INVESTIGATION ON THE OF 2,4 – DICHLOROPHENOXYACETIC ACID (2,4-D) ON THE PROCESS OF CALLUS FROM IN VITRO CULTURES *Echinocactus* (Pfiff.) *mihanovichii*

Vidican Iuliana Teodora*

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

Abstract

Hostility is offset sharp spikes in *Echinocactus mihanovichii*, cactus chlorophyll-deficient, not only by tenderness and beauty of the flowers that fascinates the viewer and totally unexpected appearance of skin coloring. This last feature is kept only by cloning. Thus the establishment of in vitro cultures of *Echinocactus mihanovichii*, we harvested coastal portions that have fragmented so that each explant hold 3-4 areola. The culture medium used was composed of macronutrients, Murashige-Skoog (1962) with the addition of growth regulators, micronutrients Heller (1953), supplemented with 2.5 mg / l 2,4 - dichlorophenoxyacetic acid (2,4-D) .

Vitroculturilor progress was monitored for 90 days. At the end of the experiment proved *Echinocactus mihanovichii* explants reaction was different in their level yielding, depending on the composition of the culture medium or buds or callus, rootedness is absent.

Keywords: cacti, vitrocultures, 2,4 - dichlorophenoxyacetic acid, callus, buds.

INTRODUCTION

Echinocactus mihanovichii plant native to Paraguay (Copăcescu, 2001), is part of chlorophyll-deficient cacti. It is a cactus with red skin with exceptional decorative qualities, being highly appreciated appearance of spectacular. The pigmentation is caused by the appearance of spontaneous, in cultures of mutations largely influenced by temperature and light (Shemorakov, 2003). Due to reversible mutation plastids during meiosis (Shemorakov, 2003) by generative reproduction chances that these plants to keep the color is minimal (Kornilov, 2008), thus it was concluded that plants can retain color only reproduced by cloning. This has led, as now, to seek new technologies for rapid multiplication as economically efficient these plants (Son, 2000, Lee et al., 2003).

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.

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 mihanovichii Echinocactus.

MATERIAL AND METHODS

Disinfection plant material was achieved by submersarea explants for one minute in 96° alcohol, after which they were coated with a solution of 0.8% sodium hypochlorite mixed with water in a ratio of 1:2, and the surfactant is added three drops of Tween 20. During this operation, which was 20 minutes, 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.

Plant material, such disinfect was deposited on filter paper discs (previously sterilized in the oven) in Petri capsules in a laminar flow hood, horizontal air sterile operation, operation followed by inocula future size and removing necrotic parts thereof. Knowing that if in vitro cultures of cactus induction of roots, shoots or callus, gives the best results if you use large explants, which have at least three areola (Dabenkausse et al., 1991), where.

The experimental current in inside the laminar flow hood, horizontal air sterile in operation, we cut along the ribs strain (longitudinal) (Fig., a). After this operation we obtained a fragment of strain in a semicircle (which have side with nipples) I removed tissue from the middle portion leaving to experts about 0.7 to 1 cm parenchymal tissue (Fig., b), and moved to portioning future inoculated explant so that each hold 3-4 areola, and sizes to fit into already established (Fig., c).

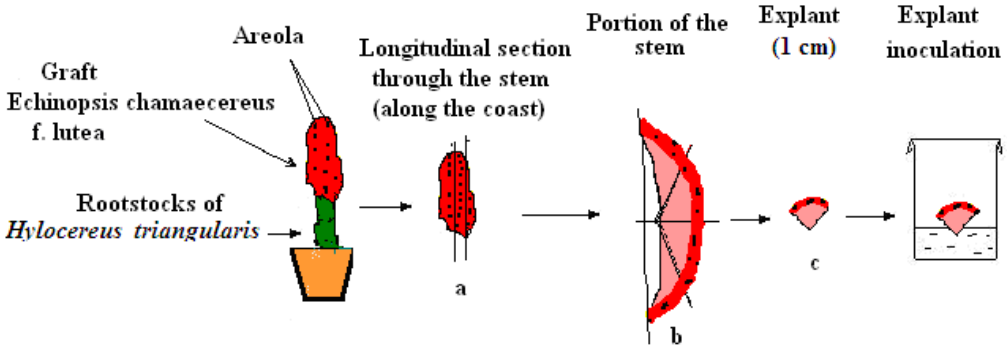

Fig. 1. Explantare schemetică representation of how the buds and fragments of Echinocactus (Pfiff.) mihanovichii and slicing their ribs to obtain inoculum.

