# STUDY ON THE REGENERATIVE AND ORGANOGENIC CAPACITY OF *Echinopsis (ZUCC.) chamaecereus f. lutea* IN VITRO CULTURE ON AN ADDITION MEDIUM OF 3-indolilbutiric (AIB)

**Vidican Iuliana Teodora*, Lazăr AndraNicoleta, Stanciu Alina Ștefania**

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

***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. Inoculationwas madealkalinemineralculture mediumMurashige-Skoog (1962) with -macroand micronutrientsHeller(1953), without growth regulators($V_0$), version control,andsupplemented with3indolilbutiricacidindifferent concentrationsas follows:1mg/lIBA($V_1$), 1.5 mg/lIBA($V_2$) and 2mg/lIBA($V_3$).

After 90 days, it was noted that supplementation of the culture medium with 1 mg/l 3-indolylbutyric acid ($V_1$) and 1,5 mg/l 3-indolylbutyric acid ($V_2$) did not stimulate any organogenic potential response of the explants. The caulogenetic and calusogenesis process was observed in inoculums grown on nutrient medium devoid of growth regulators ($V_0$), while supplementing the culture medium with 2 mg/l AIB ($V_3$) stimulated callus formation.

**Keywords:**vitro cultures, 3 - indolyl butyric acid (AIB), the callus, the newly formedstems.

## 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. AfterSkulkin (2000) plantsmaintained at a temperaturelower thannormalandshadow,growingslowly, 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.

Plant hormonesor growth regulators are organic compounds in concentrations much lower than those nutrients or vitamins, stimulates or inhibits growth and morphogenesis, respectively regulate different physiological processes in the tissues and organs of the plant (Davies, 2004).

3 indolilbutiric acid (AIB) is a class of synthetic auxin, but apparently can be found in nature, but only in some plant species (after Moore, Cachiţă et al., 2004). Auxins are commonly used in tissue culture with stimulants rootedness process and with cytokinins, and they play a major role in the proliferation and growth of plant cells. Fito inoculated tissues synthesized, as well, auxin - or IBA - in the apical meristems, and by adding to the culture medium of the phytohormone produced by synthesis, are favored rootedness. At vitroculturile of *Opuntia ellisiana* on culture media with added acid 3 indolilbutiric (AIB), the percentage of rooting explants was 100%, considering that the cumulative effect of endogenous auxin in the intake of exogenous auxins leads getting a large number of roots (Juárez et al., 2002).

**MATERIAL AND METHOD**

Biological material used inour experimentsconsisted ofseedlingsregeneratedstrains*Echinopsischamaecereus* f. *lutea*(figure 1).The explantswereabout1 cmlong,0,5cmthickand a diameter of0,5-1,5cm, depending on the areawhich washarvested(figure 2).

The plant material, seedlings of*Echinopsischamaecereus* f. *lutea*wassterilized byintroducing, for oneminute,96ºalcohol, followedbycoatingwithsodiumhypochlorite solution0,8%, mixed with waterin a ratio of1:2; in a disinfecting solutionbeing added-as asurfactant-threedrops ofTween20(Cachiță et al., 2004).

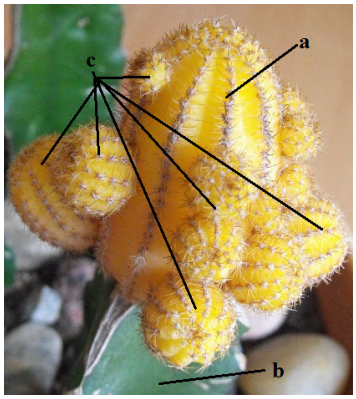


Fig.1. The plant*Echinopsis chamaecereus f. lutea*young, grown in greenhouses(where: a-graft;b-rootstock;c-formations caulinare)

