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The effect of growth regulators on callus colors and production of somatic embryos In Vitro of Rosemary Plant (Rosmarinus officinalis L.)

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Abstract: *The study included the use of young leaf explants of rosemary (Rosmarinus officinalis L.) for showing the effect of growth regulators on callus colors and production of somatic embryos In Vitro by using different concentrations of NAA (naphthalene acetic acid) and BA (Benzyladenine) at 16hrs light and 8hrs dark.*
Keywords *- color callus, grown regulators, rosemary, somatic embryos.*

I. INTRODUCTION

Rosmarinus officinalis L. belongs to the class Dicotyledon order Tubiflorae family Lamiaceae. Rosemary (*Rosmarinus officinalis* L.) is an economically important plant of family Lamiaceae. It is a woody perennial, aromatic herb, native to the Mediterranean region, but is now grown worldwide. The Lamiaceae family seems to be a rich source of plant species containing large amounts of phenolic acids, so it is considered to be a promising source of natural antioxidans[1].

Rosemary is considered one of the most important sources for the extraction of phenolic compounds with strong antioxidant activity.

The chemical constituents of essential oil of rosemary include flavonoids such as carnasol, carnosic acid and rosmarinic acid, and volatile oils[2].

This specie grows worldwide and has been cultivated since long ago, in ancient Egypt, Mesopotamia, China and India[3].

Rosemary extracts, enriched in phenolic compounds are effective antioxidants due to their phenolic hydroxyl groups but they also possess plenty of other beneficial effects like antimicrobial, antiviral, anti-inflammatory, anticarcinogenic activities and is also known to be an effective chemopreventive agent[4]. Rosemary extract formulations are the only ones commercially available for use as antioxidants in the European Union and the United States, and they are marketed in an oil-soluble form, as a dry powder, and in water-dispersible or water-miscible formulations[5]. Also, rosemary is widely used as a culinary spice and is also used for its fragrance in soaps and cosmetics. The leaves of rosemary contain 1.0-2.5% essential oils and such composition may vary according to the chemo type and the development stage at which the plant has been harvested. The essential oil is almost colorless to pale yellow liquid with a characteristic refreshing and pleasant odor[6].

Due to the increase in the demand of this plant worldwide in pharmaceutical, cosmetic and food industry[7] there is a need of propagation methods which can increase its production. At present, the propagation of rosemary is carried out essentially through cuttings as its seeds have poor germinating ability[8]. The field grown plants are exposed to variations of seasonal stresses which significantly alters the level and quantity of the essential oils[9]. Plant tissue culture has been used as a tool for the conservation and rapid micropropagation of medicinal plants and also for providing a source of secondary metabolites[10].

The introduction of the paper should explain the nature of the problem, previous work, purpose, and the contribution of the paper. The contents of each section may be provided to understand easily about the paper.

II. MATERIALS AND METHODS

The study was conducted at the micropropagation laboratory of the Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest during the period October 2013 - June 2014 on the rosemary plant (*Rosmarinus officinalis* L).

2.1 Explants preparation and sterilization

Young leaf explants were taken from rosemary plants that were container grown. For buds sterilization, before the *in vitro* stabilization, two types of chemical sterilizers were used: ethanol (C_2H_5OH) and sodium hypochlorite (NaOCl). The ethanol was used in concentration 70% for 30 seconds. The leave sterilization with sodium hypochlorite (NaOCl) was applied following the same experimental design with six concentrations (1, 2,

3, 3.50, 4, 4.50%) and five treatment periods (5, 10, 15, 20 and 25 minutes respectively). After the end of the sterilization process, the leave were washed with distilled sterilized water for three times.

2.2 Preparation of nutrient medium

Murashige & Skoog medium (MS, 1972) supplemented with sucrose as a source of energy as well as vitamins (Walkey Vitamins) and plant growth regulators was used for explants inoculation. As growth regulators, 6 x 6 combinations of benzyl adenine (BA) in concentration of 0.0, 0.5, 1.0, 1.5, 2.0 and respectively, 3.0 mg/l and naphthalene acetic acid (NAA) in concentration of 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l, were added to the culture medium to stimulate callus formation. The autoclave was used for 20 minutes at 120°C and pressure of 104 bar for sterilization. Then, the sterilized leave explants were inoculated on culture media and then placed in an incubation room at 25±1°C, 1,000 Lux light intensity and 16 hours daily lighting.

