STUDY ON THE REGENERATIVE AND ORGANOGENIC CAPACITY OF Chamaecereussylvestris f. aurea IN VITRO CULTURE ON AN ADDITION MEDIUM OF benzyladenine (BA)

Vidican Iuliana Teodora*

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

Abstract

Chamaecereussylvestris f. aurea is a yellow cactus with yellow epidermis that is part of the chlorophyll-deficient the group of cacti. The yellow color of the *Chamaecereussylvestris f. aurea* cactus is due to a mutation that occurs spontaneously in culture, which makes it impossible to synthesize the chlorophyll, so they only multiply by grafting.

In order to establish an in vitro culture of *Chamaecereussylvestris f. aurea* we took explants represented by buds (seedlings) from mother plants grown in the greenhouseInoculation of the explants was performed on a culture medium composed of macroelements and Fe EDTA Murashige-Skoog (1962), Heller microelements (1953) supplemented with different concentrations of benziladenine (BA).

The evolution*of Chamaecereussylvestris f. aurea* explants was monitored for 90 days, and their response to the presence of benziladenine (BA) culture medium was different depending on the cytokinin concentration. The best results were obtained in cultured cultures supplemented with2 mg/l BA (V_3) supplemented with 66,66% more newly formed strains with a mean baseline diameter of 50% higher than the control group V_0 (medium lacking growth regulators). The phenomenon of rhizogenesis and caulogenetic phenomenon was not only manifested by the increased explants in the absence of growth regulators (V_0).

Keywords: vitroculture,benziladenine (BA), calus, newly formed stems, roots.

INTRODUCTION

*Chamaecereussylvestris f. aurea*is a deficient chlorophyll deficient cactus with yellow skin (Copacescu, 2001), lacking the ability to synthesize chlorophyll chloroplast due to small, about 1/3 of all plastids (Shemorakov, 2003).

Pigmentation is caused by spontaneous emergence in mutation cultures influenced to a large extent by temperature and light (Shemorakov, 2003). After Skulkin plants (2000), the plants were kept at a lower temperature than normal and shadows, slowly increasing, if any, such mutations. Due to the reversible mutations of mutants during meiosis (Shemorakov, 2003), by generative reproductive chances, these plants keep the color minimal (Kornilova, 2008), so it is concluded that plants can preserve the color reproduced only by cloning.

Russian scientists have shown a particular interest for the chlorophyll cactus with deficiency species, so they have made the skin color

classification (Shemorakov, 2003). This has led, as now, to the search for new technologies for the rapid multiplication of these plants as economically efficient (Son, 2000, Lee et al., 2003).

According to Shemorakov (2001), the reversible plastid mutation during meiosis causes the reproduction generation to be *Chamaecereussylvestris f. aurea*has little chance of preserving its color (Kornilova 2008)thus, concluded that plants can keep this private property reproduced only by cloning.

Cytokinins that are plant hormones in the absence of undisturbed cells and tissue cultures that stimulate cell division and formation processes resist stalking strains (Mauseth, 1976) also prevents senescence, auxin exerts an antagonistic effect that annihilates the dominance apical, favoring cell dedifferentiation, etc. (Cachiţă et al., 2004).

Escobar et al. (1986) reported that the most effective plant growth regulator tested for in vitro cultures of cactus for multiplication of plant material is benziladenine-BA added to the culture medium to generate a larger number than neoformation.

MATERIALS AND METHODS

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

The plant material, seedlings of *Chamaecereussylvestris f. aurea* was sterilized by introducing, for oneminute,96ºalcohol, followed by coating with sodium hypochlorite solution 0,8%, mixed with water in a ratio of1:2; in a disinfecting solution being added-as a surfactant-threedrops of Tween20(Cachiță et al., 2004).

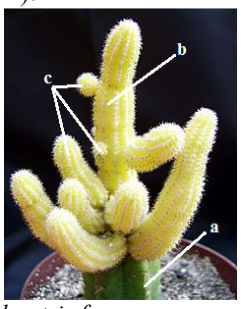

Fig.1.The plant *Chamaecereussylvestris f. aure a*young, grown in greenhouses (where: a-the rootstock; b-stems;c-buds)

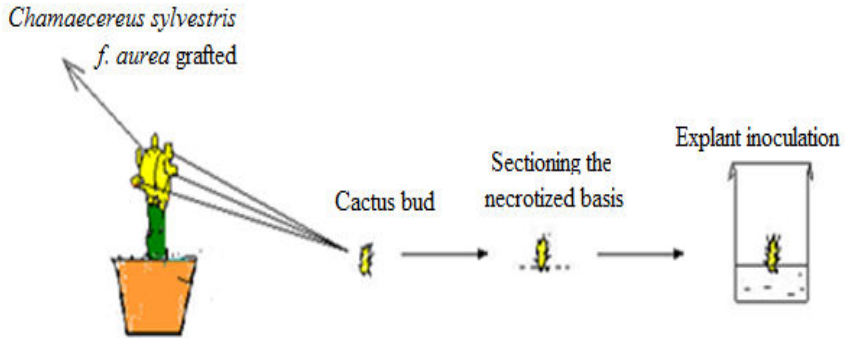

Fig. 2.Schematic representation of how operating fragments *Chamaecereussylvestris f. aurea* to be inoculated aseptic environments.