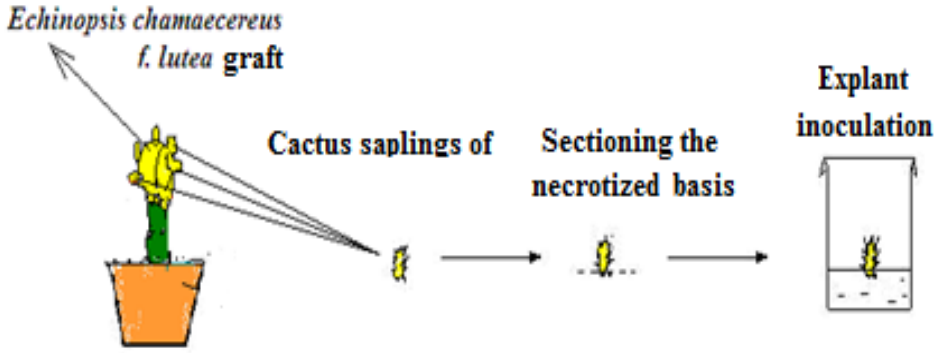


Fig. 2.Schematic representationof howoperatingfragments*Echinopsischamaecereusf. lutea*to beinoculatedasepticenvironments.

Duringsanitizingvegetativematerialwas stirredcontinuously(Cachițăet al., 2004).After20 minuteshe proceeded toremovethe disinfectant agentand went over tothe washingplant materialwithsterile water, making fiverinsestofiveminutes each.Then, the plant material was depositedunder aseptic conditionsinhorizontallaminarflow hood, sterile air, in operation, thefilter paperringssterilizedin the ovenintodusăinpetri dishes, aseptic. Next,it proceeded topostingnecrotizedpartsof futureinoculate.

The mineral mediumcultureusedin thisexperimentconsistedof: macroelements andFe-EDTA, (Murashige and Skoog, 1962),microelements (Medeiros et al.,2006),mineralmixturetowhichwere addedvitamins: HCl pyridoxine, HCl thiamineandnicotinicacid(each 1mg/l), 100mg/l m-inositol,20g/l sucrose and7g/l agar-agar, pHof the mediumwasadjustedtoa value of5.8.

In order to obtain the proposed alternatives, we added new developed nutrient medium devoid of growth regulators ($V_0$), version control, different concentrations of AIB, 1mg/l IBA ($V_1$), 1,5 mg/l IBA ($V_2$) and 2mg/l IBA ($V_3$).

Sterilization ofvialswith medium wasperformed by autoclavingata temperature of 121°C for 30minutes. The recipients with medium culture hadacapacity of15ml, andeachwereplaced5ml of themedium. Aftercooling themediaproceeded toinoculateexplants, operation conductedin aseptic cameraona laminar flowhood, horizontal, with sterile air.

Afterinoculation, explantswerevials were filled withpolyethylene folia. Conditionsin thegrowthchamberwere as follows: illuminatedwithwhite

lightemittedbyfluorescenttubes, photoperiod wasunder16 hourslight/24 h1700luxlight intensity, temperature between20-24°C.

Vitroplantletsreaction after inoculationwasmonitoredfor12weeks. Biometric assessments were taken atintervalsof 30days.Observationsconsisted frombiomeasured: vitroplantletslengthregenerated from explants, number of rotes, callusformation, determining the number ofneostems and branchesdeveloped on theinitialinocula.

**RESULTS AND DISCUSSION**

At 90 days of vitro culture, it was noted that the basal mean diameter of the main strain in the three experimental variants at which the culture medium was supplemented with AIB - added at different concentrations - is equal to 1,2 cm (Fig. 4A), which represents a 33,33% increase (Figure 3A), compared to the same parameter recorded in the control group $V_0$ (medium lacking growth regulators).

