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CONSIDERATIONS REGARDING THE EFFECTS OF GROWTH REGULATORS OVER THE „IN VITRO" MORPHOGENETIC REACTION AT *ORIGANUM VULGARIS* L.

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INTRODUCTION

Oregano is a perennial aromatic herb native to Europe and Asia, which is cultured all over the world. At present the demand of this aromatic herb is not only rising in Romania but also in other markets, capturing the interest of small and medium producers like an economic-productive alternative to be taken into account. These are versatile cultures that adapt to changing market modalities owing to their diverse uses, such as dry herbs, essential oils, etc [1,3].

Various approaches have been considered for *in vitro* multiplication of oregano (*Origanum vulgaris* L.) apical meristem and axillary bud culture, induction and development of adventitious buds and somatic embryogenesis [2,3].

Conventional techniques of vegetative propagation of *O. vulgare* based on cuttings are difficult because of the low rates of rooting. The cells and tissues cultures "in vitro" assure a unique opportunity to manipulate the morphogenesis in a perfectly controlled medium, thus offering a powerful complementary instrument that can help in overcoming such problems [4].

Therefore, the objective of this investigation was to develop a protocol for *in vitro* establishment, multiplication, rooting and acclimatization of leading oregano cultivars (*Origanum vulgaris* L) from Romania.

MATERIAL AND METHODS

The selected mother plants utilised as donor source for explants are cultivars maintained at Vegetable Research Station Bacau in controlled conditions. Young shoots of 2 cm length were excised from actively growing plants.

The defoliated shoots were first washed in tap water and the sterilized in 0.1% HgCl2 for 15 minutes, and 3 rinses in sterile distilled water.

The apexes of almost 1,5 cm were excised and inoculated on MS, 1962 culture medium supplemented with different concentrations of kinetin – 1.0 – 2.0 mg/L and 1.0 - 2.0 mg/L BAP in combination with 0.1 – 0.5 mg/L NAA and IAA.

Cultures were incubated at 25±1°C under 16 hr photoperiod of 3000-lux light intensity. The cultures were transferred each 2 weeks on fresh media, for a period of 90 days.

Observation of shoot multiplication and growth were recorded at weekly intervals. After two weeks, shoots of above 3 cm length were harvested and sub-cultured on the same medium. A part of the newly formed shoots that demonstrated a good development of leafs were transferred to rooting medium containing different concentration of NAA.

After eight weeks, the rooted plants were acclimatized and planted in a potting mixture of sterilized sand + vermiculite (1:1 ratio) in plastic cups, hardened in a mist chamber (80% relative humidity) for 2 weeks before transfer to a green house.

Experiments were set up in a completely randomized design and repeated three times, with at least 20 explants per treatment. The percentage of shoot regeneration [(number of explants with adventitious shoots/total number of explants)×100%] and the number of shoots per explant (number of adventitious shoots/total number of explants) were calculated for the explants that had been cultured for 7 weeks.

RESULTS AND DISCUSSIONS

Shoot buds got initiated on nodal segments after 6 days of culture. Immediately after the inoculation, the explants raise their volume and the peripheral parts presented a slight necrosis. The higher frequency (85%) formation of maximum number of shoots was observed in the media variant that contained BAP in combination with NAA. Initially 1 or 2 buds developed, later up to 12 shoots of above 5 cm length were formed in explants in seven weeks. The reaction of the explants on the 16 variants of nutrient medium utilised in our experiments vary depending on the hormonal formuli utilised.

The morphogenetic reaction on medium that contained BAP on lower concentration – 1 mg/L and NAA 0.05 mg/L was quite strong with a very good proliferation of shoots, while the addition of a larger concentration induced only the longitudinal growth of shoots but without bud proliferation.

A part of them degenerated in necrosis or were eliminated because of the secondary infections.

The media variants that allowed the induction of the regenerative processes were characterized through the presence of BAP in association with NAA or IAA. The replacement of BAP with other cytokinine (for example the kinetin) doesn't allow the regeneration. The results obtained by us underline once again the benefic effect that BAP has when comparing to other cytokinins. After almost 17-18 days the shoots were transferred on fresh media that supported the regenerative processes, through the determination of a good proliferation of the shoots (Figure 1).

This association between BAP and NAA also determined the apparition and developments of roots inside the media but also airing roots. This is extremely important because allow us to obtain plantlets more quickly by skipping the rooting period (Figure 2). Depending on the way that plantlets evolved, they were transferred either on a rooting media or directly to hygroponic conditions. The rooting media containing NAA determine a good development of roots, in the same time allowing also the development of foliar system.

