COMPARATIVE STUDY REGARDING THE REGENERATIVE AND ORGANOGENIC CAPACITY OF EXPLANTS OF Aylostera heliosa, CULTIVATED "IN VITRO" IN THE PRESENCE IN THE SUBSTRATE OF THE AIB (3-INDOLEBUTYRIC ACID) AND 2,4-D (2,4-DICHLOROPHENOXYACETIC ACID)

Vidican Iuliana Teodora*, Cărbunar Mihai, Vidican Oana Maria

*University of Oradea, Faculty of Enviromental Protection,11 Borsecului St., 410571 Oradea, e-mail: iuliateodora68@yahoo.com

Abstract

Aylostera heliosa, decorative cactus both the port and the flower is a difficult species propagated by grafting, but has found a viable method of multiplication in vitro micropropagation. For this cactus can be successfully acclimatized need to develop a strong root system.

In order to establish the in vitro culture we harvested buds from Aylostera heliosa stems. Inoculation of the explants was done on a culture medium consisting of macro-elements and Fe EDTA Murashige-Skoog (1962), Heller micro-elements (1953), supplemented with 1,5 mg/l auxin, respectively, AIB (3-indolylbutyric acid) and 2,4-D (2,4-dichlorophenoxyacetic acid).

The evolution of the explants was monitored for 90 days. The response of Aylostera heliosa explants to the presence in the culture medium of an equal amount of auxin, respectively 1,5 mg/l AIB and 1,5 mg/l 2,4-D, was very different; rhizogenesis was observed only in Aylostera heliosa grown on culture medium supplemented with 1,5 mg/l AIB (V_1), the largest and largest strains were recorded in plants grown on culture medium supplemented with 1,5 mg/l 2,4-D (V_2) also callus formation.

Key words: cacti, vitrocultures, 3 indolylbutyric acid, 2,4-dichlorophenoxyacetic acid, rootedness.

INTRODUCTION

Aylostera heliosa, cactus, decorative (Fig. 1) both in port - due thorns silvery-white edge aligned comb (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 rapidly and effectively by micropropagation in vitro (Karimi et al., 2010).

Cacti are considered to be extremely susceptible to the differentiation process when they are grown in mineral environments rich in growth regulators (Copăceacu, 2001), invariably inducing organogenesis processes.

In this experiment, our aim was to study the reactions of the phytoinoculi of *Aylostera heliosa* to the existence in the culture medium of an auxin, respectively, of 3-indolylbutyric acid (AIB) and 2,4-dichlorophenoxyacetic acid (2,4-D), added at the same concentration of 1,5 mg/l. We thus obtained the following variants: V_0 or control group (medium without growth regulators), V_1 with 1,5 mg/l 3-indolylbutyric acid (AIB) added and V_2 with 1,5 added mg/l 2,4-dichlorophenoxyacetic acid (2,4-D).

Fig.1. *Aylosteraheliosa*stems andflowers

Auxins are phytohormones or growth regulators commonly used in tissue cultures, having stimulatory action on rhizogenesis, considering that the cumulative effect of endogenous auxin with the input of exogenous auxins, results in the greatest number of roots (Juárez et al., 2002). 3-Indolylbutyric acid (AIB) is part of the synthetic auxin class, but it seems to be found in nature, but only in some plant species (according to Moore, from Cachiţă et al., 2004).

Among the synthetic auxins, 2,4-dichlorophenoxyacetic acid (2,4-D) is also included, this being the most used and recommended growth regulator that stimulates the generation of calus in explants. According to Johnson et al. (1979b), callus formation was observed in vitrocultures of *Mammillaria elongata* by addition of auxin 2,4-D in the culture medium.