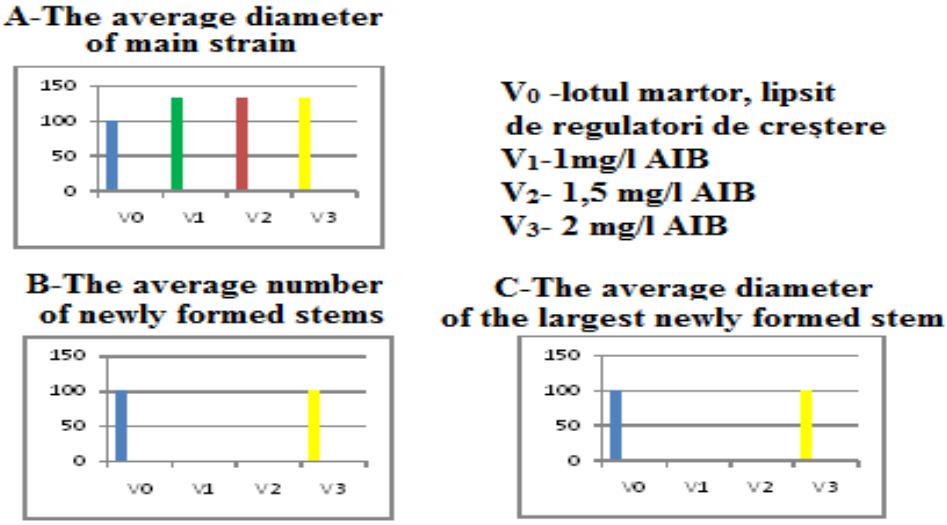


**Fig.3.**Graphical presentation of the mean values corresponding to the readings at the level of *Echinopsis (Zucc.) chamaecereus f. lutea*, on aseptic base modified by us - (variant $V_0$) - with addition of 1 mg/l AIB (variant $V_1$), 1,5 mg/l AIB (variant $V_2$) or 2 mg/l AIB (variant $V_3$), data expressed in absolute values(where: A - mean diameter of the main strain, B - average number of causal neoformations, C - mean diameter of the largest cauline neoporosis).

Of the four variants of culture medium studied, this experiment found that only the explanations belonging to the control group $V_0$ (medium lacking

growth regulators) generated new strains in an average of 0,3 stem/variant (Figure 4B) with a mean base diameter of 0,4 cm (Figure 4C).

Rhizogenesiswas not noted in any of the experimental variants studied, a phenomenon that we observed at *Echinopsis (zucc.) chamaecereus f. lutea* explantes, and in supplementing the culture medium with 2,4-dichlorophenoxyacetic acid (Vidican et al, 2015)

Callus induction was observed in the explants of *Echinopsis chamaecereus f. lutea* was inoculated and grown on culture medium devoid of growth regulators ($V_0$) and supplemented with 2 mg/l AIB ($V_3$), a phenomenon noted for *Aylostera heliosa* on the same culture medium (Vidican, 2013). Both the average number of calluses / variant (0,3 calusions/variant) (Figure 4D) and the average diameter of 0,5 cm (Figure 4E) were equal in both cases.