During sanitizing vegetative material was stirred continuously (Cachiță et al., 2004). After 20 minute she 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 ovenin to taken in petri dishes, aseptic. Next, it proceeded to posting necrotized parts of future inoculate.

The mineral medium culture used in this experiment consisted of: macroelements 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 1mg/l), 100mg/l m-inositol, 20g/l sucrose and7g/l agar-agar, pH of the medium was adjusted to a value of 5,8.

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

Sterilization of vials with medium was performed by autoclaving at a temperature of 121°C for 30 minutes. The recipients with medium culture had a capacity of 15ml, and each were placed 5ml of the medium. After cooling theme proceeded to inoculate explants, operation conducted in aseptic camera on a laminar flow hood, 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 under16 hours light/24 h1700 lux light intensity, temperature between 20-24°C.

Vitro plant lets reaction after inoculation was monitored for12 weeks. Biometric assessments were taken at intervals of 30 days. Observations consisted from biomeasured: vitro plant lets length regenerated from

explants, number of rotes, callus formation, determining the number of neostems and branches developed on the initial inocula.

RESULTS AND DISCUSSION

Based on the observations made at 90 days after the initiation of the *Chamaecereussylvestris f. aurea* bud culture on a culture medium supplemented with benziladenine (BA), we obtained similar in vitro results to other *Cactaceae* species (Vidican, 2013), thus the mean basal diameters of the major strains recorded an increase directly proportional to the amount of cytokinin added to the nutrient substrate.

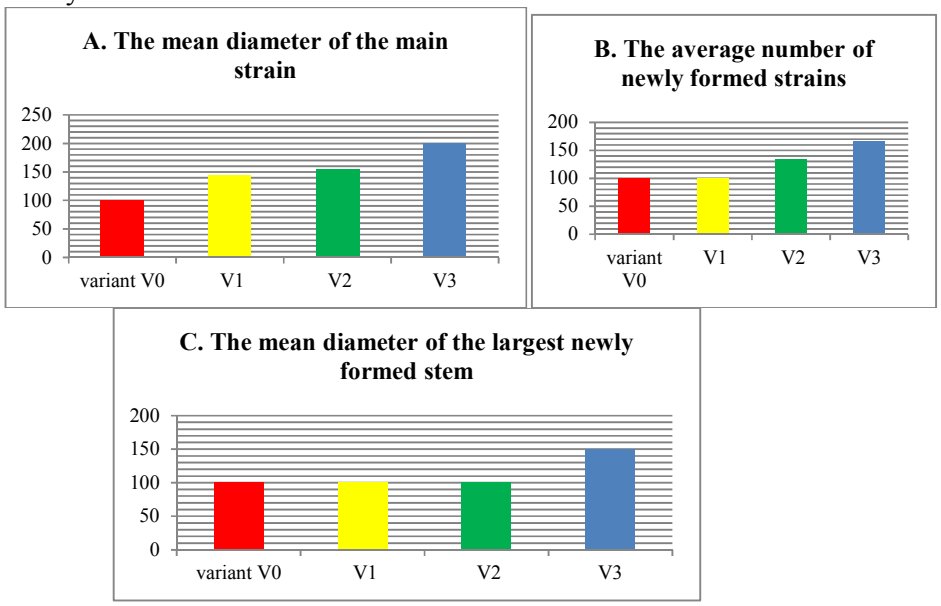

Fig. 3. Graphical presentation of the mean values corresponding to parameters at the level of *Chamaecereussylvestris f. aurea*, on aseptic base modified by us, with 1 mg/l BA (variant V_1), 1,5 mg/l BA (variant V_2) or 2 mg/l BA (variant V_3), data expressed as a percentage obtained from the reporting of biometric values to the results recorded at the respective parameters monitored in the control group (V_0), lacking growth regulators, values considered as 100%; (where: A- The mean diameter of the main strain, B-the average number of newly formed strains, C- the mean diameter of the largest newly formed stem).

