

## INITIATION OF *OPUNTIA FRAGILIS* VAR. *FRAGILIS* IN VITRO CULTURES

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**ABSTRACT.** *Opuntia* is one of the most widespread and important cactus, primarily due to their edible fruit and vegetable mass used as feed. Moreover, species of genus *Opuntia* are used successfully in combating desertification of land at an arid climate. Demand for young plants of *Opuntia* made it necessary to find a rapid method of multiplication of the cactus, the safest method consisting in vitro micropropagation of species belonging to this genus. To establish a vitroculture of *Opuntia fragilis* var. *fragilis* from mother plants grown in greenhouses, we have taken explants from cladodes level who have been fragmented into segments of 1 / 1 cm, 0.5 cm thick, which were placed on agarized medium culture, with macro-elements and Murashige-Skoog (1962) FeEDTA, Heller (1953) microelements, by adding sucrose, vitamins, m-inositol and growth regulators or with 1 mg / l BA (bensiladenine), with 1mg / l AIB (β-indolilbutiric acid) or mixed - equal parts - between these growth hormones. Vitrocultures evolution was followed for 90 days. Explants reaction was different depending on the nature of growth regulators present in the medium culture. Finally, it appeared that, inocules of *Opuntia fragilis* var. *fragilis* showed a caulogenesis phenomenon in the medium BM-MS variant, with the addition of 1 mg/l BA and for risogenesis the variant medium culture MB-MS, with the addition of 1 mg/l AIB. Using a mixture of 1 mg/l BA with 1 mg/l AIB, has proved to be beneficial in the micropropagation of this species; calusogenesis has been not observed in any of the tested variants.

**Keywords:** cactuses, vitrocultures

### INTRODUCTION

From the 130 genera of the *Cactaceae* family, *Opuntia* genus is one of the most studied of the world (Griffith, 2001a, b; Pinkava, 2002). There is evidence that the species of *Opuntia* has been used by humans, at least 9,000 years (Kiesling, 1998).

Cacti of the genus *Opuntia* are the most widespread and important cacti (Nobel 2002, Nobel et al., 2002), they are valuable not only for edible fruit, but as valuable as a vegetable or plant mass as fodder (Kluge and Ting, 1978, Casas and Barbera, 2002). This plant is recognized as a good indicator of noxious (Nobel, 1994).

Due to the spread of *Opuntia* species in arid and in the semi-arid areas (Felger, 1979, Russell and Felker, 1987), devoid from herbaceous vegetation (Le Houérou, 2000, Juárez and Passera, 2002), plants shall constitute in an important tool to combat desertification land in Sudan, which occupies approximately two thirds of the country's area (after El Gamri, 2004), but also from: Mexico, USA, Chile, Argentina, Israel, Italy, and South Africa (Flores -Valdez, 1994).

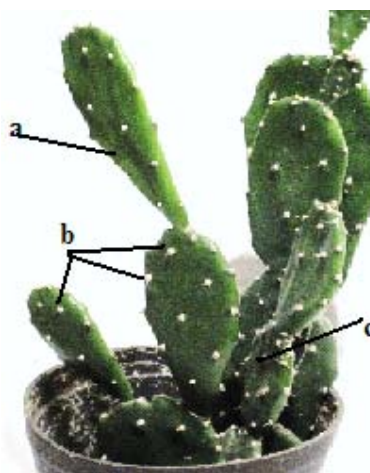
Efficient and rapid multiplication of these plants is done by "in vitro" micropropagation (Johnson Emino, 1979, Escobar et al., 1986; Rubluo et al., 1996, Smith et al. 1991).

The aim of our study was to initiate an in vitro culture of *Opuntia fragilis* var *fragilis*, and studying the explants response to different concentrations of growth regulators in culture medium aseptically placed respectively in the presence of cytokines like bensiladenine (BA) or auxinei: β-indolilbutiric acid

(AIB) administered separately or mixed in the medium culture.

### MATERIALS AND METHODS

In order to initiate vitrocultures of *Opuntia fragilis* var. *fragilis* (Fig. 1), from plants grown in greenhouses, we collected young cladodes in which the spines were not fully trained.