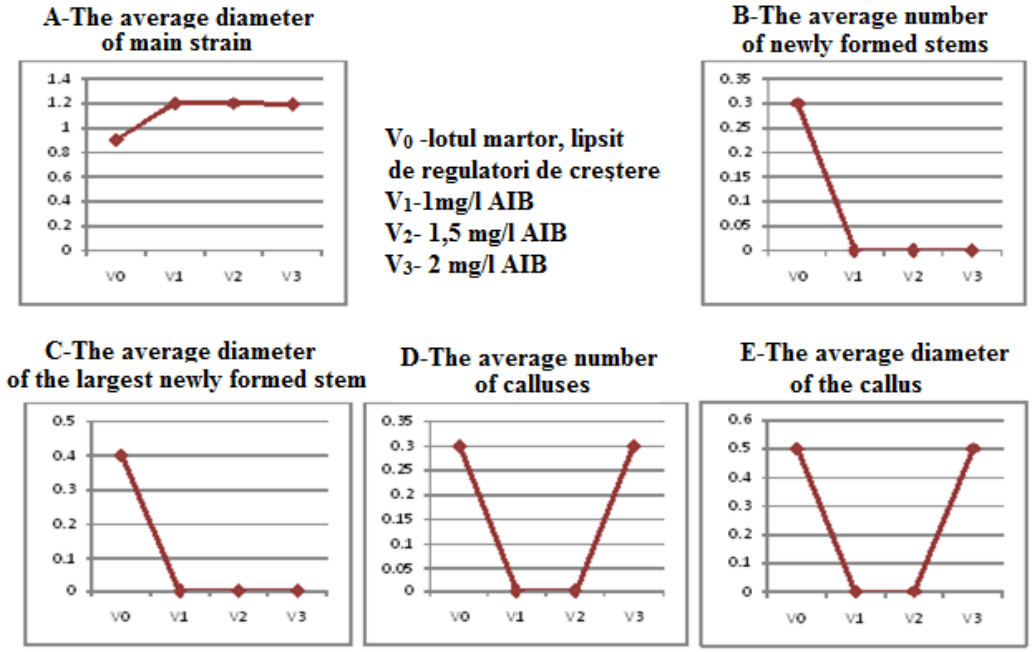


**Fig.4.**Graphical presentation of the mean values corresponding to the readings at the level of *Echinopsis (Zucc.) chamaecereus f. lutea*, on aseptic base modified by us - (variant $V_0$) - with addition of 1 mg/l AIB (variant $V_1$), 1,5 mg/l AIB (variant $V_2$) or 2 mg/l AIB (variant $V_3$), data expressed in absolute values; (where: A - mean diameter of the main strain, B - mean number of newly formed stems, C - mean diameter of the largest newly formed stem, D - mean callix, E - average caliber diameter).

Hese results are in line with those reported by Corneanu et al., (1994), which reported that the explants of *Dilochothele longimamma* fragments,

cultivated in vitro on the Murashige-Skoog medium (1962), without growth regulators, can generate both shoots and calus. In most of the species cultivated in vitro the process of rhizogenesis, it is easy on the MS medium, supplements with endogenous auxins, but species with a slow growth rate, create special problems in rooting (Copăcescu, after Corneanu, 2001).

From Figures 5, it is noted that both areoles and pine nuts grown on the medium with or without the addition of growth regulators are normally developed. It is also noted that both the explanations of the four experimental variants and the cauline neoformations generated at $V_0$ (medium lacking growth regulators) have remained yellow.

