STUDY ON THE REGENERATIVE CAPACITY AND ORGANOGENIC OF *Echinopsis* (Zucc.) *chamaecereus* f. *lutea* EXPLANTS, IN THE PRESENCE OF 2,4 - DICHLOROPHENOXYACETIC ACID (2,4-D) IN CULTURE MEDIUM

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Abstract

Cactus with yellow skin, Echinopsis chamaecereus f. lutea, is part of a cactus chlorophyll - deficient, which occur spontaneously in culture because of mutation, they are unable to synthesize chlorophyll only survive if they are grafted.

In order to establish a culture in vitro of Echinopsis chamaecereus f. lutea, we have taken explants represented by seedlings from mother plants grown in the greenhouse. Inoculation of explants I did it on a culture medium consisting of macro Murashige-Skoog EDTA and Fe (1962), micronutrients Heller (1953), supplementation with medium supplemented with 2,5 mg/l 2,4 - dichlorophenoxyacetic (2,4-D).

Explants evolution was monitored for 90 days. Echinopsis chamaecereus f. lutea explants the presence in the culture medium 2,5 mg/l of 2,4 - dichlorophenoxyacetic (2,4-D) demonstrated the beneficial effect of auxins on the generation of callus, finding a plus 66,66%, and in terms of their size, this parameter marked an increase of 140% compared to control group values V_0 (medium lacking growth regulators). The remercat that in this experiment, the rootedness not occurred.

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

INTRODUCTION

Echinopsis chamaecereus f. lutea is a cactus chlorophyll - deficient, with yellow skin (Copăcescu, 2001), deprived of the opportunity to synthesize chlorophyll chloroplasts due to the small, about 1/3 of all plastids (Shemorakov, 2003).

The process of discoloration is caused by spontaneous mutations in culture (Shemorakov, 2001) greatly influenced by temperature and light. After Skulkin (2000) plants maintained at a temperature lower than normal and shadow, growing slowly, if at all, such mutations. Russian scientists showed great interest in the species chlorophyll - deficient cactus, so they made their classification based on the color of skin (Shemorakov, 2003), that, *Echinopsis chamaecereus f. lutea* is part of a single color.

After Shemorakov (2001) reversible plastid mutation during meiosis makes the reproduction generation to *Echinopsis chamaecereus f. lutea* have little chance for it to retain color (Kornilov 2008). thus it concluded that plants can retain this particular property only reproduced by cloning.

2,4 - dichlorophenoxyacetic or (2,4-D) is an auxin with a high efficiency in the formation of callus and rootedness. In moderate concentrations, it stimulates cell division at the bill, but becomes toxic at higher concentrations. The callus culture, ensure high friability auxin, facilitating separation of cells in cell suspensions and somatic embryogenesis.

The purpose of the experiment subject of this article is to analyze the variability of reaction vitro cultures of *Echinopsis chamaecereus f. lutea* in the culture medium in the presence of 2,5 mg/l 2,4 - dichlorophenoxyacetic (2,4-D).

MATERIALS AND METHODS

Biological material used in our experiments consisted of seedlings regenerated strains *Echinopsis chamaecereus* f. *lutea* (figure 1). The explants were about 1 cm long, 0,5 cm thick and a diameter of 0,5-1,5 cm, depending on the area which was harvested (figure 2).

The plant material, seedlings of *Echinopsis chamaecereus* f. *lutea* was sterilized by introducing, for one minute, 96º alcohol, followed by coating with sodium hypochlorite solution 0,8%, mixed with water in a ratio of 1:2; in a disinfecting solution being added - as a surfactant - three drops of Tween 20 (Cachiță et al., 2004).

Fig.1. The plant *Echinopsis chamaecereus f. lutea* young, grown in greenhouses (where: a-graft; b-rootstock; c-formations caulinare)

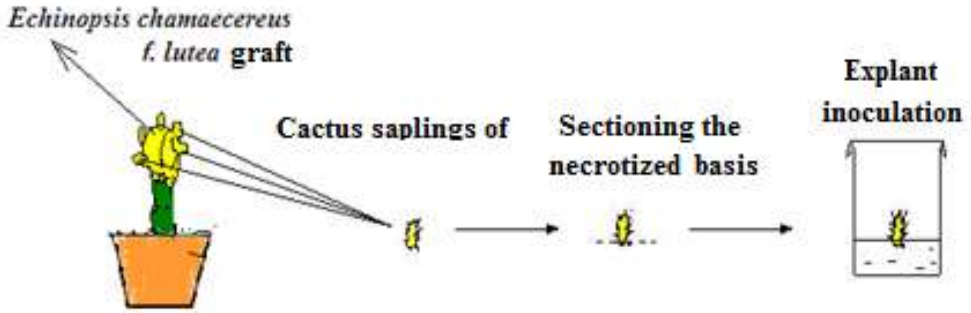

Fig. 2. Schematic representation of how operating fragments *Echinopsis chamaecereus f. lutea* to be inoculated aseptic environments.