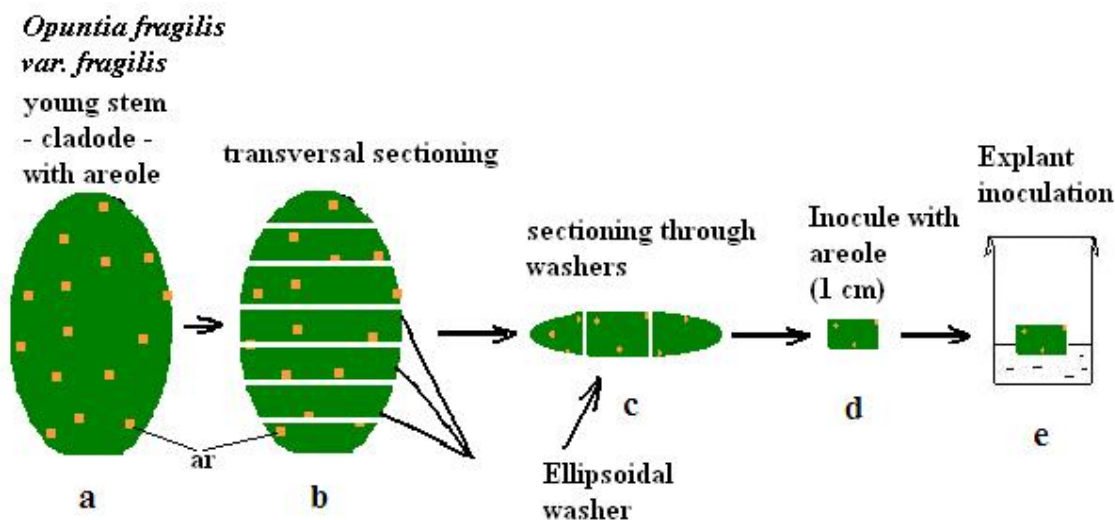


**Fig. 1** Young plant of *Opuntia fragilis* var. *fragilis*, grew in greenhouse (where: a- cladodes, b-areoles, c-spins)

Cladodes were designed into slivers of ellipsoidal shape (fig. 2b), which was fragmented so that the central area (fig. 2c) - about 1 cm long and 0.5 cm

thick, which held 2.3 areoles (Fig. 2d) - was defined as explant which was inoculated in vitro. Areola (*Cactaceae* family are different between plants and

other succulent plants), is a bearing structure and maintain a place of thorns, buds and fluff, most cacti are located in the outer coast (Copăcescu, 2001).



**Fig. 2** Schematic representation of *Opuntia fragilis* var. *fragilis* cladodes, and its slicing way into ellipsoidal slivers, central fragments were inoculated in aseptic medium culture, extreme areas of cladodes or washers were removed from the experiment (ar - areoles)

The sterilization of plant material, represented by cladodes harvested from *Opuntia fragilis* var. *fragilis* strains was done by submersing these fragments in 96° ethylic alcohol for one minutes, a procedure that was followed, by covering them with a solution of sodium hypochlorite 0.8%, mixed with water in 1:2 ratio, which were added three drops of Tween 20 as surfactant (Cachita et al., 2004).

For 20 minutes, during sterilization of plant material, it was stirred continuously. The next stage of work, after decanting disinfectant, the plant material was washed with sterile distilled water, in five consecutive rinsings, to one every five minutes, the operation performed to remove chlorine.

Plant material after sterilization was deposited in Petri capsules, placed on slivers of filter paper (previously sterilized in the oven), in laminar flow hood, horizontal, sterile air, in operation, after that the plant material was fragmented and removed the necrotic parts.

The used medium culture for explants growth it was consisted from: macro-elements Murashige-Skoog (1962) and Fe-EDTA, Heller (1953) microelements, mineral mixture to which were added vitamins: pyridoxine HCl, thiamine HCl and nicotinic acid (1 mg/l, each), m-inositol - 100 mg/l, sucrose - 20 g/l and agar 7 g/l pH of medium culture was set to a value of 5.8, for autoclave.

To the presented basic medium culture (BM), it was added growth hormones, as follows:

- V0 – witness variant, BM- MS without growth hormones,

- V1 – BM-MS with an adding of 1mg/l BA (bensiladenine)

- V2 – BM-MS with an adding of 1mg/l IBA ( $\beta$ -indolilbutiric acid)

- V3 – BM-MS with an adding mixture of 1mg/l IBA and 1mg/l BA

The medium culture was placed in a glass vial with a capacity of 15 ml (in each container were placed 5 ml of medium). Medium vials were sterilized by autoclave, for a period of 30 minutes at a temperature of 121°C. After cooling media has been done the explants inoculation, processes within the aseptic chamber, laminar flow hood with sterile air. To cover the containers for the inocules we used polyethylene foliage, immobilized with elastic.

