STUDY ON THE ABILITY OF REGENERATIVE ORGANOGENOUS EXPLANTS *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis*, CURRENTLY IN THE CULTURE MEDIUM dichlorophenoxiacetic 2,4 (2,4-D)

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Abstract

Due to the economic importance of the genus Opuntia cacti have there is a growing demand in terms of quantity of virus-free planting material, achievable only in vitro micropropagation (Johnson and Emino 1979, Escobar et al., 1986; Rubluo et al., 1996; Smith et al. 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), supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) at a concentration of 2,5 mg / l 2,4-D (V_1).

Evolution vitro cultures was monitored for 90 days.Their response was different, so the presence in the culture medium of 2,5 mg/l 2,4-D (V_1) has positively influenced callus formation, achieving a plus of 240%, also the number and length of roots formats, but the presence of auxin 2,4-D herbicide defoliant effect, was an inhibitor training and new strains length formats.

Keywords: vitro cultures, 2,4-dichlorophenoxyacetic acid (2,4-D), the newly formed stems, 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.

It is known that the presence in the culture medium of 2,4-dichlorophenoxyacetic acid (2,4-D) plays an important role in growth and cell metabolism, introduction into the environment is sufficient to induce callus (Sandra Aparecida et al., 1996). Formed callus explant can be detached, cut and then transferred to fresh culture medium to obtain broods.

After Griffith, 2001a and Pinkava, 2002 genus *Opuntia* 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 (Valdez-Flores, 1994; 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).

Cacti are considered to be highly susceptible to the process of differentiation when grown in mineral-rich medium growth regulators (Copăceacu, 2001) invariably induces organogenesis processes.

The purpose of this research was to study how they influence the presence of culture medium to a concentration of 2,5 mg/l 2,4 - dichlorophenoxyacetic acid (2,4-D) on explants cultured in vitro *Opuntia fragilis* var. *fragilis.*

MATERIAL AND METHODS

The research was conducted in 2015 in the laboratory of plant biotechnology at the Faculty of Sciences at the University of Oradea, Department of Biology.

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. 1).

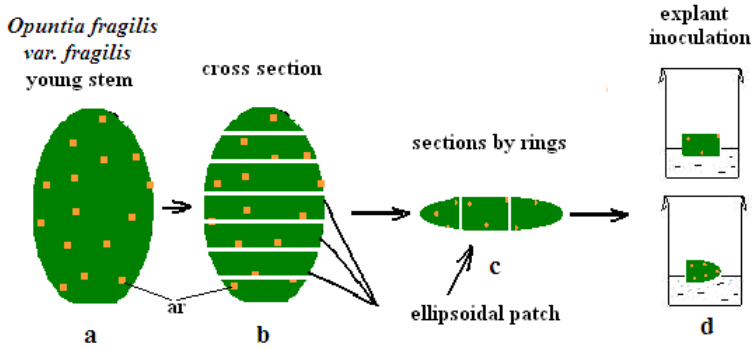


Fig. 1. Schemetic representation of *Opuntia fragilis* var *fragilis* young stems (a, b), and how slicing it into rings ellipsoid (c) and lateral explants inoculated on media centers and aseptic (d), where: ar - areola.

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 - the control, medium lacking growth regulators and V_1 - medium supplemented with 2,5 mg/l 2,4-D

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 DISCUSSIO

After 90 days vitro cultures, the explants *Opuntia fragilis* var. *fragilis* grown on medium supplemented with 2,5 mg/l 2,4-D (V_1) noted a smaller increase average length of main stem, number and average length of the longest newly formed strains as compared with the same parameters recorded explants cultured on medium lacking growth regulators (V_0). This is due to a very good and fast development of explentelor inoculated control group compared explentele V_0 grown on medium supplemented with 2,4-D (2,5 mg/l), which had slower growth (Fig.2).