2.3 Measurement of the callus size (volume)

In order to measure the size, the mass callus was extracted using a sterile forceps and was then washed with distilled sterile water several times to remove the remaining culture medium. The block callus was then put inside a graduated cylinder containing sterile distilled water at a given volume and the supplementary volume was recorded and expressed in mm^3.

III. RESULTS AND DISCUSSION

3.1 The effect of different concentrations of NAA and BA and their interaction on callus volume (mm^3) grown at 16hrs light and 8hrs dark

Data shown in „Table 1" indicate that the levels of BA and NAA and overlap of two, significantly affected the volume of formed callus. The concentration of 1.5 mg/l BA and 1.5 mg/l NAA gave the highest volume of callus (14.4 mm^3). It is noted in the same table that the lowest volume (0.9 mm^3) at 0.5 mg/l BA.

By increasing the concentrations of NAA to 2.0 mg/l or more, the callus size reduced and this shown that high concentrations led to inhibit the callus growth. The variants free of NAA determined the severe inhibition of cells, leading to weak growth.

3.2 Effect of NAA and BA and overlap in the estimation of color callus after 30 day

„Table 2" shows that, the treatment with BA and NAA have led to a significant effect on the color callus. As we can see 0.5 mg/l BA concentration influenced the green coloring. The concentration of 1.5 mg/l BA and 1.5 mg/l NAA determined a pure white color callus.

2.0 and 3.0 mg/l BA concentration made the callus look from light brown to brown.

3.3 The impact of BA and NAA (mg/l) and the overlap between them in the percentage of somatic embryos after 45 days of cultivation Rosemary plant leaves in the MS medium

The results shown in „Table 3" show that the treatment of BA and NAA have determined a significant increase in somatic embryos after 45 days of cultivation Rosemary plant leaves in the MS medium. The concentration of 2.0 mg/l BA had a significant effect – 10.18, while the lowest rate (1.61) was registered in the control treatment.

Similar results, with a value which stood above 8.1, were registered at a concentration of 1.5 mg/l NAA.

IV. FIGURES AND TABLES

Table 1. Effect of different concentrations of NAA and BA and their interaction on callus volume (mm^3) grown at 16hrs light and 8hrs dark

NAA (mg/l) / BA (mg/l)	0.0	0.5	1.0	1.5	2.0	3.0	Average BA
0.0	0.0	2.0	2.2	2.4	1.9	1.6	1.68
0.5	0.9	2.6	2.9	3.5	4.4	2.3	2.76
1.0	1.2	3.1	3.7	4.3	6.1	2.9	3.55
1.5	1.6	6.2	7.4	14.4	11.5	9.3	8.40
2.0	1.3	3.3	6.9	6.2	7.5	8.2	5.56
3.0	1.1	2.6	5.4	3.5	5.4	7.5	4.25
Average NAA	1.1	3.3	4.8	6.1	6.1	5.3	
LSD≤ 0.05	BA = 0.1532		NAA = 0.1532		BA x NAA = 0.3754		

Table 2. Effect of NAA and BA and overlap in the estimation of color callus after 30 days

NAA **BA**	**0.0**	**0.5**	**1.0**	**1.5**	**2.0**	**3.0**
0.0	----------	Weightish	White	White	White	White
0.5	Green	Green	Green	Green	Green	Green
1.0	White	White	White	Pure white	White	White
1.5	Pure white	White	White	Pure white	White	White
2.0	Light brown	Light brown	Light brown	Brown	Brown	Light brown
3.0	Light brown	Brown	Brown	Brown	Light brown	Light brown

Table 3. The impact of BA and NAA (mg/l) and the overlap between them in the percentage to be somatic embryos after 45 days of cultivation Rosemary plant leaves in the MS medium

NAA (mg/l) BA (mg/l)	0.0	0.5	1.0	1.5	2.0	3.0	Average BA
0.0	0.0	1.3	2.1	2.3	2.2	1.8	**1.61**
0.5	2.2	2.4	3.7	6.6	3.5	6.4	**4.50**
1.0	2.5	3.2	5.4	7.5	5.3	7.9	**6.20**
1.5	6.3	7.3	7.2	10.5	8.4	10.3	**8.33**
2.0	8.8	7.0	8.1	13.4	12.2	11.6	**10.18**
3.0	3.5	6.6	6.4	8.3	7.4	9.7	**6.98**
Average NAA	3.9	4.6	5.5	8.1	6.5	8.0	
LSD≤ 0.05	BA = 0.1637	NAA =0.1 637		BA x NAA = 0.401			

Fig 1. Callus color output from the plant rosemary leaves on the nutrient medium(MS) resulting from various combinations of growth regulators (BA) and (NAA).

