-dichlorophenoxyacetic acid (2,4-D) added at a concentration of 2,5 mg/l (V_1) stimulated the induction of callus formation in both explants *Opuntia fragilis* var *fragilis* as well as those of *Aylostera heliosa*, in the first case the number of calluses being 1,4 calluses/variant (Fig.3) reaching an average diameter of 2,4 cm (Fig.4); while in the second case the average number of calluses/variant was 1,6 (Fig.3) higher in the case of phytoinocles belonging to variant V_2 (average supplemented with 2,5 mg l 2,4-D), compared to the values of the same parameter recorded in the control group V_0 (medium without growth regulators) these having an average diameter that exceeded the control V_0 by 2,9 cm.

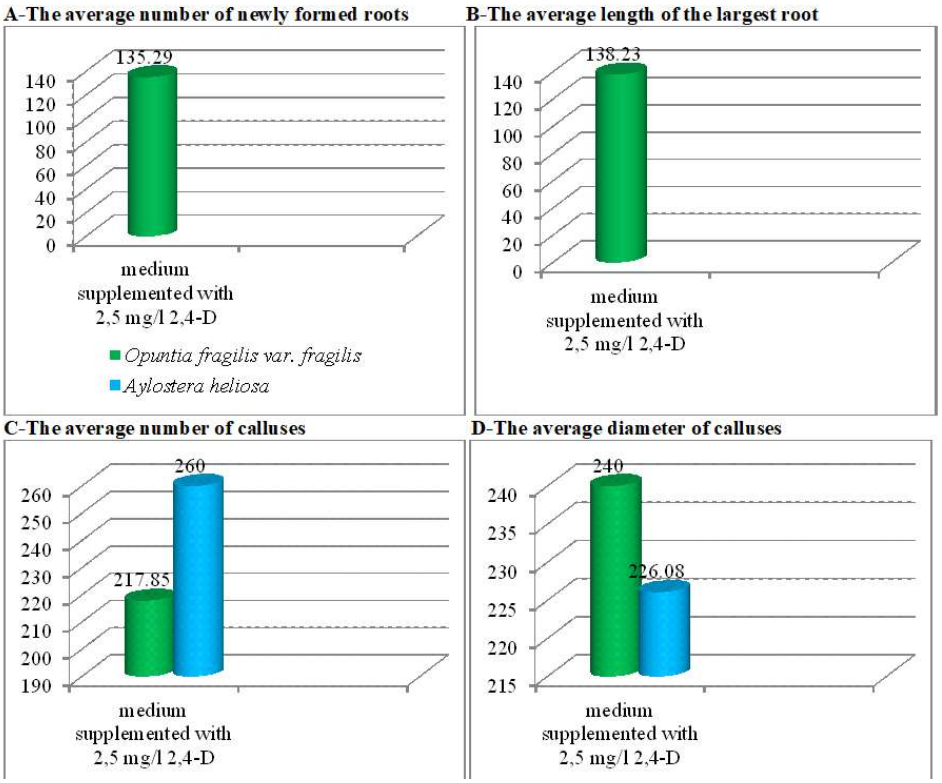

Fig.4. Graphic presentation of the average values corresponding to the biometric parameters at the level of vitropultures of *Opuntia fragilis* var *fragilis* and *Aylostera heliosa* on aseptic medium modified by us, with the addition of 2,5mg/l 2,4-D, data expressed in percentages, obtained after reporting the values read at the results recorded at the respective parameters biometrized to the control group (V_0), without growth regulators, values considered to be 100%; (where: A-the average number of newly formed roots; B – the average length of the largest root; C-the average number of calluses; D-the average diameter of calluses).

In terms of percentage in *Opuntia fragilis* var *fragilis* the average number of calluses formed showed an increase of 217,85% (Fig.4) with no increase in their diameter of 240% (Fig.4) compared to the control V_0, and in *Aylostera heliosa* there was an increasing trend, so the values recorded at control V_0 were exceeded by an increase of 260% in the number of calluses formed (Fig.4) and of 226,08% in their average diameter (Fig.4). these results, from a statistical point of view, are considered to be very significant (Table 1).

Parametrul / Varianta	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
60 ZILE												
V0	1,70±0,29	0,0800	***	3,40±0,29	0,0863	***	0	0		0	0	
V1	2,30±0,53	0,2763	**	4,70±0,75	0,5631	***	1,40±0,25	0,0632	**	2,40±0,30	0,0895	***
90 ZILE												
V0	0	0		0	0		1,00±0,65	0,4211	NS	2,30±1,21	1,4526	
V2	0	0		0	0		2,60±0,41	0,1684	***	5,20±0,58	0,3368	***

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

Table 1. Results of the biometric evaluation 90 days after inoculation of the explants on V_0 or control group (medium without growth regulators), V_1 in which we cultivated inoculi of *Opuntia fragilis* var *fragilis* on a medium supplemented with 2,5 mg/l 2, 4 - dichlorophenoxyacetic and V_2 in which we cultured *Aylostera heliosa* inocula on a medium supplemented with 2,5 mg/l 2,4 - dichlorophenoxyacetic.