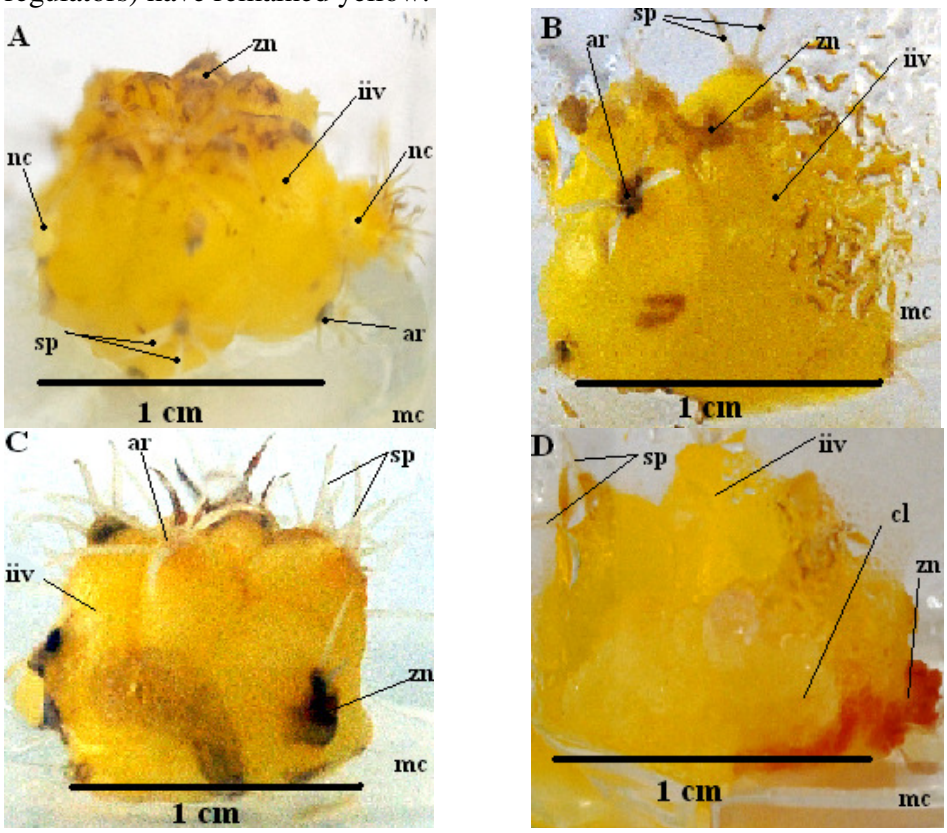


Fig. 5.*Echinopsis (Zucc.) chamaecereus f. lutea*inocula, 90 days after in vitro explant inoculation, where: A-on aseptic base modified by new and lacking growth regulators ($V_0$); B-base medium with 1 mg/l AIB ($V_1$); C-base medium with 1,5 mg/l AIB ($V_2$); D-base medium with addition of 2 mg/l AIB ($V_3$); (iiv–viable initial culture medium, mc-culture medium, nc-new buds, ar-arthales, sp-spines, cl-clusters, zn-necrosis zones).

**CONCLUSIONS**

1. After 90 days it is noted that the results obtained by in vitro cultivation of *Echinopsis chamaecereus f. lutea* are not satisfactory by supplementing the culture medium with 3-indolylbutyric acid (AIB) at a concentration of 1 mg/l AIB ($V_1$) and 1,5 mg/l AIB ($V_2$) no response was received regarding the organogenic potential of the explants.

2. *Echinopsis chamaecereus f. lutea* cultivated and grown on nutrient medium lacking growth regulators ($V_0$), generated both buds and callus.

3. The presence in culture medium of 2 mg/l AIB ($V_3$), in our case, has been shown to be the variant supplemented with growth regulators on which calus was generated.

**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. Corneanu M., Corneanu G., Badica C., . Minea R, Bica D., Vekas I. , *In vitro* organogenesis of *Aloe aborescens,* 1994, Revue Roumaine de Biologie Vegetale, 39, pp. 45-52
5. Davies, P. J., 2004, "*The plant hormones: their nature, occurrence, and function*," in Plant Hormones:
6. Juarezi, M.C., Passera C.B., 2002, In vitro propagation of *Opuntia ellisiana Griff.* And acclimatization to field conditions. Biocell, vol. 26, pp. 319–324
7. Kornilova L.P., 2008, *Grafting on Pereskiopsis*, Cultivar, publicat online: 20 decembrie. Biosynthesis, Signal Transduction, Action , ed. P. J. Davies (Dordrecht: Kluwer Academic Publishers), pp. 1–15.
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. Medeiros L., Ribeiro R., Gallo L., Oliveira E., Demattê M., 2006; *In vitro* propagation of *Notocactus magnificus.* Plant Cell, Tissue and Organ Culture, Springer, vol. 84, nr. 2, p. 100147-100151;
10. Murashige T., Skoog F., 1962, A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, vol. 15, p. 473 –497;
11. Shemorakov, N., 2001, Plastid mutations. Cultivar, vol. 2, nr. 3, p. 11-20.
12. Shemorakov N., 2003, Cultivar's classification by stem color. Cultivar, vol. 2, nr. 18, p. 68-76.
13. Skulkin I. M., 2000, The History of Biological Discoveries, Yekaterinburg.

14. Vidican I.T., Study on theinfluenceof 3indolilbutiric(AIB), addedin different concentrationsinthe culture medium, theregeneration capacityofexplants*Aylostera* (speg.) *heliosa,* 2013, Analele Universităţii din Oradea, Fascicula Protecţia Mediului, Simpozion Internaţional, Vol. XX, anul 18, ISSN: 1224-6265, p. 72-85.
15. Vidican I.T., Lazăr A.N., Stanciu A.Ş., Study onthe regenerative capacitandorganogenicof*Echinopsis (zucc.) chamaecereus f. lutea explntes*, inthe presence of 2,4-dichlorophenoxyacetic acid (2,4-d) in culture medium, 2015,Analele Universităţii din Oradea, Fascicula Protecţia Mediului, Simpozion Internaţional, Vol. XX, anul 18, ISSN: 1224-6265, p. 72-85.