COMPARATIVE STUDY ON THE REGENERETIVE AND ORGANOGENIC CAPACITY OF *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis*, IN THE PRESENCE IN CULTURE MEDIUM 2,5 mg/l 3- indolylbutyric acid (AIB) and a 2,5 mg/l 2,4dichlophenoxyacetic acid (2,4-D)

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Abstract

Of all the cacti, the Opuntia genus has the greatest economic importance, which makes the demand for virus-free propagating material to be higher, which can only be achieved by in vitro micropropagation. (Johnson si Emino 1979, Escobar et al., 1986; Rubluo; şi colab., 1996; Smith şi colab. 1991).

Opuntia fragilis var. fragilis to initiate vitro cultures of lime strains prelevet hold with areolas mature, secţiont fragments of about 1 cm long and 0,5 cm thick but at least 2-3 areola. Hold the strains have been deposited on the sterilized culture medium consists of macro and Fe EDTA Murashige-Skoog (1962) Heller microelements (1953), supplement with two auxins, respectively 2,5 mg/l AIB (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 presence in the culture medium of auxin 3-indolylbutyric acid (AIB) or dichlorophenoxyacetic acid (2,4 D), in both cases new stems and roots were generated, but the presence in the culture medium of 2,5 mg/l AIB determined the highest number of newly formed strains 2,2 (157,14%) but also of 5,0 roots (294%), The genesis of the horse was noted only in explants grown on medium with the addition of 2,5 mg/l 2,4 D (V_2), on average 1,4 calluses/variant (140%) with an average diameter of 2,4 cm (240%).

Keywords: indolylbutyric acid (AIB) 2,4dichlophenoxyacetic acid (2,4-D), newly formed stems, roots, callus.

INTRODUCTION

Phytohormones are endogenous stimuli, but can be added to the culture medium, the exogenous form of synthetic compounds that have the ability to mimic the effects of natural growth regulators. In callus culture, auxin ensures high friability, facilitating the separation of cells in cell suspensions and somatic embryogenesis (Cachiță et al., 2004).

The presence in the culture medium of auxins substances with direct action on cell division, rhizogenesis and callus formation (Cachiță et al., 2004). 3 Indolylacetic acid (AIB), the only natural auxiliary is a less thermally stable compound and oxidizes in light, thus reducing its efficiency, is formed in the growth tips of the stems, but is also present in

meristems, it is known that the presence in the culture medium of 2,4-dichlorophenoxyacetic acid (2,4-D) plays an important role in cell growth and metabolism, the introduction into the environment being sufficient to induce callus (Sandra Aparecida et al., 1996).

According to Griffith, 2001 Pinkava, 2002 the genus of cactus *Opuntia* (fig.1) includes the most studied species in the world, due to the economic importance of this cactus. *Opuntia* cactus is an economic asset, it is consumed as a vegetable, but there are also edible fruits, 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 an important tool to combat desertification (El Gamrat, 2004).

Fig.1 - Cactus *Opuntia fragilis* var. *fragilis*. Where: a - plant, b and c – fruit

The purpose of this experiment was to study how *Opuntia fragilis* var *fragilis* reacts to supplement the V_0 culture medium with two auxins, 2,5 ml/l AIB (V_1) and 2,5 ml/l 2,4 D (V_2).

MATERIAL AND METHOD

To initiate *in vitro* cultures of *Opuntia fragilis* var *fragilis* 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).

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.