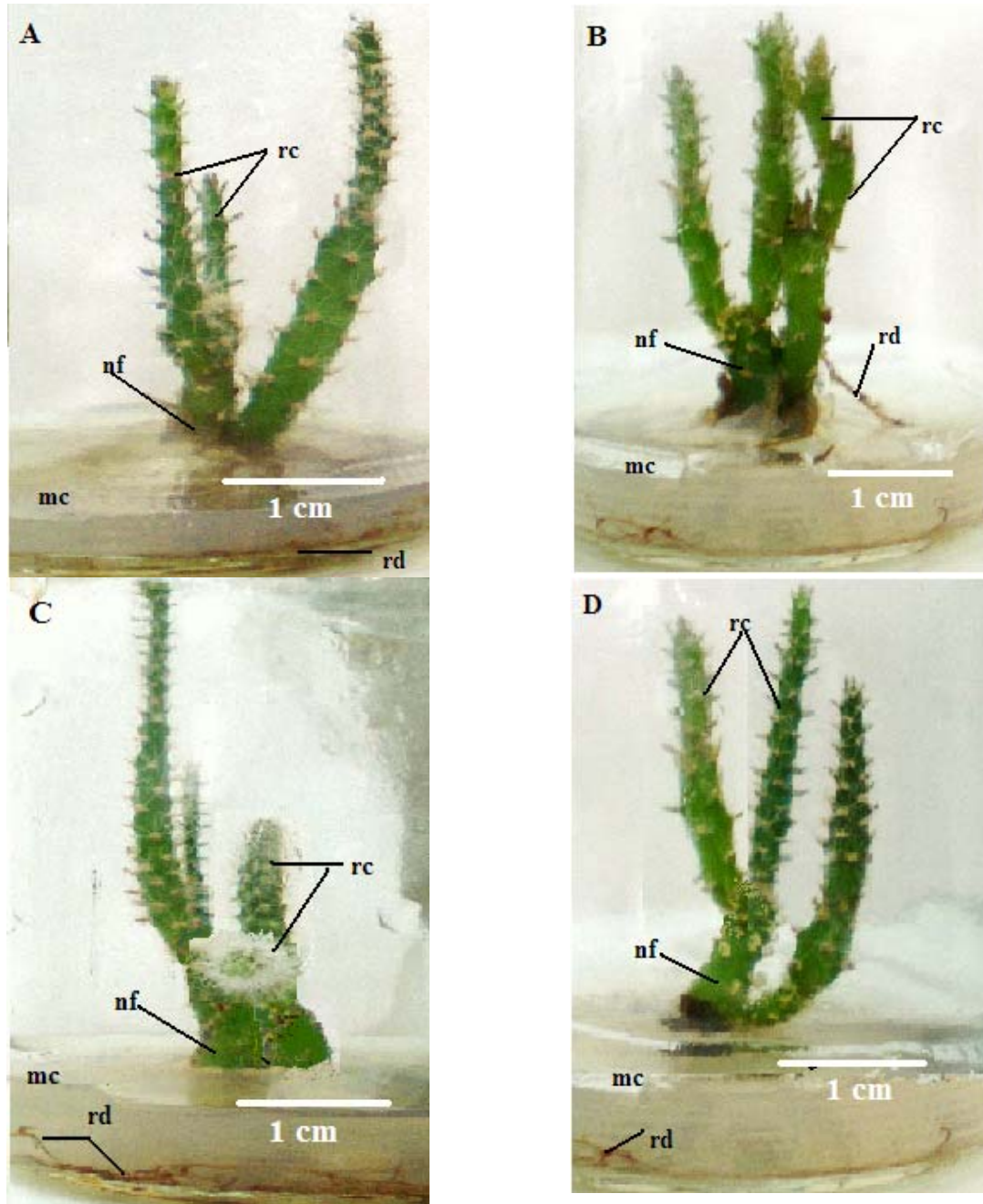
The recipients with the inocules were transferred to growth chamber under the following conditions: temperature varied between 24°C and 20°C, during illumination and the lighting was done in 16 hours light/24h photoperiod regime, lighting of the culture was realized with white light, emitted by fluorescent tubes, with intensity of 1700 lux.