MATERIAL AND METHOD

In this experiment to order to initiate the Aylostera heliosa in vitro culture, the plant material consist ed from youngs tems harvested from mother plants. The materialwas sterilized byplacingforoneminute, in alcohol 96º, followed by asubmersionoperation, inasodiumhypochlorite solution0,8% in proportionof1:2with water(one partsodium hypochlorite, 2partssterile water), which hwereadded threedropsofTween20, shaking continuously (Cachiţă et al., 2004). After 20 minutes, the removal of disinfectant agent was achieved by washing the plant material insterile water, in five consecutive rinses, of five minutes each, after which the plant material was deposited on aseptic filter paperrings, introduced in sterile Petri dishes. Sizing future inocula was performed under aseptic condition sin horizontal laminar flowhood, with sterile air. Young stem were cut into spherical slices, which has the following dimensions: about 1 cm long,0,5cm thi kanda diameter of 0,5-1,5 cm, depending on the area from

which they were harvested. Explantsmodeling(Fig. 2) were done so that each has at least 2-3areolae(Karimi et al., 2010).

The mineral medium culture used in the experiment consisted of: microelements 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, 20g/l sucrose and 7g/l agar-agar, pH of the medium was adjusted to a value of 5,8.

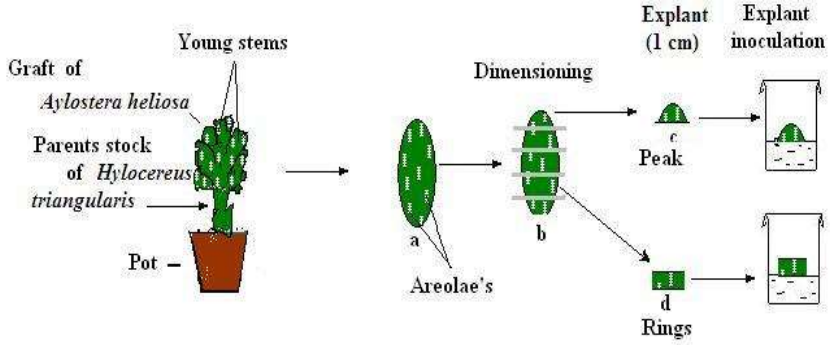

Fig. 2. Schematic representationof sectioning method of theyoung step to obtain *Aylostera*(Speg.)*heliosa*explants(where: a-young strain, b-sizing of youngstems, c-explantrepresentedfromyoungstemd-explant represented as spherical rings)

In order to obtain the proposed alternatives, we added new developed nutrient medium devoid of growth regulators (V_0), version control, and variant V_1 to which 1,5 mg/l 3-indolylbutyric acid (AIB) was added and variant V_2 to which 1,5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) were added. .

Sterilization of vialswith medium wasperformedby autoclavingata temperature of 121°C for 30 minutes. The recipients with medium culture 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 laminar flow, 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.

Vitro plantlets reaction after inoculation was monitored for 12 weeks. Biometric assessments were taken at intervals of 30 days.Observations consisted from biomeasure: vitro plantlets leng regenerated from explants,number of callus formation, determining the number of neo stems and branches developed on the initial inocula.

RESULTS AND DISCUSSION

At 90 days after the initiation of the experiment, the absolute value of the basal mean diameter of the Aylostera heliosa strains remained constant, respectively 1,3 cm (Fig. 3A), in the phytoinoculars belonging to the control group V_0 (medium lacking growth regulators) as well as those grown on mediums supplemented with 1,5 mg/l AIB (variant V_1) and 1,5 mg/l 2,4 D (variant V_2).