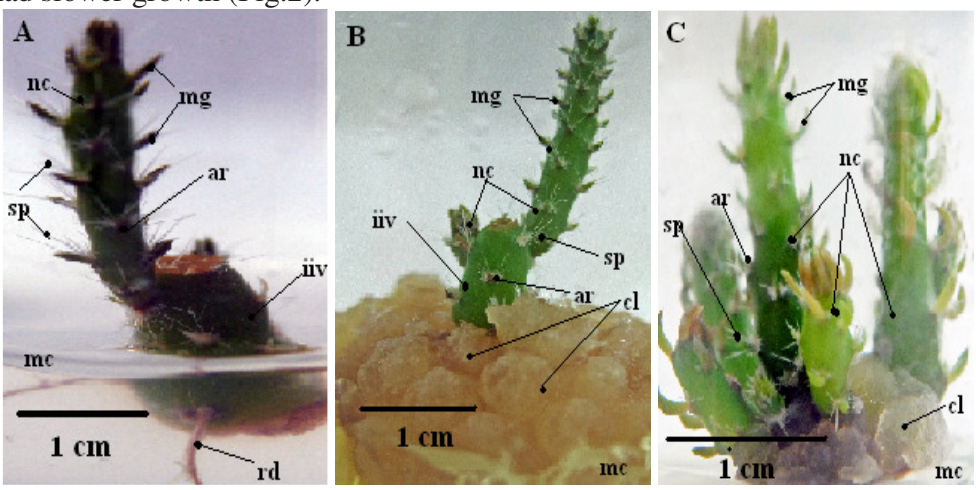
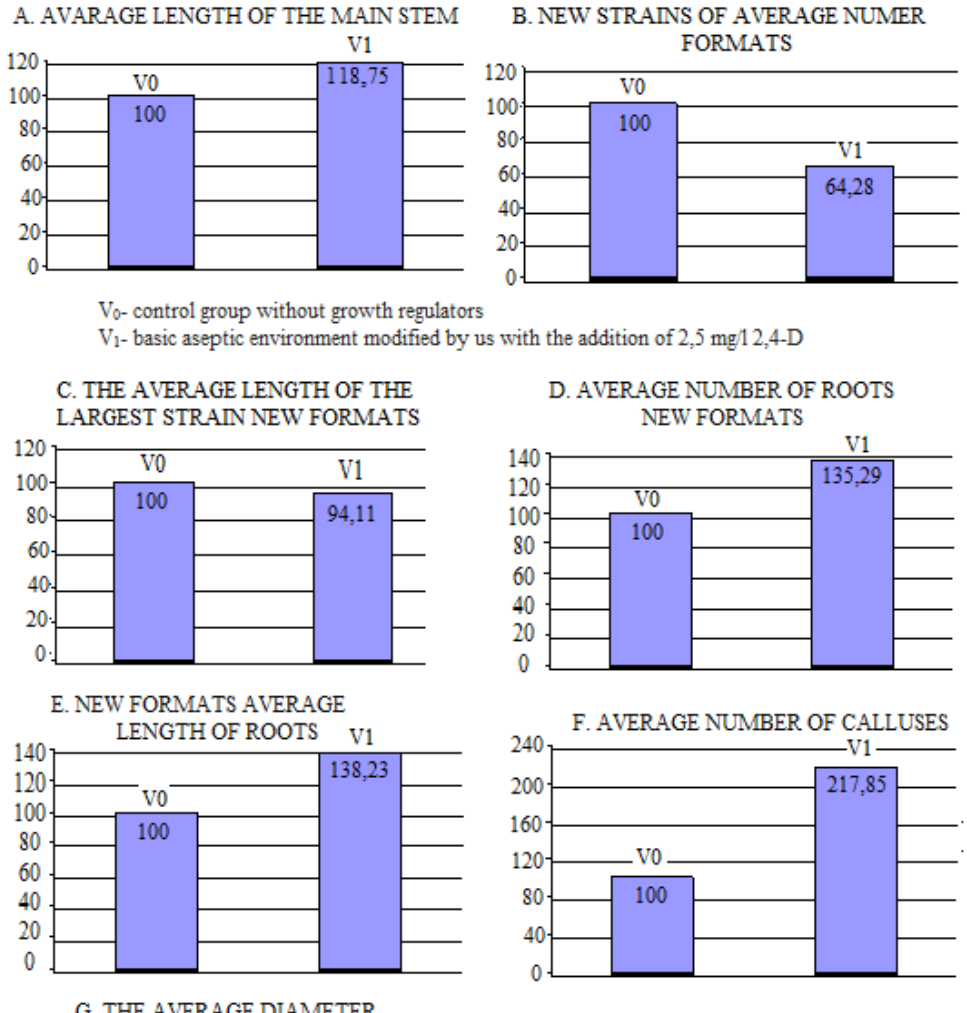