Phytoinocules reaction and explants evolution was followed for 90 days. At 30 days after inoculation we determined the percentage of explants survival. Recorded biometric values were registered to control group (V0, inocules grew on basic medium culture, without growth regulators), were considered as reference, 100%, they were reported - to every trait - all averaged readings to every experimental variant.

## RESULTS AND DISCUSSIONS

Images of *Opuntia* vitroculture, captured at 90 days after explants inoculation on the four variants of culture media, are shown in Figure 3 and Figure 4, are illustrated graphically as histograms, in percentage values, the results from made biomeasures performed

at the regenerated organ level of *Opuntia* vitrocultivated explants, the next explants parameters were monitored: survival percentage, average stem length, average number of caulinar branches, average length of branches caulinar, the average number of roots, average length of roots.



**Fig. 3** Images of *Opuntia fragilis* var. *fragilis* vitrocultures, 90 days after explants inoculation on aseptic medium culture, where: A - explants inoculated on Murashige-Skoog (1962) basic medium culture, without hormone regulators (variant V0); B - explant inoculated on basic MS medium with the addition of 1mg / l BA (variant V1); C - explants inoculated and grown on basic MS medium culture with the addition of 1mg / l IBA (variant V2), D - explants inoculated and grown on basic MS medium culture with a mixture addition of 1 mg / l BA and 1mg / l IBA (variant V3) (NF- caulinar neoformation, rc - caulinar branch; rd - root, mc- aseptic culture medium).

At 30 days after inoculation, the survival rate was 100% at all explants (Fig. 4), in contrast, after 60 days, respectively 90 days after vitrocultures initiation, part of the inoculated explants on basic medium (changed BM-MS), without growth regulators (V0) were necrotic and therefore the rate of survival - in all variations with aseptical substrate consisted from basic medium plus growth regulators (variants V1-V3), were much more than on the control variant (Fig. 4).

In this examination, performed one month after inoculation, the average length of stems (fig.4B) in all experimental variants was located below the stems waist from the control group-V0-regarded as representing 100% (fig.4B) thus, the variant V2 (BM - MS medium supplemented with 1mg / l IBA), this parameter was lower than the control variant by 10.4%, while the variant V1 (MS medium with the addition of 1 mg / l BA), and V3 (BM - MS medium supplemented with 1 ml / l BA + 1 ml / l IBA), was a decrease by 18.7% and 20.8% of this parameter.

From the four variants of culture media taken in the study, to ascertain that only the BM - MS medium culture supplemented with 1 ml / l BA (V1), from those explants were regenerated caulinar ramifications in a percentage 20%, higher than the control group (V0), (fig. 4C). To note the fact that from any experimental variation - this time - we did not noticed any risogenesis or calusogenesis processes.

At 60 days after inoculation, observations have shown that survival rate has exceeded 25% (fig.4) parameter determined in the control group (V0).

The BM-MS medium culture supplemented with 1 mg/l BA, the rate of growth of vitrostem exceeded the witnesses with 5.8%, while the variant V2 (medium BM-MS supplemented with 1 ml/l AIB) and the V3 (medium BM-MS supplemented with a mixed add of 1 ml/l BA + 1 ml/l IBA), the size of neoformed stem of initial explant was 9.7% lower than the regenerated shoot of the inoculum grown on medium without growth regulators (V0) considered as reference group (fig. 4B).

As resulting from Figures 3 and 4 of this series of observations, in all experimental variants, it has been fully manifested a phenomenon of caulogenesis and risogenesis. Thus, the average number of caulinar branches (fig. 4C), the V1 variant (changed medium BM-MS and supplemented with 1 mg/l BA), the exclusive presence of BA in the culture medium resulted from the excess of 60% of the control group followed the same process at variant V0, the variant V2 (same BM-MS changed but with the addition of 1 ml/l IBA), this parameter has matched the witness, and the variant V3 (culture medium BM - MS supplemented with 1 ml/l BA + 1 ml/l IBA), the average of this parameter was by 36% below the witness (V0).

The average length of caulinar branches (fig. 4D), the version V1 (MB-MS, with the addition of 1 mg/l BA), has exceeded 41.5% V0 witness variant, while the variant V2 (medium BM-MS, with the addition of 1

ml/l IBA) and V3 (medium BM - MS with addition of 1 ml/l BA + 1 ml/l IBA), the registered values in this parameter was by 19.3%, respectively with 44.6 % below its value.