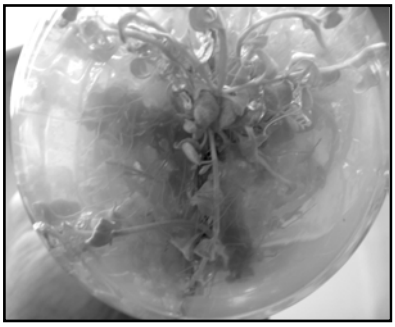
Table 1. Effect of different types and concentrations of plant growth regulators in MS media. Experiments were carried out 3 times

Growth regulators (mg/L)	Regeneration frequency (%)	No. of shoots/explant	Average length of shoots
kinetin			
0.1	38.55	1.5	4.0±0.6
0.5	35.20	1.7	4.0±0.5
1.0	43.33	2.9	4.1±0.1
2.0	36.28	3.0	4.5±0.5
BAP			
0.1	50.45	5.6	5.1±0.7
0.5	50.20	5.7	5.2±0.8
1.0	62.46	6.5	5.6±0.4
2.0	54.46	6.1	5.2±0.1
BAP+NAA			
1.0 + 0.1	78.93	10.2	5.0±0.3
1.0 + 0.5	82.69	11.9	5.2±0.1
2.0 + 0.1	85.36	12.1	5.9±0.3
2.0 + 0.5	83.12	10.3	6.0±0.2
BAP+IAA			
1.0 + 0.1	58.91	8.3	4.8±0.5
1.0 + 0.5	64.25	8.8	4.8±0.2
2.0 + 0.1	71.54	9.6	5.0±0.1
2.0 + 0.5	65.30	8.5	5.7±0.3

Table 2. Effect of different types and concentrations of plant growth regulators in MS media on root induction

Growth regulators (mg/L)	Rooting frequency (%)	No. adventious roots/shoots
NAA		
0.4	88.30	5.15±0.47
0.6	91.80	6.28±0.18
0.8	77.51	3.59±0.33
1.0	60.23	3.30±0.32

a



b

Figure 1 a-b - Neo-formation of the plantlets at the basis of the initial shoot

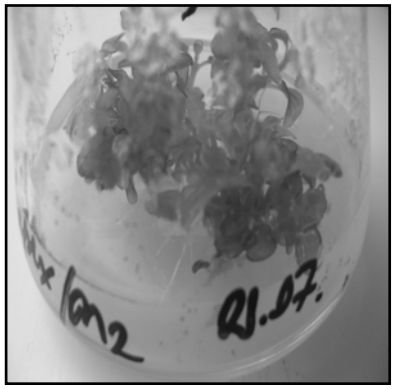


Fig. 2 - The apparition of the airing roots at the plantlets regenerated on BAP and NAA combination media

Roots were observed as early as 2 weeks after placing the microshoots (2-3 cm) on rooting medium. Most of the shoots had developed roots by week 4.

The highest frequency of roots formation was induced in MS supplemented with 0.6 mg/L NAA (Table 2).

Shoots exposed to higher concentrations of NAA (2.0 mg/L or more) became necrotic and lost leaves and the shoot tips died gradually.

The plants that presented a well developed rooting and foliar system were transferred directly in hydroponic conditions for their acclimatization (figure 3). Due to the fact that the humidity must be gradually reduced over time because tissue-cultured plants are extremely susceptible to wilting, the plants were covered with plastic folia for three days and then gradually discovered.

After the plants were fully adapted to the environmental conditions (almost 10 days), we passed them to soil substrate in plastic recipients (figure 4) and then utilized it in the breeding activity in open-field or greenhouses.

Fig. 3 - The acclimatization stage

Fig. 4. Fully adapted plants, in plastic recipients

The potential of in vitro propagated O. vulgaris plantlets to be used for ex vitro establishment was investigated with plantlets transferred to soil pots after 2 weeks of initial hardening under culture-room conditions. Almost 93% of these regenerants survived and showed new branch development. These may be useful for the production of somaclonal variants for breeding programs

ABSTRACT

Origanum vulgare L. – oregano, is a perennial plant of 0.6 - 0.8 m, that belongs to the *Lamiaceae* family. Oregano is an important aromatic plant utilised both as culinary and medicinal plants. Tissue culture „in vitro" is a useful method for large scale production of pathogen-free plants. In this study in order to determine the best hormone variant that allows the obtaining of a large number of plants, apical shoots of young plants grown in controlled conditions were utilised. The explants were cultured in solid MS medium supplemented with different concentrations of kinetin – 1.0 – 2.0 mg/L and 1.0 - 2.0 mg/L BAP in combination with 0.1 – 0.5 mg/L NAA and IAA. Multiple shoots were obtained from the apical explants, the higher frequency (85%) formation of shoots was observed in the media variant that contained BAP in combination with NAA. Initially 1 or 2 buds developed, later up to 12 shoots of above 3 cm length were formed in node in two weeks. Shoots were multiplied by subculture on the same medium. The shoots rooted on the same media. The rooted plantlets were hardened and successfully established in pots at 85% success rate.

The reported experimental dates represent viable methods of plant regenerations of *Origanum vulgaris* L. through shoot tip culture.

CONCLUSIONS

- the results obtained in the present work showed that the micropropagation of *Origanum vulgare* L. „in vitro" is a viable tool for the production of identical pathogen-free plants for agriculture;
- the higher frequency (85%) formation of maximum number of shoots was observed in the media variant that contained BAP in combination with NAA. The replacement of BAP with other cytokinine (for example the kinetin) doesn't allow the regeneration of plants;
- the maximum number of shoots/explant was observed on hormonal formuli with BAP 2.0 mg/L and NAA 0.1 mgL, the increase in the quantity of hormons determined a decrease in the number of shoots as some of them become necrotic;
- the highest frequency of roots formation was induced in MS supplemented with 0.6 mg/L NAA.
- almost 93% of these regenerants survived and showed new branch development. These may be useful for the production of somaclonal variants for breeding programs.

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