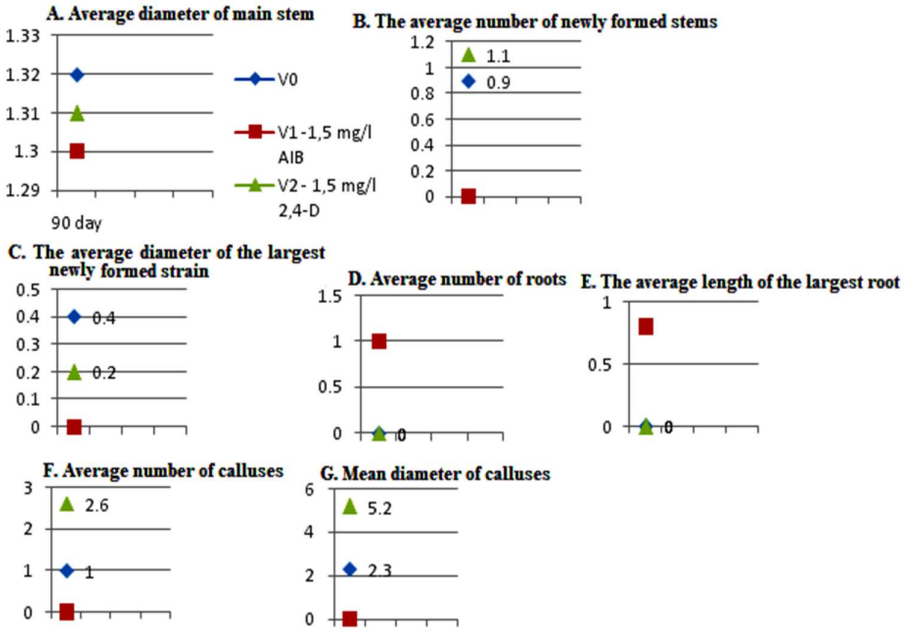

Fig. 3. Graphical presentation of the average values corresponding to the biometric parameters in the viticulture cultures of Aylostera (Speg.) Heliosa, on the aseptic base modified by us - (V0 variant) - with 1.5 mg / l AIB (V1 variant) and 1, 5 mg / l 2.4 D (variant V2), data expressed in absolute values; (where:A - Average diameter of main stem; B - The average number of newly formed stems; C - The average diameter of the largest newly formed strain; D-Average number of roots; E - The average length of the largest root;F-Average number of calluses; G-Mean diameter of calluses).

Analyzing the tracked data it is found that inoculated and raised explants on mediums supplemented with 1,5 mg/l AIB (variant V_1) did not generate strains, these being generated on explants belonging to variant V_2 (medium supplemented with 1,5 mg/l 2, 4-D) wave with 0,2 buds/variant (Fig. 3B) compared to the same parameter recorded in control V_0 (0,9 buds/variant), represents an increase of 22,22%.

Regarding the basal average diameter of the newly formed strains, we notice new shoots generated at the level of explants inoculated and raised on a medium lacking growth regulators (V_0), which with a value of this parameter of 0,4 cm (Fig. 3C) they marked a 50% increase (Fig. 3C), compared to the values recorded in the explants grown on the medium supplemented with 1,5 mg / l 2,4-D (V_2).

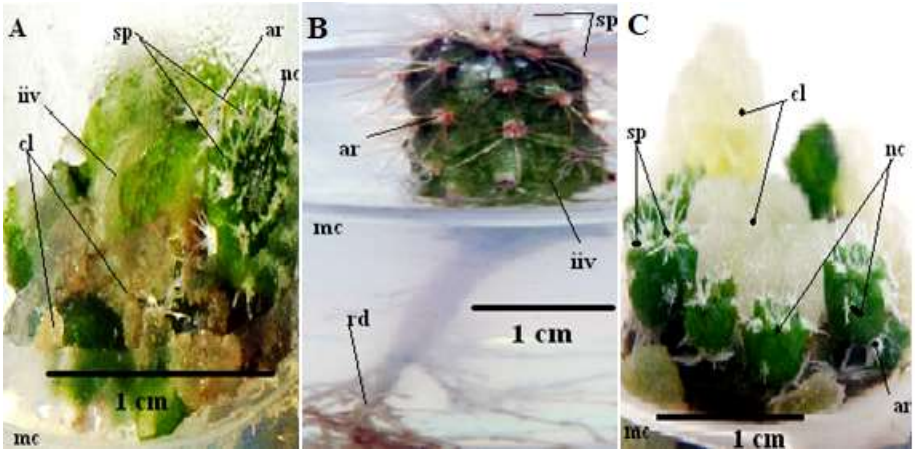

Fig. 4. Inoculates of *Aylostera (Speg.) heliosa*, 90 days after inoculation of explant "in vitro", where: A-on basic medium modified by us and lacking growth regulators (V_0); B-on basic medium with addition of 1,5 mg/l AIB (variant V_1); C-on basic medium with 1,5 mg/l 2,4-D (V_2) addition; (iiv-inoc initially viable; nc-newly formed stems; ar-areola; sp-thorn; cl-callus; mc – culture medium).

