ROSMARINUS OFFICINALIS VITROCULTURE INITIATION

Simona Ana GABOR-POTOR*, Liviu POP*

*University of Oradea, Faculty of Science, Department of Biology, Romania
simona_potor@yahoo.com

Summary: In this experiment we have initiated a *Rosmarinus officinalis* vitroculture, on different growth media. As biological material we used apexes, taken from an only plant. The development medium have consisted in Murashige and Skoog standard mixture, where growth regulators were added, resulting 4 experimental variants: V_0 – control variant – basic medium (BM), V_1 – BM + 2mg/l BA + 1mg/l IBA, V_2 - BM + 2mg/l BA + 1mg/l IAA, V_3 –BM + 2mg/l BA + 1mg/l NAA. The experiment lasted for 90 days. We have found that the initiation of *Rosmarinus officinalis* vitroculture is possible, the best growth medium for this purpose being the basic one (V_0) - Murashige and Skoog without growth regulators.

Keywords: *Rosmarinus officinalis*, vitroculture, growth regulators, initiation

Abreviations: MS=Murashige and Skoog; BM=basic medium; IBA= indole 3-butyric acid; BA= benzyladenine; NAA=α-naphtylacetic acid;

INTRODUCTION

Rosemary *(Rosmarinus officinalis* L. 1758*)* is a pleasantly aromatic, Mediterranean "subarbust". Its name originally comes from the Roman (ros=dew and marinus=sea), etymologically meaning "sea dew". Numerous studies have shown that rosemary one of the strongest antioxidants which beneficially contribute to cancer prevention. The useful parts of the rosemary are the flowers and leaves. The flower is harvested in bloom and the leaves which are perennial can be harvested throughout the year. "Rosemary water" has been used from medieval times to the present for skin cleansing and toning.

IN VITRO REPRODUCTION

In vitro cellular cultivation was created independently by Gautheret (1959) and White (1963).

Currently, this process can be effectively utilized by using large-capacity bioreactors and even through cloning, by starting with a single cell or isolated cells which can regenerate somatic embryons.

Certain plants of economic importance are being reproduced through this methodology since it leads to the creation of disease-free plants. The maximum productivity in the cultivation of the plants was obtained by determining the optimal environmental conditions and soil composition which was most beneficial to reproduction.

In this experiment, we studied the "in vitro" inception of the *Rosmarinus officinalis* and noted the development of the plantlets, specifically their regenerative capacity, over 90 days.

MATERIALS AND METHODS

The plant material used for this experiment consisted of apical meristems, collected from the plants of *Rosmarinus officinalis* , grown in pots in a greenhouse. The biologic material used was 5 mm in length. It was kept under cold, running water for several hours and then sterilized in a sterile environment.

The basic medium used (BM) consisted of microelements and macroelements as well as Fe EDTA Murashige-Skoog (1962) (MS), mineral supplements to which I added the following vitamins:B1, B2 and B6 (1 mg/l from each one), plus m – inozitol - 100 mg/l, suchrose - 20 g/l and agar - 7 g/l.

To this medium we have also added growth regulators which led to the following experimental variants:

- V_0 (control variant) – BM without growth regulators;
- V_1 – BM - with 2mg/l BA + 1mg/l IBA;
- V_2 – BM - with 2mg/l BA + 1mg/l IAA;
- V_3 – BM - with 2mg/l BA + 1mg/l NAA.

(BA – benzyladenine; IBA – indole 3-butyric acid; IAA – indolilacetic acid; NAA –α-naphtylacetic acid)

The experiments consisted of the "in vitro" inoculation of apex-type plants in the above-mentioned media.

The sterilization of the cultivation environment, which consisted of several 8x3 cm receptacles, was achieved through heating to a temperature of 121 degrees Celsius for 30 minutes (Cachiţă 2004). After cooling, the shrubs were inoculated in the sterile room under aseptic conditions.