With the highest value of 1,8 cm (Fig.4A) of this parameter we can notice the explanations of variant V_3 (medium supplemented with 2 mg/l BA) which shows a 100% increase compared to the values of the same parameter registered in the control group V_0 (Fig.3A). Smaller values of this parameter were recorded in variants V_1 (medium supplemented with 1 mg/l BA) or V_2 (medium supplemented with 1,5 mg/l BA), which with 1,3 cm

and 1, respectively, 4 cm marked an increase of 44,44% in the first case and 55,55% in the second case.

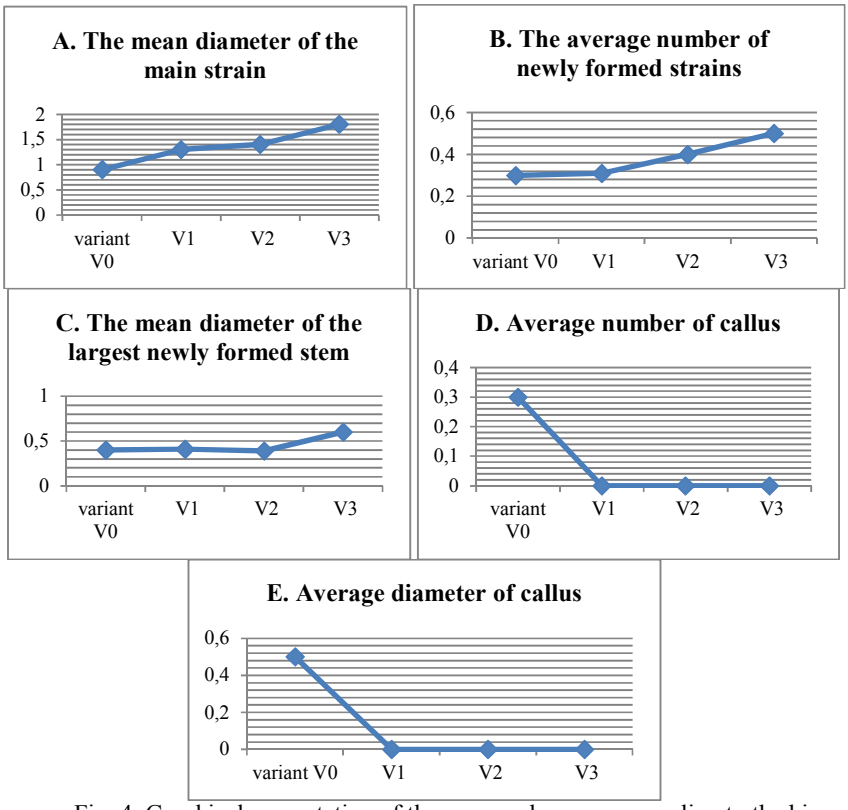

Fig. 4. Graphical presentation of the mean values corresponding to the biometric parameters at the *Chamaecereussylvestris f. aurea*, on aseptic base modified by us (variant V_0), with addition of 1 mg/l BA (variant V_1), 1,5 mg/l BA (variant V_2) or 2 mg/l BA (V_3), expressed in absolute values; (where: A - mean diameter of the main strain, B - average number of newly formed strains, C - mean diameter of the largest newly formed stem, D - average number of callus, E - average diameter of callus).

After 90 days of explant culture on cultured culture supplemented with BA in different concentrations, the neogenesis of the buds is noted. The average number of newly formed strains was of 0,2 buds/variant V_1 variant (medium supplemented with 1 mg/l BA) which equaled the control, while in variant V_2 (medium supplemented with 1,5 mg/l BA) with 0,4 buds/variant (Fig.4B)a 33,33% increase was dropped (Fig.3B). Also, in this experiment we can see that the increased culture on medium supplemented with 2 mg/l BA (V_3) with 0,6 buds/variant varied by 66,66% compared to the values of the same parameter recorded in the matrix V_0 environment lacking growth regulators).

In terms of the mean basal diameter of the newly formed stems, this parameter with a value of 0,4 cm (Fig.4C) equals the witness to the variants of the variants V_1 (medium supplemented with 1 mg/l BA) or V_2 (medium supplemented with 1,5 mg/l BA), but it exceeds by 0,2 cm this value in variant V_3 (medium supplemented with 2 mg/l BA), which represents a 50% increase compared to the V_0 control (Fig.3C). Only the values obtained in variant V_3 (medium supplemented with 2 mg/l BA). The results of the present experiment are consistent with those published by Starling (1985a), which found that the *Cactaceae* species in vitro, cultured on BA-supplemented media at different concentrations, generated the largest number of stems/variant.