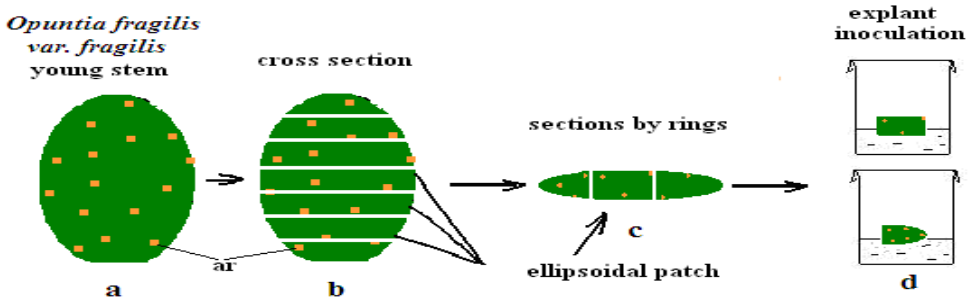


Fig. 2. Schemetic representation of *Opuntia fragilis* var *fragilis* young stems (a, b), and how slicing it into rings ellipsoid (c) and lateral explants inoculated on media centers and aseptic (d), where: ar - areola.

Medium free of growth regulators (V_0) and baseline media supplemented with 2,5 mg/l AIB (V_1) and 2,5 mg/l 2,4-D (V_2).

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, explants 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

Comparing the results of the biometric measurements performed on the 90th day after the initiation of the present experiment, compared to the mature variant V_0 (medium without growth regulators), a slowing of the growth rate was observed in terms of the average length of the main strain at variants V_1 (supplemented with 2,5 mg/l AIB) by 0,3 cm, which is due to a very good and rapid development of the explants inoculated on the control group, while at explants cultured on V_2 (mean supplemented with 2,5 mg/l 2,4-D) the absolute values of this parameter increased by 0,5 cm (Fig.4)

compared to the values of V_0 (1,6 cm), which which in percentage values represents a deficit of 18% in the first case and an increase of 18% (Fig.5) in the second case (Fig.4). Values that, statistically, are considered to be distinctly significant (Table 1).

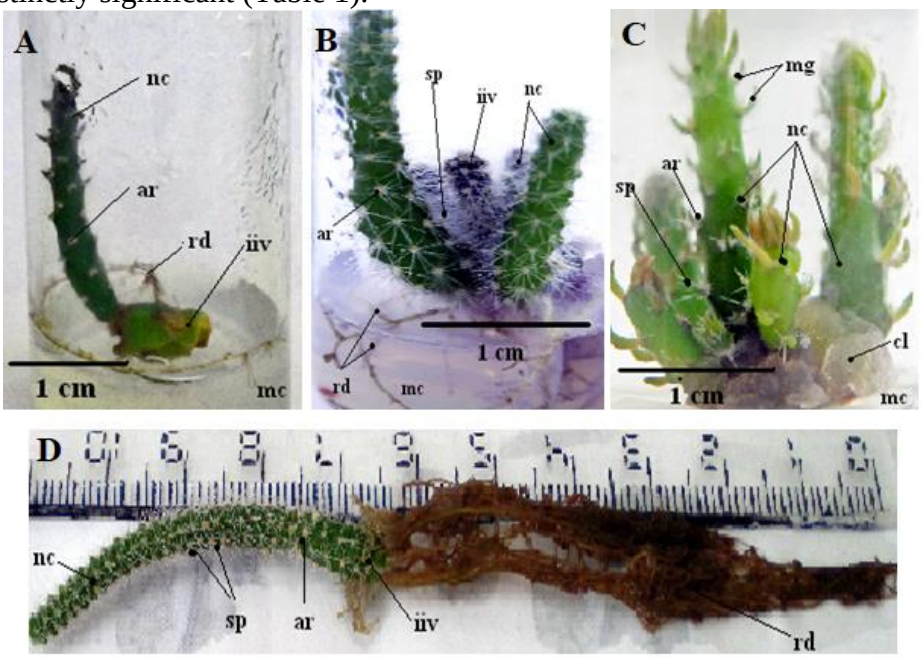


Fig.3. Inoculi of *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis*, 90 days after inoculation of the "in vitro" explant. A-on basic environment modified by us and lacking growth regulators (V_0); B-on basic medium with the addition of 2,5 mg/l AIB (V_1); C-on base medium with the addition of 2,5 mg/l 2,4-D (V_2); d-roots formed on inocula grown on basic medium with the addition of 2,5 mg/l AIB. Where: iiv – the viable initial inoculum; mc – culture medium; nc-newly formed strain; rd-root; ar-areoles; sp-spines; cl-calus; mg-buds.