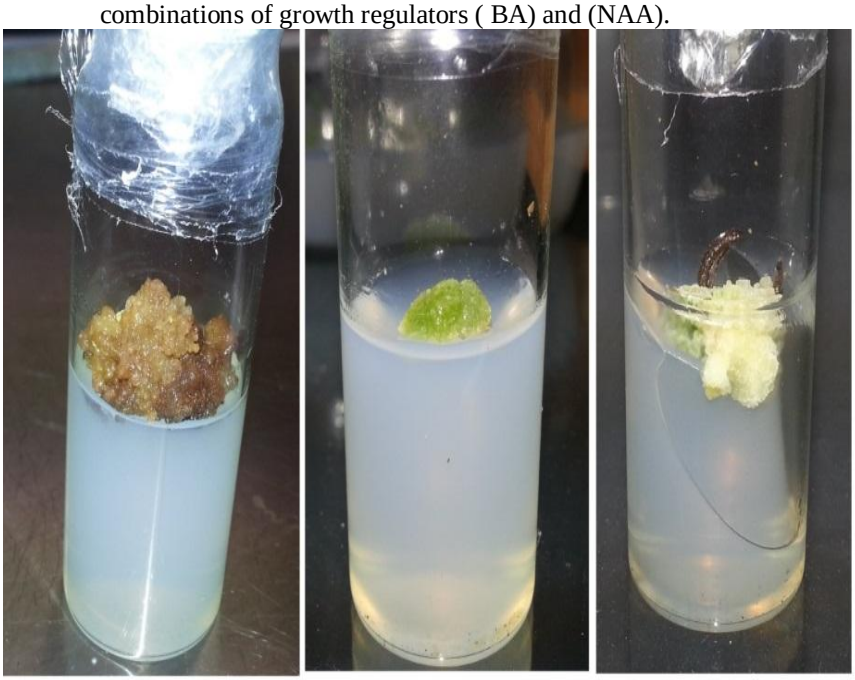
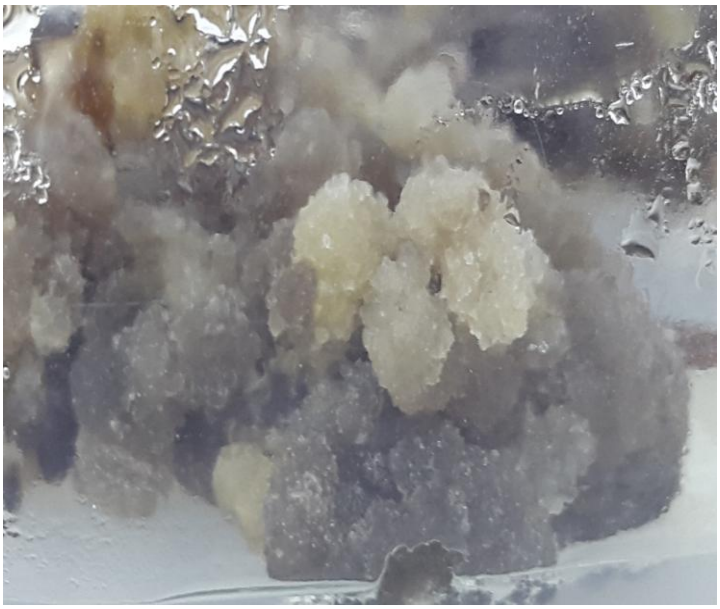


Fig 2. Callogenesis and somatic embryogenesis from leaf explants of Rosmarinus officinalis. a Maximum callogenic response from leaf explants.



V. CONCLUSION

The effect of 1.5 mg/l BA and 1.5 mg/l NAA gave the highest volume of callus (14.4 mm^3), grown at 16hrs light and 8hrs dark.

The study show that the treatment with BA and NAA have led to a significant effect on the color callus, from green, to white or brown. The concentration of 1.5 mg/l BA and 1.5 mg/l NAA determined a pure white color callus.

The best percentage of somatic embryos after 45 days of cultivation Rosemary plant leaves in the MS medium was registered at he concentration of 2.0 mg/l BA had a significant effect – 10.18.

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