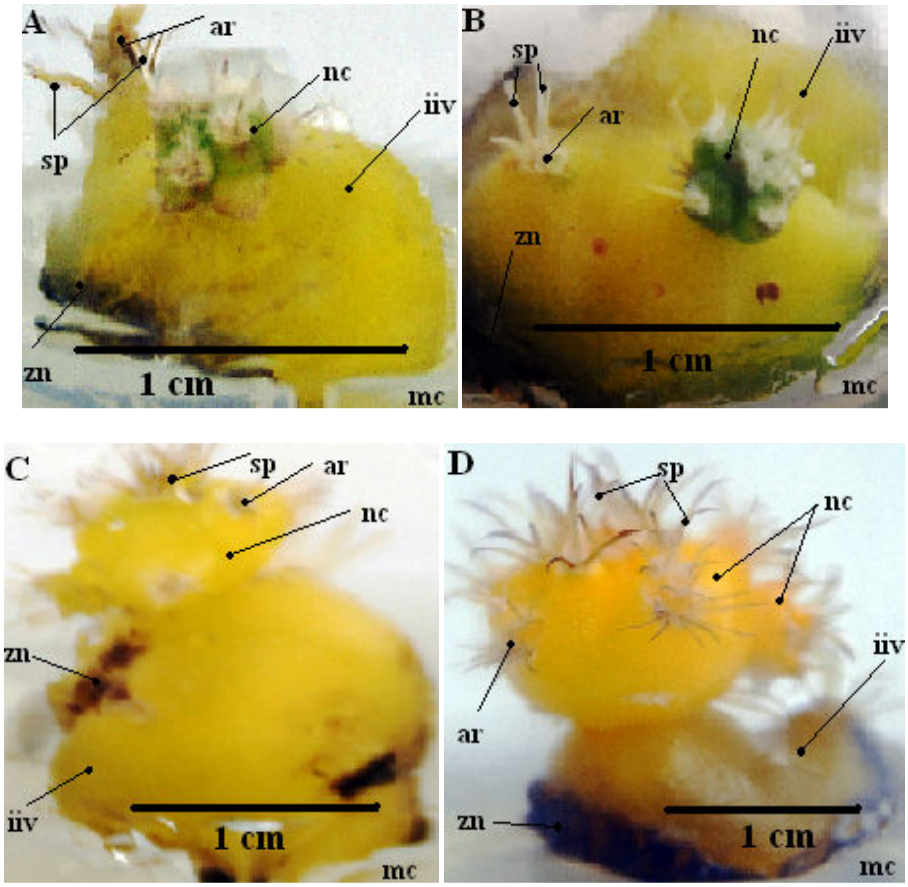

Fig. 5. Inoculum of *Chamaecereussylvestris f. aurea*, 90 daysafter in vitro explant inoculation, where: A-on aseptic base modified by non-growth regulators (V_0); B-on base medium with 1 mg /l BA (V_1) added; C-on a basic medium with 1,5 mg/l AB (V_2) added; D-on the base medium with 2 mg/l BA (V_3); (iiv-the initially viable inoculum;mc-culture medium; nc-newly formed stems;ar-areoles; sp-thorns; zn-necrotic area).

It can be seen that newly budded buds inoculated and grown on culture medium lacking growth regulators (V_0) or with addition of 1 mg/l BA (V_1) are green in color (Fig.5A,B) unlike those to which the substrate was supplemented with 2 mg/l BA (V_3) having an orange tinge (Fig.5C). The only new strains that retain the mother-yellow plant color (Fig.5D) are regenerated at the cultured explants of culture medium supplemented with 2 mg/l BA (V_3). As a common feature it is noted that in the contact area of the explants with the nutrient substrate, regardless of its composition, the neocosis appeared; while at the level of the newly formed buds, regardless of the growing environment of the inoculum, areoles and thorns are well developed as well.

Until this date, none of the experimental variants recorded the phenomenon of rhizogenesis, and calusogenesis was recorded only at the explants of control variant V_0 (medium lacking growth regulators) where an average number of 0,3 callus/variant (Fig.4D), with an average diameter of 0,5 cm (Fig.4E).

CONCLUSION

1. Analyzingthe experimental data recordedafter 90 days of vitro culture in *Chamaecereussylvestris f. aurea*, onecannotice the beneficial influence of supplementation of the culture medium with 2 mg/l BA (V_3) on the manifestation of the caulogenetic phenomenon.

2. Growth extensions on the culture medium supplemented with 2 mg/l BA (V_3) recorded 66,66% more newly-formed strains with a mean basal diameter of 50% greater than that of control group V_0 (environment lacking growth regulators).

3. The presence of benziladenine (BA) in the culture medium didnot stimulate theprocesses of rhogenesis

and calusogenesis in *Chamaecereussylvestris f. aurea* in vitro cultures, phenomena manifested only at elevated explants in the absence of growth regulators (V_0).

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