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

At this time it can be seen that the growth of explants of *Echinocactus mihanovichii* is different depending on the composition of the culture medium. An accelerated growth stands in explants inoculated and grown on medium without growth regulators (V_0) that after 90 days of culture in vitro, have a basal medium dimetru the main stem of 1.1 cm (Fig. 3A).

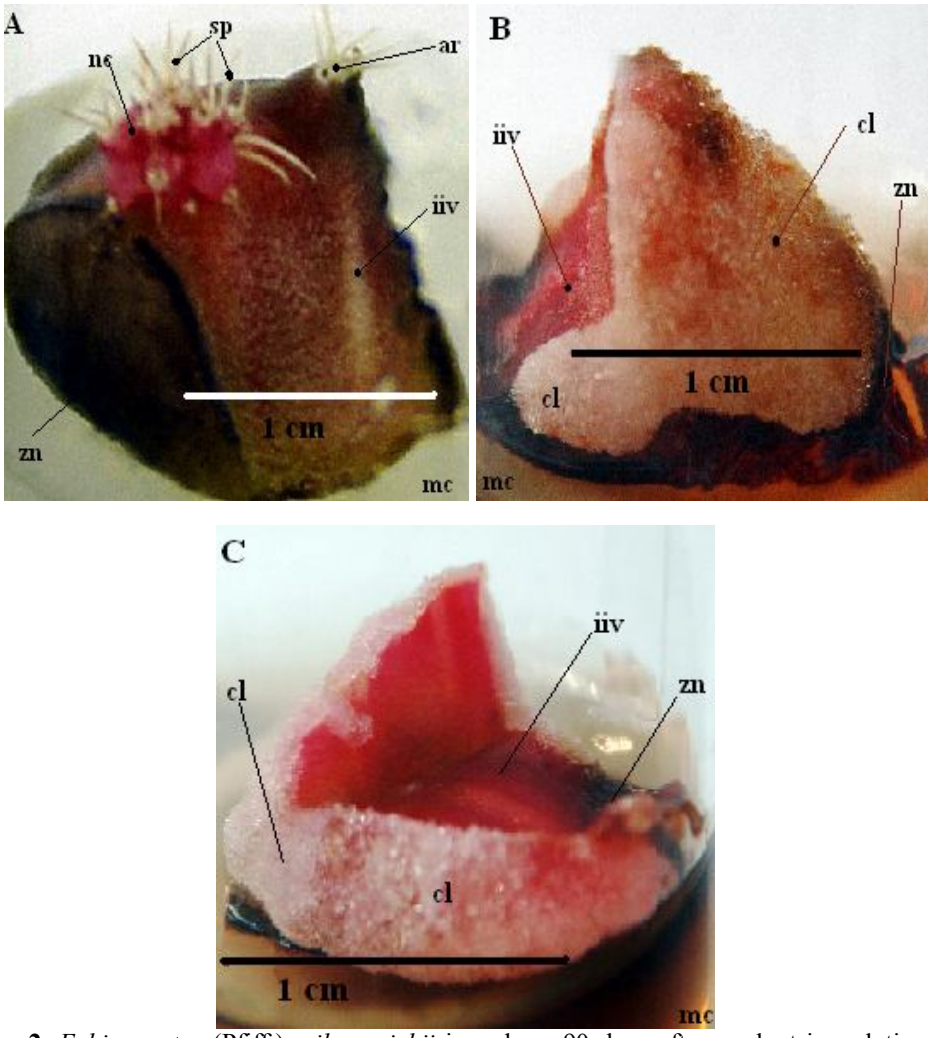

Fig. 2. *Echinocactus* (Pfiff.) *mihanovichii* inoculum, 90 days after explant inoculation in vitro, where A, the new modified basic medium without growth regulators (V_0), B, C, the average basic with addition of 2.5 mg / l 2,4-D (V_1) (iiv-viable initial inoculum, culture medium mc, nc-neoformation caulinară, sp-thorns; would-areola, cl-gagging, zn-necrotic area).