The plants were inoculated under normal polarity. After the inoculation, the receptacles which contained the plants (covered in plastic foil and sealed with a rubber band) were placed on shelves with a temperature varying between 20 and 24 degrees Celsius; light exposure was 16 hours out of a 24 hour period and the light intensity was approximately 1700 lux, white light coming from fluorescent tubes.

At intervals of 30 days from inoculation, we noted the regeneration of the plants which developed from the initial inoculation.

RESULTS AND DISCUSSIONS

The biometric parameters used during the experiment were the length of the stems regenerated from the shrubs, the forming of the roots, their number and length, the forming of cali, as well as the number of new stems regenerated due to the initial inoculation and their length.

The data was computed mathematically and the results were compared to the control sample V_0, which served as reference for comparison. These values are represented graphically.

Sixty days after the in vitro cultivation, it was noted that there was significant development of the plants in sample V_1-MS with IAB 1mg/l and BA mg/l compared to the others. When compared to the original sample, the above sample developed longer stems, richer caulogenesis and more buds, as well as branching of ramifications on the main stalk of phytoinoculs, from the main stem. We have also found cali formation, which was also noted in this sample.

Ninety days after the "in vitro" cultivation, it was noted that negative results were obtained in regards to vitroplantlets rhysogenesis, because it never took place; thus in sample V_3-MS with NAA 1 mg/l and BA mg/l, the plants did not survive.

The longest stems were obtained in the sample V_2 (MS with 1 mg/l IAA + 1 mg/l BA) when compared to the original sample, proving this would be the most favorable cultivation environment to develop *Rosmarinus officinalis*

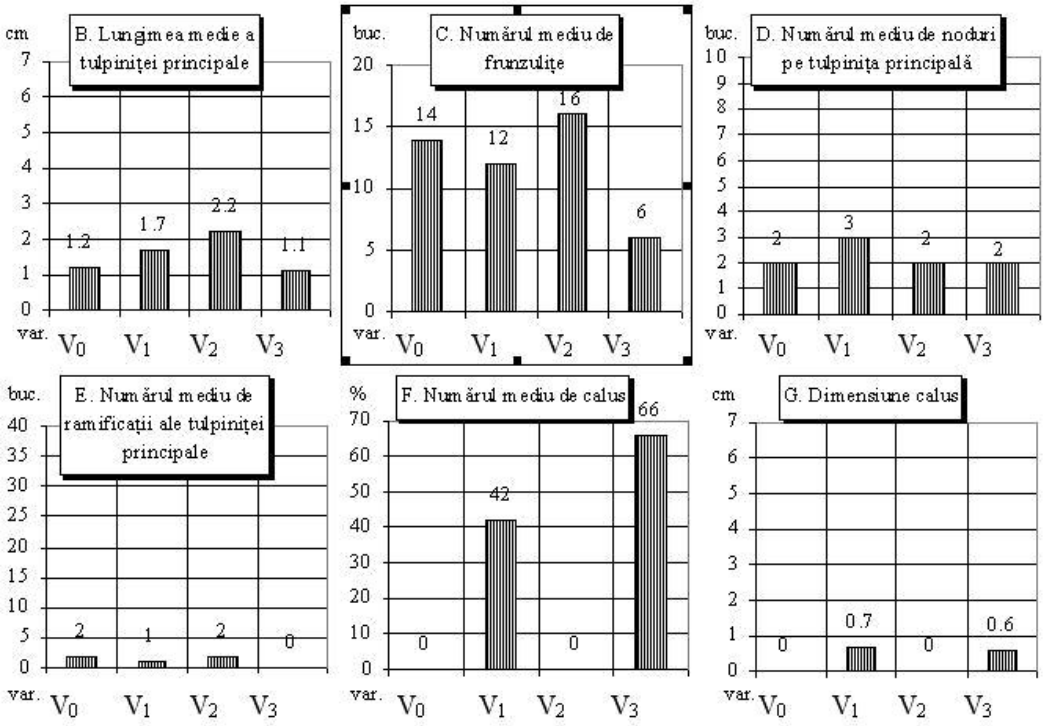

Figure 1. The "in vitro" plantlets of *Rosmarinus officinalis* at 30 days after inoculation.