Regarding the rhizogenesis, this phenomenon was manifested only in the phytoinoculi belonging to variants V_1 (medium supplemented with 1,5 mg/l AIB), the average number of roots/variant being 1. In this case, due to the species characteristics, *Aylostera heliosa* has a pivoting root system, the newly formed roots, unlike those generated in explants of *Opuntia fragilis var. fragilis* grown on the same culture medium, are much thicker (Vidican, 2012).

The addition of 1,5 mg/l AIB (V_1) in the culture medium exerted a stimulatory effect on root growth (Vidican, 2013), the value of the average length of the longest root - in this case - was 0,8 cm.

Calus induction did not occur in *Aylostera heliosa* explants grown on medium supplemented with 1,5 mg/l AIB (variant V_1), but it is noted that in explants belonging to variant V_2(medium supplemented with 1,5 mg/l 2,4-D) the average number of calluses/variant is 1,6 (Fig. 3D) higher than the values of the same parameter registered in control group V_0 (average lacking growth regulators), which in percentage values represents an increase of 160% (Fig. 3D). And as for the average diameter of the calusus,

the highest values were also recorded in explants inoculated and raised on medium supplemented with 1,5 mg/l 2,4-D (V2), which exceeded control V_0 by 2,9 cm (Fig. 3E), which represents an increase of 126,1% (Fig. 3E).

The callus generated from the explants cultivated on a medium lacking growth regulators is located on the surface of the explant but also on the level of the culture medium, it shows signs of early senescence, a fact indicated by its creamy color or even its light brown (Fig. A). At the level of explants inoculated on culture medium supplemented with 2,5 mg/l 2,4-D (V_2) the callus was friable, snow-white (Fig. 4B), and due to abundance it covered the entire surface of the culture medium. (Vidicanet al., 2011).

It should be noted that although characteristic of *Aylostera heliosa* is the comb-like alignment of the marginal spines that are white - silver, with a thickened basal area and a dark brown color, in the case of phytoinocles grown on culture medium supplemented with 1,5 mg/l AIB (V_1), the central spines were bristled, long and reddish, depending on the characteristic aspect of the species (Fig. 4C and D). In the case of phyto-inoculants grown on culture medium without growth regulators (V_0), the spines maintained their characteristics in terms of shape, size and color (Fig. 4A).

These results allow us to assume that the presence in the culture environment of a certain amount of growth regulator enhances the great morphological plasticity of the catfish *Aylostera heliosa*. It should be mentioned that, as regards the characteristics of spines in phytoinoculi belonging to variants V_1 (medium supplemented with 1,5 mg/l AIB), they are specific to the traits of the genus *Aylostera*, in which the central or marginal spines are very different from one species to another, in terms of shape (needles, straight, curved, etc.), their length (0,1-2,0 mm), areola position (seamed, combed in alignment) or their color (from transparent white to black) , passing through a wide range of shades of the other colors).

CONCLUSIONS

1. Comparing the data obtained from the evaluation of the evolution of *Aylostera heliosa* explants at 90 days of viticulture, we noticed that, by supplementing the culture medium with 1,5 mg/l auxin, respectively AIB (variant V_1) and 2,4 D (variant V_2) there were significant differences in the response of phytoinoculi to the composition of the nutritional environment. Thus, explants cultivated on a medium lacking growth regulators (V_0) were noted by regeneration of new stems and by induction of callus, but did not form roots.

2. The number of newly formed strains at the level of explants grown on culture medium supplemented with 1,5 mg/l 2,4-D (V_2), was higher than the values registered by the same parameter in control group V_0 (medium

lacking growth regulators), by 22,22%, while their average basal diameter was 50% smaller than in the control.

