Abstract

Although *Rosmarinus officinalis* propagates in vegetative way in its natural state, the propagation rate is too slow to meet the demand of high-quality planting material for commercial cultivation. The field grown plants experience environmental stress and thus results in low yield, so, the propagation of Rosemary through cutting are taking place widely nowadays. Plant tissue culture technique has been used as a potential tool for rapid micropropagation to enhance the quantity and quality of essential oils.

Micropropagation method for elite selection of *Rosmarinus officinalis* by axillary branching method using shoot tip as explant was standardized. An alternative method with more consistent results to obtain phenolic diterpenes from Rosemary is a biotechnological approach, specifically, plant tissue culture.

In this research, different culture types (callus cultures, suspension cultures, and shoots from *in vitro* propagated plants) of Rosemary

The study included the use of tissue culture technique in Rosemary (*Rosmarinusofficinalis*L.) plant propagation starting from leaves, apical buds, axillary bud explants for the production of callus of *Rosmarinus officinalis* L., one of the most important sources for the extraction of phenolic compounds with strong antioxidant activity.

The first experiment consisted in the apical buds explants sterilization using ethanol 70% for 30 seconds (C_2H_5OH), mercuric chloride ($HgCl_2$) and sodium hypochlorite (NaOCl). The mercuric chloride ($HgClO_2$) gave the maximum number of healthy sterile Rosemary leaves (5.10) for 6 minutes sterilization time at the concentration of 0.06 mg/l. The highest number of healthy Rosemary leaves (2.72) on average was obtained when the sterilization period for 15 minutes the sodium hypochlorite (NaOCl) was used at the amount of 0.75 mg/l. After sterilization with sodium hypochlorite, the highest average number of healthy buds (3.24) was obtained when the duration of sterilization was 20 minutes. The values drop to 2.70 healthy buds at the duration of 15 minutes while the lowest number of 1.18 buds was obtained at 5 minutes sterilization time.

After the explants sterilization, the effect of six concentrations of benzyl adenine (BA) and six concentrations of naphthalene acetic acid (NAA) and the overlap between them on the callus formation were studied. The study showed that the highest callus volume (10.2 mm^3) was produced by the overlap between BA and NAA in the concentration of 2.0 and 1.5 mg/l. Callus fresh and dry weight (g) was significantly influenced by the combination of BA and NAA. Best results were obtained at concentrations of 2.0 mg/l BA and 2.0 mg/l NAA.

This study also 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. The combination of 1.5 mg/l of NAA and 1.5 mg/l of BA concentration produce pure white color callus. After 45 days of cultivation the best percentage of somatic embryos, Rosemary plant leaves at the concentration of 2.0 mg/l BA a significant effect of 10.18 has been recorded in the MS medium. When the concentrations of NAA is increased to 2.0 mg/l or more, the size of callus reduced which explained that high NAA concentrations of NAA reduce or even inhibit the growth of the callus. However, the medium free of NAA showed the severe cells development inhibition, which leads to weak growth. The largest effect of the overlap between NAA and BA on the average number of vegetative branches from callus was obtained at 2.0 mg/l BA and 1.50 mg/l NAA with the average number of shoots (1.39). The concentration of 2.0 mg/l BA showed an increase in the average number of vegetative branches that is 1.39.

The effect of explant age, plants growth regulators and culture conditions on somatic embryogenesis and rosmarinic acid production from leaf explants of *Rosmarinus officinalis* collected in Bucharest was investigated. Embryogenic callus with numerous spherical somatic embryos could be induced on explants derived from both species and cultured for 3 weeks on a Murashige and Skoog (MS) medium supplemented with 2 mg/l BA and 1.5 mg/l NAA.

The yield of Rosemary (*Rosmarinus officinalis* L.) from active compounds was investigated. The yield of callus tissue was compared with the intact plant production. Callus was induced in leaf explants and maintained on Murashige and Skoog medium (MS) supplemented with NAA and BA. Maximum callus fresh weight was obtained in the combination of 2 mg/l NAA and 1.5 mg/l BA under 16/8 hrs photoperiod, which reached 1780 mg. The GC-MS analysis of leaf extracts revealed the most abundant componentswere α-Pinene (16,33%), eucalyptol (10,54%), camfor (7,45%) and isobornyl formate (8,87%). The composition of rosemary leaf extracts was qualitatively different to those obtained by other authors. The chemical analysis of callus ethanolic extracts showed the most compounds were α-Pinene (8,74%), eucalyptol (2,86%), camfor (2,20%) and a metabolite different from the leaf extract, respectively tetracontane (58,16%). but at higher percentages than in leaf extracts.