The average number of newly formed strains (Fig.3) was above the values recorded in the control sample (V_0), with a difference of 0,8 new strains/
variant, in explants inoculated and grown on culture medium supplemented with 2,5 mg/l AIB (V_1) which represents an increase of 57,14%, while in the explants belonging to variant V_2 (medium supplemented with 2,5 mg/l 2,4 D) there was a decrease of 0,5 cm (Fig.4) Compared to the control (1,4 cm), so a deficit of 35,72% (Fig.5).

At this time, the average length of the largest newly formed strains (Fig.3) was, at absolute value, at V_1 (mean supplemented with 2,5 mg/l AIB) by 0,4 cm (Fig.4), Above the values of control V_0 (3,2 cm) and 0,2 cm in the explants of variant V_2 (mean supplemented with 2,5 mg/l 2,4 D), which in the first case represents an increase of 12% and 6% in the second case (Fig.5), results confirmed in our 2015 research (Vidican, 2015)

Table 1.

Results of evaluations of the values read in the vitroplants of *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis*, performed 90 days after inoculation of explants on basic aseptic media (V_0) with the addition of 2,5 mg/l AIB (V_1) and 2,5 mg/l 2,4 D (V_2)

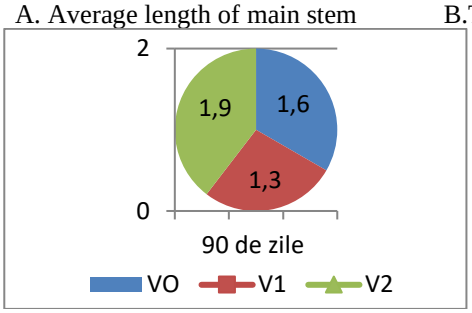
Parametrul / Varianta	Lungimea medie a tulpinitei principale ± abaterea standard	Varianța	Semnificația	Numărul mediu al neoformațiunilor caulinare ± abaterea standard	Varianța	Semnificația	Lungimea medie a celei mai mari neoformațiuni caulinare ± abaterea standard	Varianța	Semnificația	Numărul mediu 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
90 ZILE																					
V0	1,60±0,15	0,0211	***	1,40±0,45	0,2042	**	3,40±0,32	0,1053	***	1,70±0,29	0,0800	***	3,40±0,29	0,0863	***	0	0		0	0	
V1	1,90±0,40	0,1537	**	0,90±0,33	0,1079	*	3,20±0,84	0,7084	**	2,30±0,53	0,2763	**	4,70±0,75	0,5631	***	1,40±0,25	0,0632	**	2,40±0,30	0,0895	***
V2	1,30±0,29	0,0842	**	2,20±0,45	0,2000	**	3,60±0,41	0,1653	***	5,00±0,92	0,8421	**	9,80±0,88	0,7633	***	0	0		0	0	

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

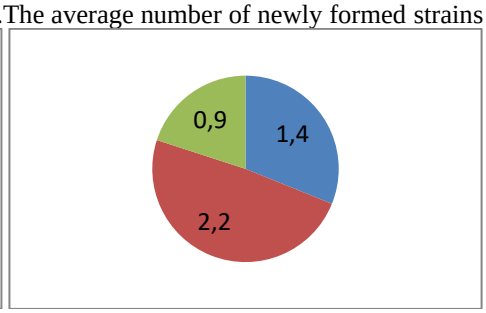
Regarding the average number of newly formed roots (Fig.3), it exceeded the control in all experimental variants, thus with an increase of 3,3 roots/variety (Fig.4), Recorded in the explants of variant V_1 (average supplemented with 2,5 mg/l AIB) an increase of 194% was reported compared to the control group V_0 (culture medium without growth regulators), and an increase of 0,6 cm roots/varied at V_2 (average supplemented with 2,5 mg/l 2,4 D), so an increase of 35% (Fig.5).