Fig.2. Explente of *Opuntia* (Tournef.) Mill. *fragilis var. fragilis*, 90 days after the inoculation of the explant "in vitro", where: **A**-modified basal medium, the new and lacking growth regulators (V_0); **B, C**-basic medium with the addition of 2,5 mg/l 2,4-D; (iiv-viable initial inoculum; mc-culture medium, nc-strains newly formed; rd-root; ar-areola; sp-thorns; cl-callus; mg -buds).

The average length of the stem prinipale at explentelor variant V_1 (medium supplemented with 2,5 mg/l 2,4-D) was 0,3 cm over the same parameter recorded in the control group V_0 (free environment growth regulators), which represented an increase of 18,75% (Fig. 3A).

At this time the experimental variant V1 (medium supplemented with 2,5 mg/l 2,4-D) registered an average of 0,9 new strains formats / variant, thus



V_0- control group without growth regulators
V_1- basic aseptic environment modified by us with the addition of 2,5 mg/l 2,4-D

Fig. 3. The graphical presentation of average values of parameters corresponding to the track vitro cultures of *Opuntia* (Tournef.) Mill. *fragilis* var. *fragilis*, modified based on new aseptic environment with the addition of 2,5 mg/l 2,4-D (V_1), data expressed as a percentage, obtained by reporting the results achieved biometrizate values to those parameters biometrizaţi the control group (V_0), lacking growth regulators, values considered as 100%; (Where: A-average length of main stem; B-average number of strains newly formed, C-average length of most strains newly formed, D-average number of roots newly formed; E-average length of the largest root newly formed, F-the average number of calli, G-average diameter of the calli).

registering a deficit of 35,72% (Fig. 3B) relative to the same parameter values recorded in the control group V_0. And in terms of the higher average length stems newly formed was recorded minus 0,2 cm of the control V_0 (medium lacking growth regulators), a deficit of 5,89% (Fig. 3C).

Both strains newly formed in the explants cultivated in the medium intake of 2,5 mg/l 2,4-D (V_1) and the formats in media lacking growth regulators (V_0), maintain green color, intensity, and areolas and thorns are normally developed. Note that in the control group (V_0), lacking growth regulators, strains present a much larger number of thorns (Fig. 2A) compared to new strains formed on explants cultured in medium with 2,5 mg/l 2,4-D (V_1) at which the spins - modified leaves - were more rare, but quite long which could be a consequence of the action of "defoliation" that auxin 2,4-D - a herbicide having a defoliant - to be exercised in this case (Fig. 2A and B) also is aware of their level of large numbers of newly formed buds.

The average number of roots generated at explentalor grown on medium supplemented with 2,5 mg/l 2,4-D (V_1) was 0,6 roots/alternative over a parameter value recorded in the control group V_0 (free environment growth regulators), which is an increase of 35,29% (Fig. 3D). The presence of 2,4-D auxin at a concentration of 2,5 mg/l (V_1) in the culture medium had a positive effect and increase in root length, thus the average length of the roots of the newly formed higher - in absolute terms - surpassed the recorded value of this parameter in the control group to 1,3 cm, thus marking an increase of 38,23% (Fig. 3E).

Action 2,4-D added in a concentration of 2,5 mg /l (V_1) stimulated the induction of callus formation from explants of *Opuntia fragilis* var. *fragilis*, and their number is 1,4 calluses/variant, reaching an average diameter of 2,4 cm. Results lead us to consider that, added to the culture medium of 2,5 mg/l 2,4-D (V_1), is sufficient to induce callus explants *Opuntia fragilis* var. *fragilis*, estimates are consistent with those reported by Sandra Aparecida et al., (1996), the cultural *Cereus peruvianus* practiced "in vitro".

If our experiment callus generated from the explants *Opuntia fragilis* var. *fraglis* inoculated and grown in culture medium supplemented with 2,5 mg/l 2,4-D (V_1) or because of the abundance of nutritional covered the entire surface of the substrate (Fig. 2B), where early signs of aging - in fact cream color highlighted or - strains was located at the bottom of the newly formed tissue in the form of a cloudy, brittle, pale green (Fig. 2C).