During sanitizing vegetative material was stirred continuously (Cachiță et al., 2004). After 20 minutes he proceeded to remove the disinfectant agent and went over to the washing plant material with sterile water, making five rinses to five minutes each. Then, the plant material was deposited under aseptic conditions in horizontal laminar flow hood, sterile air, in operation, the filter paper rings sterilized in the oven intodusă in petri dishes, aseptic. Next, it proceeded to posting necrotized parts of future inoculate.

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. In basic medium (MB) I added a concentration of 2,5 mg/l 2,4-D getting two experimental variants, namely:

- V_0 - version control, medium without growth regulators and
- V_1 - medium supplemented with the addition of 2,5 mg/l 2,4-D.

Culture medium thus obtained was placed in a glass vial with a capacity of 15 ml (each container was placed 5 ml of medium). Medium vials were sterilized by autoclaving for 30 minutes 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 were inoculated Transferred to room for growth, under the Following Conditions: temperature ranged from 24ºC in the range of light and 20°C during the phase of darkness and light was the regime fotoperiodic 16 hours with light/24h,

lighting Achieving cultures with the white light emitted by fluorescent lamps, the intensity of 1700 lux.

Explants and explants reaction progress was monitored for 90 days. In this time period were conducted periodic observations and readings every 30 days. Values thus obtained in the 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