The average length of the largest newly formed root (Fig.3) also showed the largest increase in phytoinocules of variant V_1 (average supplemented with 2,5 mg/l AIB), which with an absolute average value of this parameter of 9,8 cm (Fig.4), Recorded an increase of 188% (Fig.5). Compared to the control V_0 (3,4 cm), significantly lower values were recorded for variants V_2 (average supplemented with 2,5 mg/l 2,4 D) which recorded at this parameter an average of 4,7 cm, (Fig.5), so an increase of 38%.

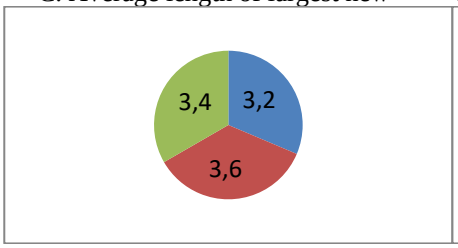
A. Average length of main stem



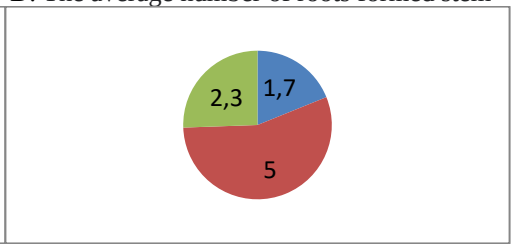
B.The average number of newly formed strains



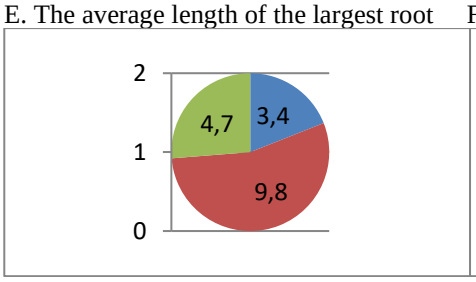
C. Average length of largest new



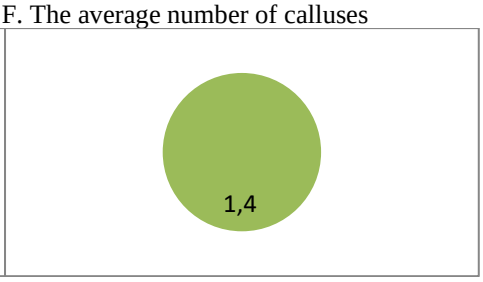
D. The average number of roots formed stem



E. The average length of the largest root



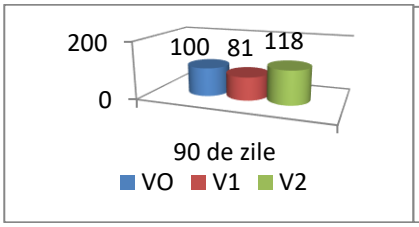
F. The average number of calluses



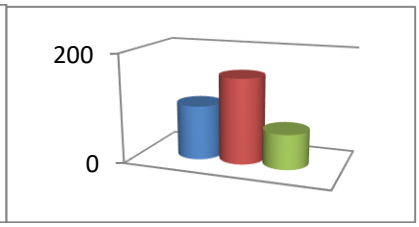
G. The average diameter of the calluses

Fig.4. Graphic presentation of the average values corresponding to the parameters read in the in vitro cultures of *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis*, on basic aseptic medium modified by us - (variant V_0) - with the addition of 2,5 mg/l AIB (V_1) and 2,5 mg/l 2,4-D (V_2), data expressed in absolute values ; (where: A – the average length of the main stem; B – the average number of newly formed stems; C – the average length of the largest newly formed stem; D – the average number of roots; E – the average length of the largest root; F -the average number of calluses, G-the average diameter of the calluses.