Average number of roots (fig.4) was higher with 10% on variant V2 (medium with the addition of 1 ml/l AIB), compared with V0 witness variant, but average values of this parameter were below its value by 46%, to variants V3 (medium with the addition of 1 ml/l BA + 1 ml/l IBA), respectively 60%, and V1 (medium with the addition of 1 mg/l BA), which occurred the risogenesis counter effect of IBA by BA, producing an exclusion of auxins from the medium culture and the presence of cytokines BA in the substrate of the medium culture.

The average length of roots (fig. 4F), was 1.7% above the value of V0 witness variant, also on the variant V2 (medium BM-MS supplemented with 1 ml/l IBA), while the variant V1 (identical medium culture, but with the addition of 1 mg/l BA) was 8.9% below the standard and the variant V3 (MS medium with addition of 1 ml/l BA + 1 ml/l IBA), this parameter was also, with 47.4% lower than in control variant V0.

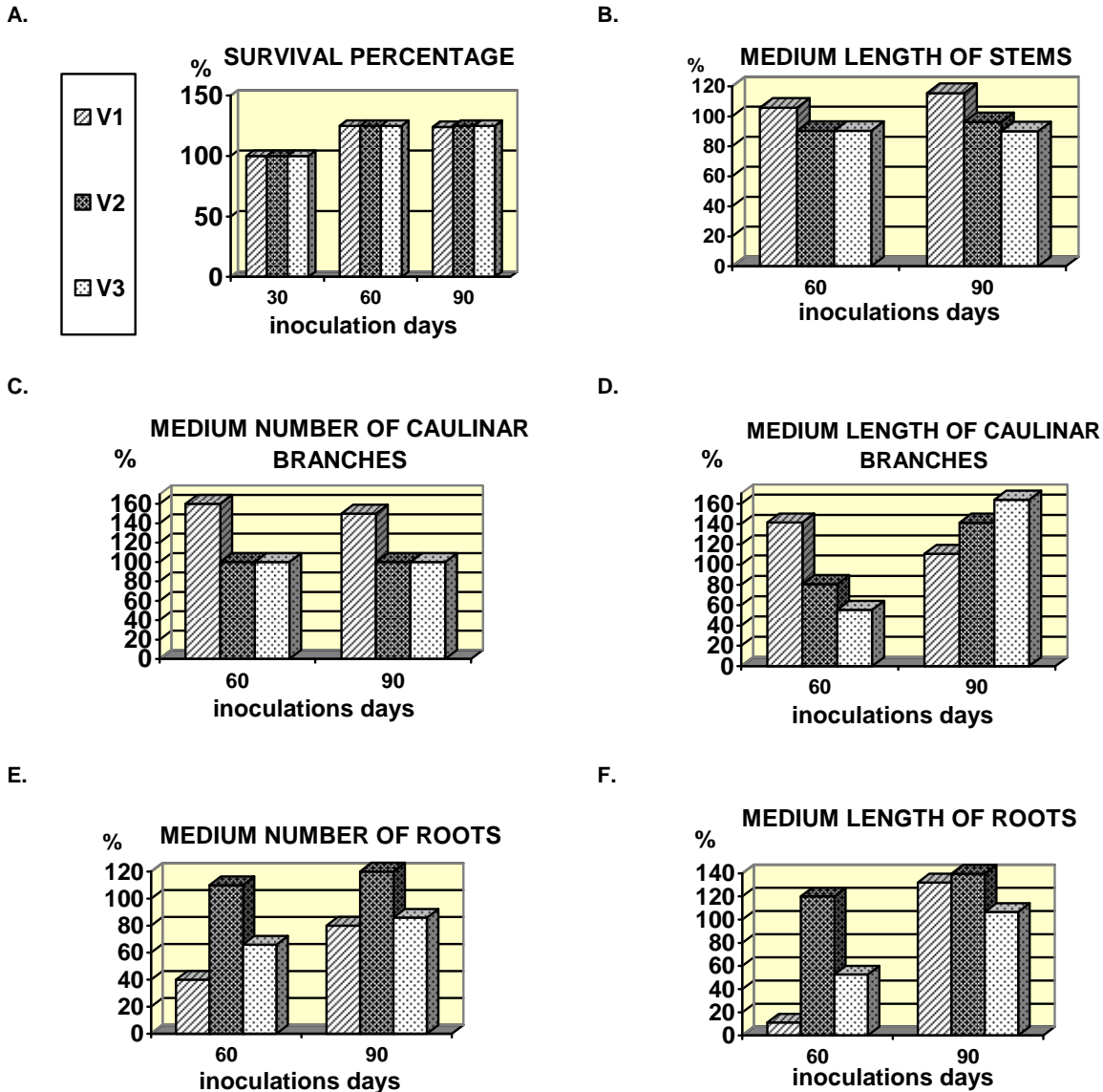
At 90 days (fig. 3), after inoculation, observations showed that survival percentage of explants was increased by 25% (fig. 4), in all experimental variants in relation to witness variant (V0).