In terms of morphogenetic response in this time, in none of the experimental variants studied phenomenon not seen rootedness, while the explants belonging variant V0 (medium without growth regulators) has been generated bud. Note that the newly formed buds kept the red color of the mother plant, which could enable multiplication cactus *Echinocactus mihanovichii* with them and shape and spin characteristics (fig. 2A).

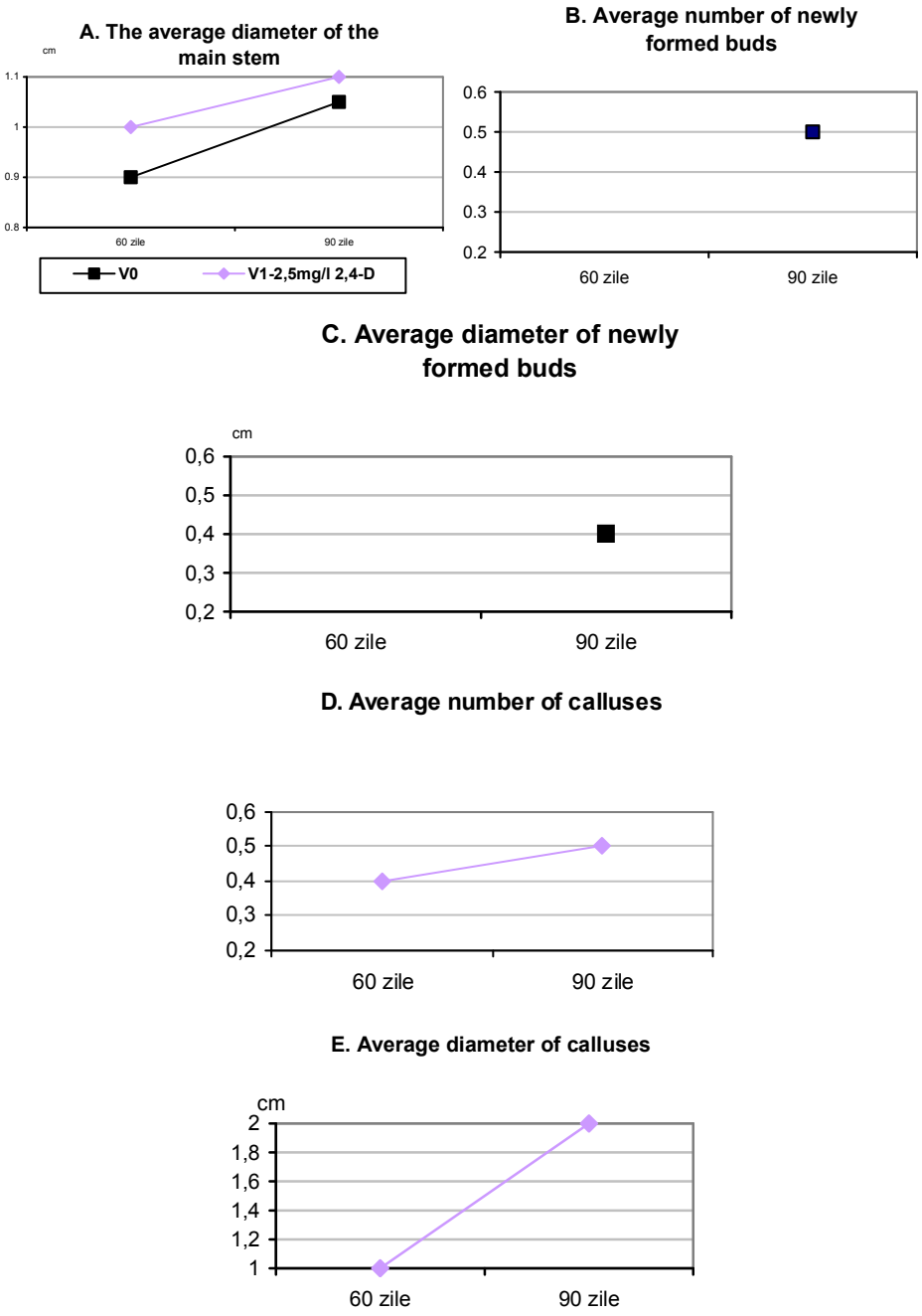

Fig. 3. Graphical presentation of mean values corresponding to the parameters biometrizați *Echinocactus* (Pfiff.) *mihanovichii* cultures in vitro on basic aseptic environment changed us - (V0 version) - with the addition of 2.5 mg / l 2,4-D (V10) data denominated in absolute values, (where A-average diameter of the main tulpinței, B-average number of neophytes-Nations to caulinare, C-average diameter of the largest neoformation caulinare, D-average number of calluses, E -average diameter of calluses).

Presence in the culture medium of 2.5 mg / l 2,4 - dichlorophenoxyacetic acid has yielded some positive results in terms of callus induction from the explants grown on the nutrient substrate. There was thus a total of 0.5 calluses / variant (Fig. 3D), their average diameter (measured at the widest area) of 2.0 cm (Fig. 3D). Analyzing data with mean values shown in the graph in figure 3 D and E we can say that, in the interval between the 60th day and 90th day of culture in vitro, there has been a dramatic increase in the average diameter of calluses, already formed, and not their number. It is noted that the explants the total lack of thorns, probably due to the action "defoliator" auxinic of 2,4 - dichlorophenoxyacetic acid, but a callus pink (Fig. 2B,C). Staining in red or shades of red callus is due to a high content in anthocyanins, which accumulate in the cells due to growth regime, species, origin and age (Cachiță, 2004), in this case it may be that this pigmentation is influenced by the red color of chlorophyll-deficient skin cactus *Echinocactus mihanovichii*. A red callus, compact, formed on the cut surface of the explants was obtained and some species of *Mammillaria*, color is due the presence of beta alanine in the cells (Pérez et al., 1998).