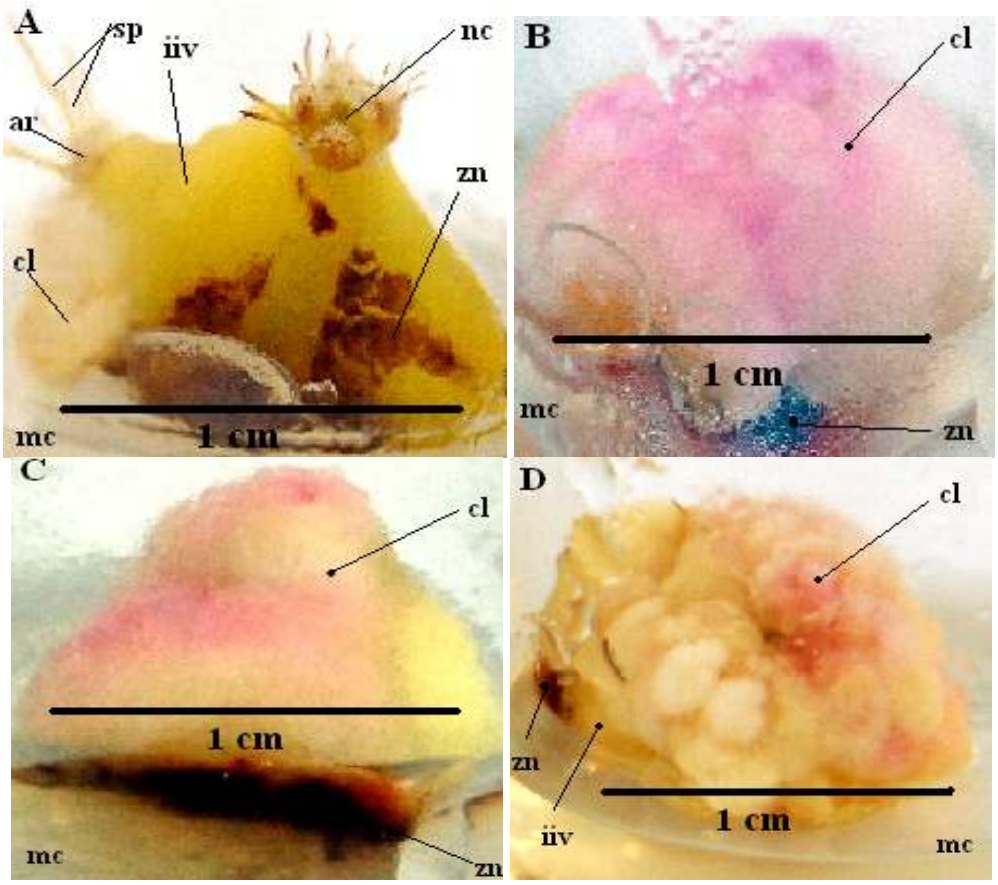

Fig.3. *Echinopsis* (Zucc.) *chamaecereus* f. *lutea* inoculum, 90 days after inoculation explant "in vitro", where: A-basic medium-changed us and devoid of growth regulators (V_0); B, C, D, the basic medium with the addition of 2,5 mg/l 2,4-D (V_1); (iiv-initial inoculum viable; nc-younger stems; mc-culture medium; sp-thorns would; ar-the areola; cl-callus; zn-area necrosis).

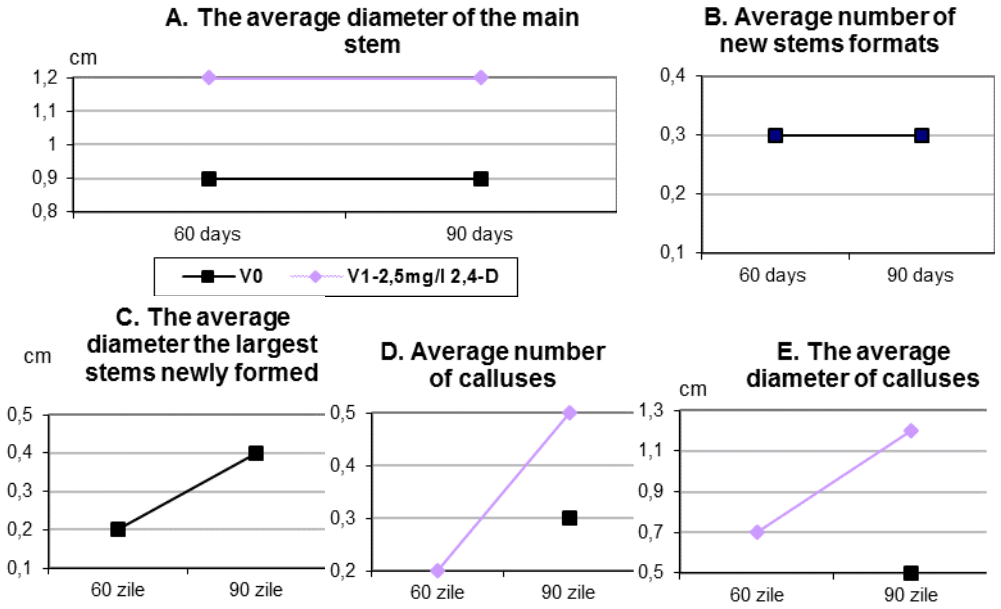

Fig. 4. Graphical representation of average values corresponding parameters of *Echinopsis* (Zucc.) *chamaecereus* f. *lutea* followed in vitro cultures, the new modified basic aseptic environment - (variant V_0) - with the addition of 2,5 mg/l 2,4-D (variant V_1), data expressed in absolute terms; (where: A-the average diameter of the main stem; B-average number of strains young; C-average diameter of the largest young stems; D- the average number of calluses, E-average diameter of the calluses).

After 90 days of initiating the current experiment basal medium diameter of the main stem was maintained at values recorded in the previous reading (fig. 4A).

The newly formed buds average number of explants inoculated control group remained unchanged, 0,3 buds/variant (fig. 4B), only their diameter increased media this parameter reaching 0,4 cm (fig. 4C) .

At this time both experimental groups generated callus, but the presence in the culture medium to 2,5 mg/l 2,4-D (V_1) has boosted its formation a greater extent; thus an average number of 0.5 calluses / embodiment (fig. 4D) to the explants grown on this substrate has been an increase of 66,66% (fig. 5B), while the average diameter of the callus (measured at the the wide) to 1,2 cm (fig. 4C), thereby finding an addition of 140% (fig. 5C). These results are consistent with those reported by Medeiros et al. (2006), which *Notocactus magnificus* grown in medium supplemented with 0,5 mg/l 2,4-dichlorophenoxy acetic acid obtained massive formation of callus.