CONCLUSIONS

1. Vitro cultures after 90 days and it was observed that synthesis of the callus was carried out only on culture medium supplemented with 2,5 mg/l 2,4-D (V_1), which was an increase of 240%.
2. Presence in the culture medium of 2,5 mg/l 2,4-D (V_1) negatively affected the number and length of new strains formats or below values recorded in the control group V_0 explants increased with 35,72% or 5,48%.
3. The number of explants formed roots grown in culture medium supplemented with 2,5 mg/l 2,4-D (V_1), exceeded the value of the same parameter recorded 35,29% in the control group, and the average length of the larger roots also ranged, with 38,23% of the batch control over the V_0.

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REFERENCES

1. Cachiţă C.D., Deliu C., Tican R.L., Ardelean A., 2004, Tratat de biotehnolo-gie vegetală. vol.I, Editura Dacia, Cluj-Napoca, p. 29-154.
2. Casas A., Barbera G., 2002, Mesoamerican domestication and diffusion. In P. S. Nobel [ed.], Cacti: biology and uses, University of California, Berkeley, California, USA, p. 143–162.
3. Copăcescu V.S., 2001, Cactuşii, monografie; Ed. Ceres, Bucuresti, p. 11-517.
4. El Gamri, T., 2004, Prospects and Constraints of Desert Agriculture. Lessons from West Omdurman, Environmental Monitoring and Assessment, vol. 99, p. 57–73.
5. Escobar H.A., Villalobos V.M., Villegas A., 1986, Opuntia micropropagation by axillary proliferation. Plant Cell Tissue Org. Cult., vol. 7, p. 269–277.
6. Garcıa-Saucedo P.A., Valdez-Morales M., Valverde M.E., Cruz-Herna-ndez A., Paredes-Lopez O., 2005, Regeneration of three Opuntia genotypes used as human food. Plant Cell, Tissue and Organ Culture, vol. 80, p. 215–219.
7. Griffith M., P., 2001a, A new Chihuahuan Desert prickly pear, Opuntia x rooneyi. Cactus and Succulent Journal (U.S.A.), vol. 73, pp. 307-310.
8. Heller R., 1953, Rescherches sur la nutrition minérale des tissus végétaux cultives in vitro. Ann.Sci. Nat. Bot. Veg. Ser.,vol. II, p. 1-5.
9. Johnson J., Emino E., 1979a, Tissue culture propagation in the Cactaceae. Cactus and Succulent Journal (U.S.A.), vol. 51, p. 275 - 279.

10. 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 Journal of Sciences, 8(2): 31-35.
11. Kluge M., Ting I.P., 1978, Crassulacean acid metabolism: an ecological analy-sis, Springer-Verlag, Berlin, Germany, Ecological studies**,** vol**.** 114, p. 324–335
12. Murashige T., Skoog F., 1962, A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, vol. 15, p. 473 –497.
13. Nobel P.S., 1994, Remarkable agaves and cacti. Oxford University Press, New York Environmental biology, p. 36-48.
14. Pinkava D.J., 2002, On the evolution of continental North American Opuntioideae. Succulent Plant Research, vol. 6, p. 59-98.
15. Rubluo A., Reyes J., Rodriguez-Garay B., Pimienta-Barrios E., Brunner I., 1996, Métodos de propagación biotecnológicos y convencionales en cactáceas para zonas áridas. In: Técnicas Convencionales y Biotecnológicas para la Propagación de Plantas de Zonas Áridas, J Izquierdo, G Palomino (eds). Santiago, Chile, vol 9, p. 345.
16. Sandra Aparecida O., Silva Machado M.F.P., Claudete Aparecida M.A. J.P., 1996, Micropropagation of Cereus peruvianus mill. (Cactaceae) by areole activation. In Vitro Cellular & Developmental Biology – Plant, Springer Berlin/ Heidelberg, vol. 32, nr. 3, p. 47-50.
17. Smith R., Burdick P., Anthony J., Reilley A., 1991, In vitro propagation of Coryphantha macromeris. HortScience, vol. 26, nr. 3, p. 315.