Legend: 1st graph: Average length of main stem; 2nd graph: Average number of leaves; 3rd graph: Average number of buds; 4th graph: Average number of branches; 5th graph: Average of cali number; 6th graph: cali dimension

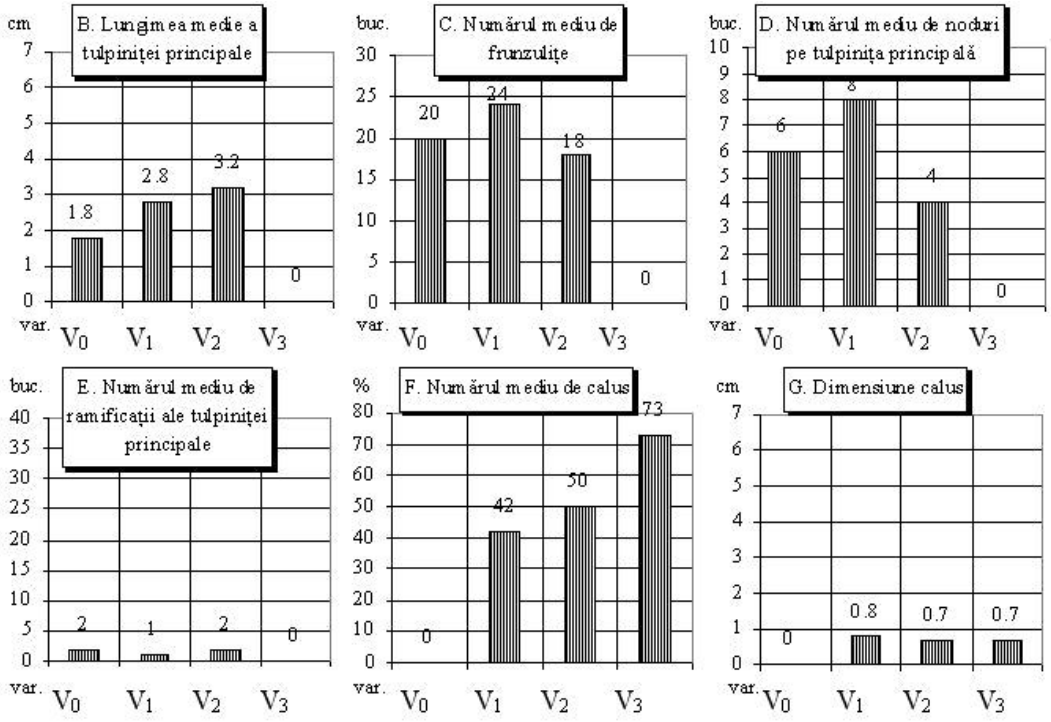

Figure 2. The "in vitro" plants of *Rosmarinus officinalis* at 60 days after inoculation.

Legend: 1st graph: Average length of main stem; 2nd graph: Average number of leaves; 3rd graph: Average number of buds; 4th graph: Average number of branches; 5th graph: Average of cali number; 6th graph: cali dimension.