The average length of stems (fig. 4B), showed an increase compared with control variant (V0), the variant V1 (changed medium culture BM-MS, and with the addition of 1 mg/l BA) and variant V3 (MS medium culture with addition of 1 ml/l BA + 1 ml/l IBA), with 11.5%, respectively 1.0%, while the variant V2 (MS medium culture with the addition of 1 ml/l IBA), this switch was placed 3.8% below the standard (V0).

Vitroplantlets of *Opuntia fragilis* var. *fragilis* on variant V1 (MS medium culture with the addition of 1 mg/l BA) were noted by an increase of 50% of the average number of caulinar branches and those from the variants V2 (medium culture MS with the addition of 1 ml/l IBA) and variant V3 (MS medium culture with addition of 1 ml/l BA + 1 ml/l IBA), have matched the witness variant V0 (fig. 4C).

The average length of caulinar branches (fig.4D), presented the witness with an increase of 64% to variant V3 (MS medium culture with addition of 1 ml/l BA + 1 ml/l IBA) (fig. 4C), and 10.6% to variant V1 (MS medium culture with the addition of 1 mg/l BA), and a decrease of 58.7% at variant V2 (MS medium with the addition of 1 ml/l IBA), from V0.

Average number of roots (fig. 3) was 20% higher on variant V2 (MS medium culture with the addition of 1 ml/l IBA) for the control group V0, but it marked a decrease of 14%, respectively 20%, to variants V3 (MS medium culture with addition of 1 ml/l BA + 1 ml/l IBA), and the variant V1 (MS medium culture with the addition of 1 mg/l BA).



**Fig. 4** Graphical presentation of survival percentage of *Opuntia fragilis* var. *fragilis* (A) and their evolution on aseptic Murashige-Skoog basic modified medium (MS) culture modified with the addition of 1mg/l BA (variant V1), or with 1 mg/l IBA (variant V2), or the mixture add of 1 mg/l BA plus 1 mg/l IBA (variant V3), data expressed as a percentage, obtained by reporting the bio-measured results values recorded in the control group bio-metrized those parameters (V0), without growth regulators, values regarded as 100% (B - average length of stems, C - average number of caulinar branches, D - average length of the caulinar branch, E - average number of roots and F - the average length of roots)

The average length of roots (fig.4F), is higher compared with control variant V0 at all experimental variants, so, with 39.6% on variant V2 (MS medium culture with the addition of 1 ml/l IBA) (fig. 4D), with 32% on variant V1 (MS medium culture with the addition of 1 mg/l BA), and 6.6% on variant V3 (MS medium culture with addition of 1 ml/l BA + 1 ml/l IBA).

The calusogenesis phenomenon has not been observed in any of the experimental variants.

## CONCLUSIONS

The vitroculture initiation of *Opuntia fragilis* var. *fragilis* has proved to be possible on all four medium culture variants whose effects were tested, even without growth regulators of the substrate, but caulogenesis and the risogenesis processes were modest in the absence of growth hormones in the culture medium. The explants evolution varied according to the nature and concentration of BA or IBA in culture medium.

At 90 days after initiation *Opuntia fragilis* var. *fragilis* vitrocultures was found that explants grown on basic MS medium variant, modified by us, and with the addition of 1 mg/l BA, were regenerated strains and bends more than the other variants.

*Opuntia fragilis* var. *fragilis* explants were grown on the same basic MS medium, but with the addition of 1 mg/l IBA, had the strongest risogenesis implemented by a regeneration of many roots and their length. Using a mixture of 1 mg/l BA with 1 mg/l IBA, has proved to be beneficial in the micropropagation of this species. It should be noted that in none of the variants used in the experiments performed by us, the calusogenesis it has been not manifested.

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