The obtained results determine us to appreciate that the addition in the culture medium of 2,5 mg/l 2,4-D constitutes a sufficient measure for the induction of callus in the explants of *Opuntia fragilis* var *fragilis* and *Aylostera heliosa*, estimates that are in agreement with those reported by Sandra A. et al., (1996), in *Cereus peruvianus* cultures practiced "in vitro".

In the case of our experiment, the callus generated in the explants of *Opuntia fragilis* var *fragilis*, inoculated and grown on culture medium supplemented with 2,5 mg/l 2,4-D (V_1) or due to the abundance covered the entire surface of the nutrient substrate (Fig.5.1.B), in which case it shows signs of early semescence - fact highlighted by the cream color - either it was located at the base of the neotulpins in the form of opalescent, friable, pale green tissue (Fig.5.1.C).

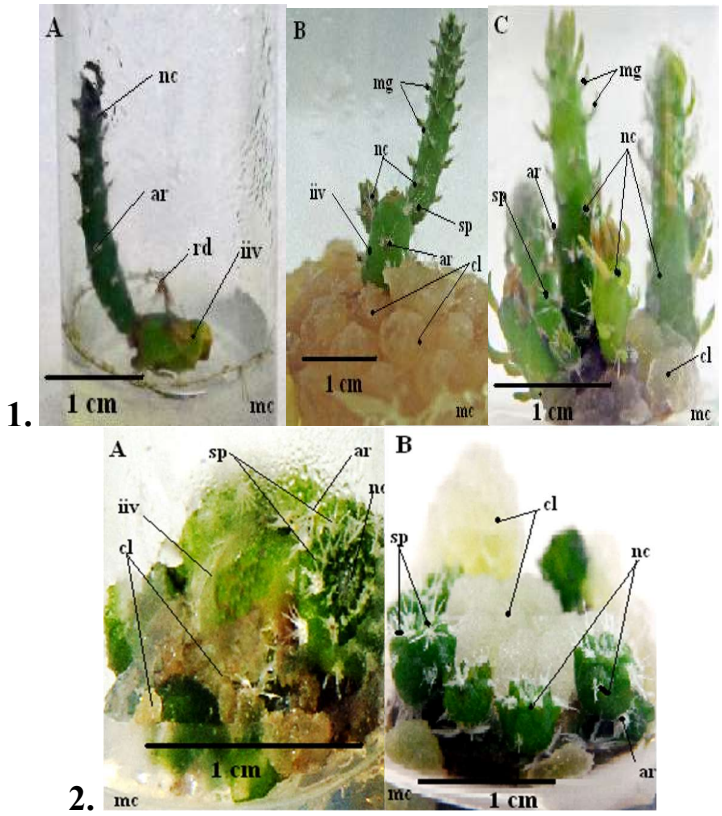

Fig.5. Inoculi of **1** - *Opuntia (Tournef.) Mill.fragilis var. fragilis* and **2** - *Aylostera heliosa*, 90 days after inoculation of the "in vitro" explant, where: A-roots; B-callus showing signs of early senescence; C-callus friable; and: iiv – viable initial inoculum; mc –Culture medium; nc-newly formed stems; rd-roots; ar-areoles; sp-spines; cl-callus; mg-buds).

Callus generated from *Aylostera heliosa* explants grown 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 color (Fig.5.2.A). 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.5.2.B), and due to its abundance it covered the entire surface of the culture medium.

CONCLUSIONS

1. Summarizing the results obtained from the observations made for 90 days on the phytoinocles of *Opuntia fragilis* var *fragilis* and *Aylostera heliosa* from the values recorded on inoculated explants and grown on medium supplemented with 2,5 mg/l 2,4-D, we can say that the response of

explants in both cases is, as expected, favorable to callus induction, thus in *Aylostera heliosa* there is a 260% increase in the average number of calluses/variant while in *Opuntia fragilis* var *fragilis* increased by 217,85%.

2. The values of the average diameter of the callus compared to the batch supplemented with 2,5 mg/l 2,4-D compared to control V_0 (phytoinocles grown on basic medium, without growth regulators) and considered as reference, registered a plus of and an increase of 226,08% in *Aylostera heliosa* and 240% in *Opuntia fragilis* var *fragilis*.

3. The presence in the culture medium of 2,5 mg/l 2,4-D stimulated the formation of roots only in the experimental variant V_1 (*Opuntia fragilis* var *fragilis*) where the number of newly formed roots exceeded by 135,29% the value the same parameter recorded in the control group, and the average length of the largest newly formed root was also 138,23% higher than that of the control group V_0, while in *Aylostera heliosa*, rhizogenesis did not occur in this interval time.

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