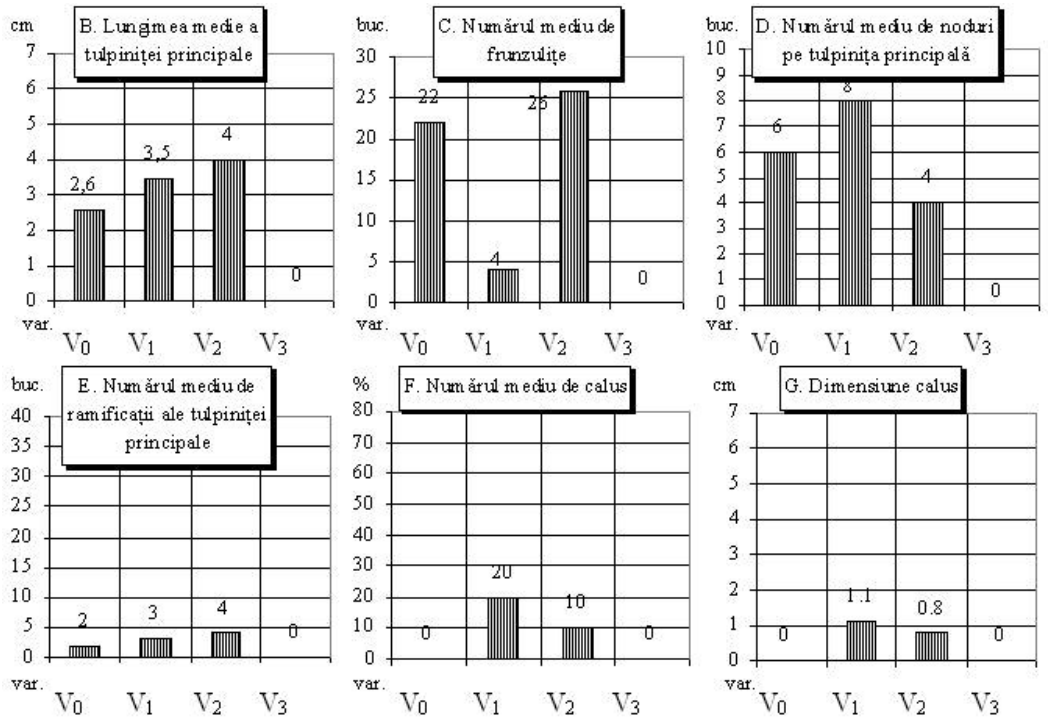

Figure 3. The "in vitro" plants of *Rosmarinus officinalis* at 90 days after inoculation.

Legend: 1st graph: Average length of main stem; 2nd graph: Average number of leaves; 3rd graph: Average number of buds; 4th graph: Average number of branches; 5th graph: Average of cali number; 6th graph: cali dimension

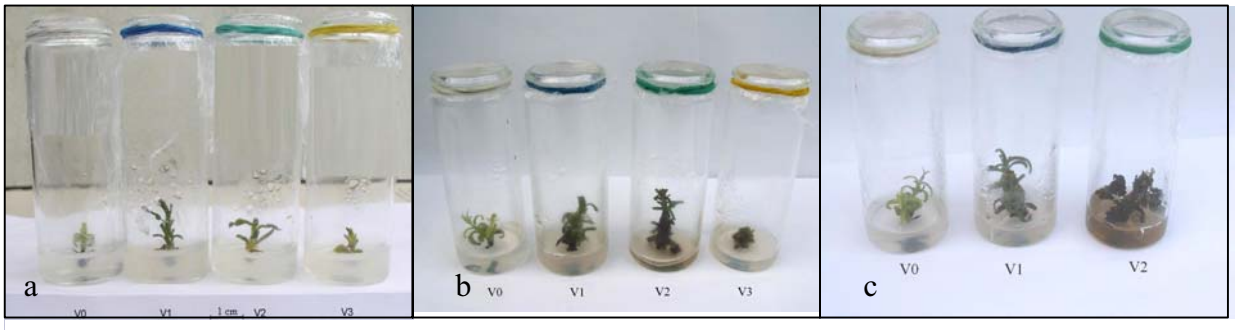

Figure 4. *Rosemarinus officinalis* vitroplantlets at 30 (a), 60 (b), and 90 (c) days from inoculation on aseptic media

CONCLUSION

Our research confirms that the "in vitro" cultivation of this ornamental plant is possible.

Best medium for elongation is V2 (BM with BM - with 2mg/l BA + 1mg/l IAA)

Best medium for obtaining buds is V1 (BM - with 2mg/l BA + 1mg/l IBA)

The mixture BM - with 2mg/l BA + 1mg/l NAA was a bad choice, because the vitroplantlets didn't survive on such a medium.

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