After the first six weeks of vitroculură, found the spreading necrosis (fig.2), leading to the death of 50-55% of inoculum, in all experimental variants, including blank, necrosis represents a common problem vitroculturile cactus (Minocha et al., 1974; Oliveira et al., 1995; Pérez et al., 2002).

CONCLUSION

1. 90 days after initiation of in vitro culture *Echinocactus mihanovichii* rete found that growth of explants is different depending on the composition of the culture medium, growth faster pointing out the environment without growth regulators (V_0).
2. Generation of buds was made all the explants grown on medium without growth regulators (V_0), it preserves the red color of the parent plant.
3. The beneficial effect of growth regulator 2,4 - dichlorophenoxyacetic acid added to the culture medium at a concentration of 2.5 mg / l (V_1) resulted obtain favorable results in terms of callus induction, both number and size.
4. By this time rootedness phenomenon not seen in any of the variants tested.

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. Cachiţă C.D., Ardelean A., 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. Dabekaussen R., Pierik R., Van der Laker J., Hoek J**.,** 1991, Factors affecting areole activation in vitro in the cactus *Sulcorebutia alba*. Rausch. Scientia Horticulturae, vol. 46, p. 283 – 294.
5. Kornilova L.P., 2008, *Grafting on Pereskiopsis*, Cultivar, publicat online: 20 decembrie.
6. Lee J.M., Oda M., 2003, Grafting of herbaceous vegetable and ornamental crops. Hort. Rev., vol. 28, p. 61-124.
7. Minocha S., Mehra P., 1974, Nutricional and morphogenetic investigations on callus cultures of *Neomammillaria prolifera Miller (Cactaceae).* American Journal of Botany , vol. 61, nr. 2, p. 168 – 173.
8. Pérez E., Perez M., Villalobos E., Meza E., Morones L., Lizalde H., 1998, Micropropagation of 21 species of mexican cacti by axillary proliferation. In vitro Cellular Development Biology Plant, vol. 34, p. 131– 135.
9. 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.
10. 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
11. Shemorakov N., 2003, *Cultivar's classification by stem color*, Cultivar 2(18), Published Online: aprilie .
12. Son B.K., 2000, The culture of cacti & succulents (in Korean). Gyeonggi Province, Korea, vol. 28, p. 61-124.