3. The presence in the culture medium of 1,5 mg/l AIB (V_2) was sufficient to stimulate the rhizogenesis, in an average number of 1,0 root / variant that reached an average length of the largest root 0,8 cm.

4. The presence in the culture medium of 1,5 mg/l 2,4-dichlorophenoxyacetic acid (V_2), favored the induction of calus, so after 90 days of viticulture in their level there is a 160% increase in the average number of calusis/variant and an increase of 126,1% of the value of the average diameter of the calusus compared to those grown on medium without growth regulator (V_0).

REFERENCES

1. Cachiţă C.D., C. Deliu, R.L. Tican, A. Ardelean, 2004, Tratat de biotehnologie vegetală. Vol.I, Editura Dacia, Cluj-Napoca, p. 29-154.
2. Cachiţă C.D., A. Ardelean, 2004, Vitroculturile vegetale în fitopatologie. In: Fiziologia celulei vegetale în regim de vitrocultură. Al XII-lea Simpozion National de Culturi de Tesuturi si Celule Vegetale, Jibou 5, Ed.Daya, Satu Mare, p. 18-29.
3. Copăcescu V.S., 2001, Cactuşii, monografie; Ed. Ceres, Bucuresti, p. 11-517.
4. Karimi N, Mofid MR, Ebrahimi M, Naderi R. 2010, Efect of areole and culture medium on callus induction and regeneration Cereus peruvianusMill. (Cactaceae), Trakia Journal of Science 8: 31–35.
5. 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.
6. 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.
7. Juarezi, M.C., Passera C.B., 2002, In vitro propagation of Opuntiaellisiana Griff. and acclimatization to field conditions. Biocell, vol. 26, p. 319–324.
8. Johnson J., Emino E., 1979b, In vitro propagation of Mammillaria elongate. HortScience, vol. 14, nr. 5, p. 605 – 606.
9. Medeiros L., R. Ribeiro, L. Gallo, E. Oliveira, M. Demattê, 2006; In vitro propagation of Notocactus magnificus. Plant Cell, Tissue and Organ Culture, Springer, vol. 84, nr. 2, p. 100147-100151.
10. Murashige T., F. Skoog, 1962, A revised medium for rapid growth and bioassays with tobacco tissue cultures. PhysiologiaPlantarum, vol. 15, p. 473 –497.
11. Myeong I.J., Ghang-Hui C., Jung-Myung L., 2004, Production and Breeding of cacti for grafting in Korea. Chronicahorticulturae, Korea, vol. 44, nr. 3, p. 7-10.
12. Pérez E., Pérez M., Davila C., Villalobos E., 2002, In vitro propagation of three species of columnar cacti from the Sonoran Desert. Hortscience, vol. 37, nr. 4, p. 693 – 696.
13. Vidican I.T., Urdea O., Study on the regenerative capacity and organogenrsis of Aylostera (Speg.) heliosa explants, in the presence of 2,4 - dichlorophenoxyacetic acid (2,4-D) IN culture medium, 2011, AnaleleUniversităţii din Oradea, Fascicula Protecţia Mediului, Simpozion Internaţional, Vol. XVII, ISSN: 1224-6265, p. 397-405.

14. Vidican I.T., Studiens on the influence of acid contraction – β indolilbutiric (IBA) on regenerative capacity and organogenesis of explants of Opuntia (Tournef.) Mill. fragilis var. fragilis,, 2012, Analele Universităţii din Oradea, Fascicula Protecţia Mediului, Vol. XIX, anul 17, ISSN: 1224-6265, p. 312 – 319.
15. Vidican I.T., Study on the influence of 3 indolilbutiric (AIB), added in different concentrations in the culture medium, the regeneration capacity of explants Aylostera (speg.) heliosa, 2013, Analele Universităţii din Oradea, Fascicula Protecţia Mediului, Simpozion Internaţional, Vol. XX, anul 18, ISSN: 1224-6265, p. 72-85.