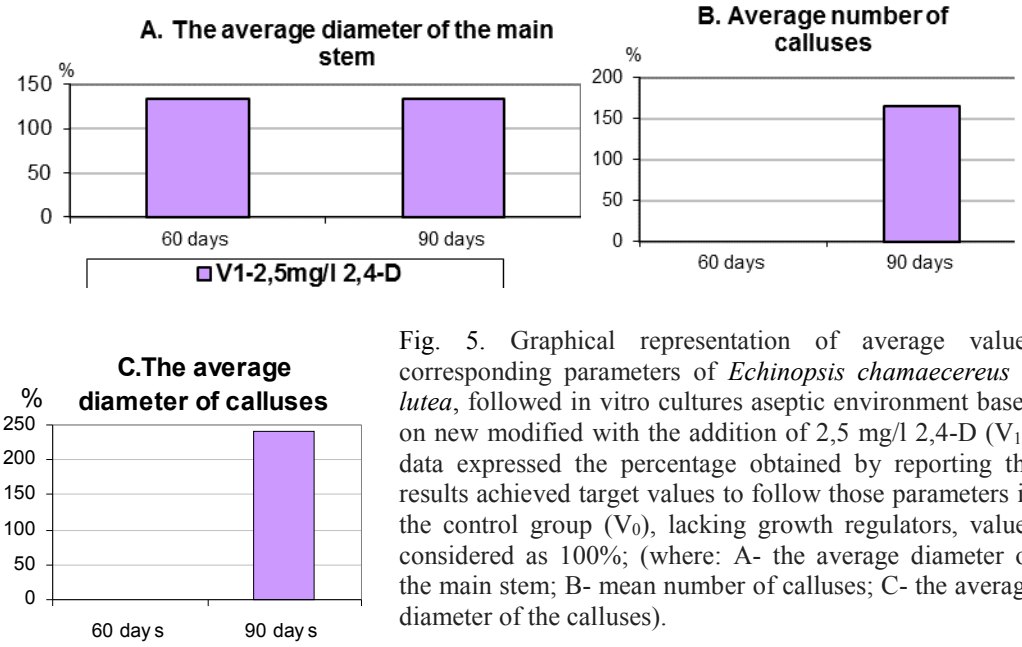

Fig. 5. Graphical representation of average values corresponding parameters of *Echinopsis chamaecereus* f. *lutea*, followed in vitro cultures aseptic environment based on new modified with the addition of 2,5 mg/l 2,4-D (V_1), data expressed the percentage obtained by reporting the results achieved target values to follow those parameters in the control group (V_0), lacking growth regulators, values considered as 100%; (where: A- the average diameter of the main stem; B- mean number of calluses; C- the average diameter of the calluses).

By analyzing images of figure 3 is noted that the level of *Echinopsis chamaecereus* f. *lutea* explants belonging variant V_0 (medium lacking growth regulators), formed callus is located on the surface thereof and has a creamy-yellowish; also areolas and thorns are normally developed special features. Noteworthy is the fact that, besides the existence explant surface necrotic areas there are colored in shades of green surfaces, actually found and the newly formed sprouts (Vidican et al, 2009).

In the explants inoculated and grown on medium supplemented with 2,5 mg/l 2,4-D (V_1) has developed a callus optically dense, compact, velvety, located at the base or on the explant, covering almost entirely and nutrient substrate; This is different color from white, yellow, pink, or shades of orange (fig. 3 B, C and D), probably due to that in the case of in vitro culture of *Echinocactus mihanovichi* on the same type of nutrient medium contents high anthocyanin and species (Vidican, 2012). Another similarity between the two species lacking chlorophyll, as of necrosis in the contact area with the culture medium (Vidican, 2012).

CONCLUSIONS

1. After 90 days of in vitro culture initiation *Echinopsis chamaecereus* f. *lutea* found that supplementation of culture medium with 2,5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) (V_1), had only beneficial effects on the generation of callus in both the number of calluses/variant, where it was found a 66,66% addition, and with regard to their size, this parameter marking an increase of 140% on the V_0 control group values (environment without growth regulators).
2. To be noted that explants belonging V_0 control group (medium lacking growth regulators) have remarked both by caulogenesis - a phenomenon manifested itself in the first 60 days of experiment - and by generation of callus.
3. Rootedness phenomenon not seen in any of the variants studied experiments

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