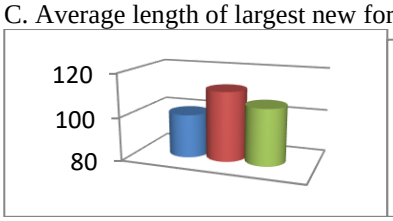
A.Average length of main stem strains



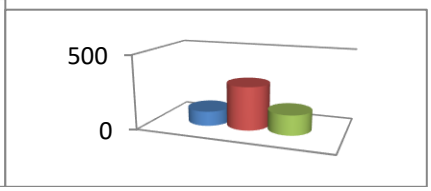
B.The average number of newly formed



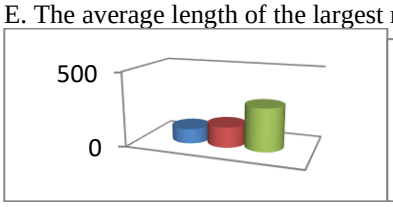
C. Average length of largest new formed stem



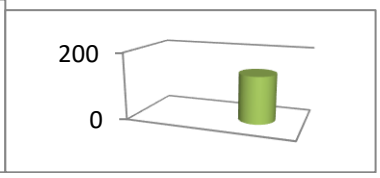
D. The average number of roots



E. The average length of the largest root



F. The average number of calluses



G. The average diameter of the calluses

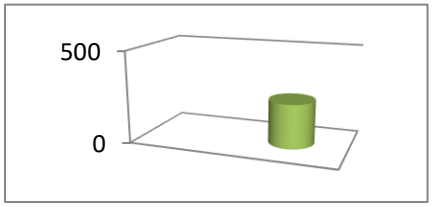


Fig.5. Graphic presentation of the average values corresponding to the parameters recorded at the level of in vitro cultures of *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis*, on a modified aseptic basis by us, with the addition of 2,5 mg / l AID (V_1) and 2,5 mg/l 2,4-D (V_2), data expressed as a percentage, obtained after reporting the values monitored at the results recorded at the respective parameters read in the control group (V_0), without growth regulators, values considered to be 100%; (where: A – average length of main stem; B – average number of main stems; C – average length of largest main stem; D – average number of roots; E – average length of largest root; F-number callus medium; G-average callus diameter).

These results attest to the special rhizogenic effect that AIB has (Fig.3), On this species of cactus, in vitro-culture regime, and especially, added in the culture medium in a concentration of 2,5 mg/l. The results obtained at this parameter are considered, from a statistical point of view, to be very significant (Table 1).

The presence in the culture medium of 2,5 mg/l 2,4-dichlorophenoxyacetic acid (V_2) stimulated the generation of callus (Fig. PP), forming on average 1,4 calluses/variant (Fig.4), It increasing to an average diameter of 2,4 cm, which represents an increase of 40% in the first case and 140% in the second case (Fig.5). These results were also confirmed in our research on *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis* in 2016 (Vidican et al., 2016).

CONCLUSIONS

1. After 90 days from the initiation of the in vitro culture of of *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis* we observed that explants grown on medium supplemented with 2,5 mg/l AIB (V_1) generated the most new strains, exceeding by 0,8 new strains/control variant, which represented an increase of 57,14% .
2. Rhizogenesis was manifested in both variants studied but with the highest values, compared to control V_0, are the explants grown on culture medium supplemented with 2,5 mg/l AIB (V_1) which with 5 roots /variant in an average length of 9,8 cm showed an increase of 194% compared to the control group V_0 (culture medium without growth regulators), in the first case and 188% in the second case.
3. The genesis of callus was manifested only in inocula grown in culture medium improved by 2,5 mg/l 2,4 D (V_2) which formed on average 1,4 calluses/variant, it increasing to an average diameter of 2,4 cm, which is an increase of 40% in the first case and 140% in the second case.

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