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Nutrition and Micropropagation of *Origanum vulgare x applii*

María A. Bracamonte[1], P. Bima[1], G. Bongiovanni[2] and Marta Goleniowski[2]

[1]Laboratorio de Biotecnología Vegetal. Facultad de Ciencias Agropecuarias, Universidad Nacionald de Córdoba, Córdoba, Argentina. [2]Agencia Córdoba Ciencia-Unidad CEPROCOR, Arenales 230, Córdoba, Argentina. e-mail: goleniow@ceprocor.uncor.edu

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 Argentina but 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.

It is one of the classic plants of the traditional herboristery. In infusion, it is used traditionally to relieve dysmenorrhea, and cough because of its antispasmodic action. Because of this action, it is useful for digestive system spasms. It also alleviates cold and asthma symptoms. As liniment, it is applied in cases of sciatica, arthritis, rheumatism, and other articulation affections (Biondi *et al.*,1993). Oregano belongs to the Lamiaceae family, in which are most of the aromatic species. One of the oregano species that grows in Argentina is *Origanum vulgare* x *applii* ('negrito'). This species was *in vitro* introduced with a good yield of seedlings (Goleniowski *et al.*, 2002). Since in the agricultural practices for field fertilization of the species is recommended to assure a contribution of the three fundamental elements (N, P, K), it is interesting to know the *in vitro* behaviour of the plantlets at different concentrations of these nutrients.

METHODOLOGY

Of the previously obtained *in vitro* cultures, uninodal cuttings were extracted, which were seeded on 10 ml Murashige and Skoog (MS) medium containing 30 g/l sucrose and sucrose 8 g/l agar (Agar-Agar), 0.1 mg/l NAA, 1 mg/l AG3, 0.1 mg/l BA. This basal medium was used as control (1). To observe the effect of different nutrient concentrations the following treatments were performed: (2) MS containing half of the basal medium K^+ concentration; (3) MS containing 50% more K^+ than the basal medium concentration; (4) MS containing half of the basal medium PO_4^{-3} concentration; (5) MS containing 50% more PO_4^{-3} than the basal medium concentration; (6) MS containing half of the basal medium NH_4^+ concentration; (7) MS containing 50% more NO_3^- than the basal medium (8) MS containing half of the basal medium NO_3^- concentration; (9) MS containing 50% more NH_4^+ than the basal medium. In the case of reduction of the ions PO_4^{-3}, K^+, NO_3^- and NH_4^+ ions in the medium, cations and anions were replaced by ClK, ClNa, $(NO_3)_2Ca$ and $SO_4(NH_4)_2$, being achieved the same equivalence levels of the basal medium (MS). The pH was adjusted at 5.8, and the media were sterilized at 121°C for 20 min. Cultures were maintained at 22°C, and subjected to a photoperiod of 16 h light (60 - 70) $E^{-2}.S^{-2}$ by means of fluorescent tubes. At 4, 8 and 12 weeks of culture, growth parameters were determined: number of branches, number of knots, main axis lenght, root occurrence, and fresh weight.

RESULTS AND CONCLUSION

In all treatments axillary buds (one or two) were able to regenerate *in vitro* oregano plants, except for treatment (9), in which plants showed necrosis syndrome and death (not shown). All treatments turned out to be statistically significant except in the case of: number of branches (Table 1) in treatments (3), (4) and (5) for 4 weeks growth. In that culture time data on the number of knots were also not significant (Table 2): Treatments (4), (5) and (6), main axis length: treatment (6) (Table 3), and in relation to root occurrence (Table 4): (4), (5) and (8).

After 8 weeks growth, treatments (2) and (8) were not significant in relation to the number of knots and branches, respectively. At 12 weeks growth, treatments
(7) and (8) were not significant only in relation to the number of branches. In relation to the number of

branches, number of knots, main axis length, the best results were obtained with treatment (1)

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Control.

On the other hand, plants grown at a lower PO_4^{-3}, and K^+ ions concentration showed yellowish leaves in the basal part, because these minerals are easily redistributed from the old leaves to the new ones showing chlorosis symptoms. Under the studied conditions a dosis increase treatments that received more NO_3^-, PO_4^{-3} and K^+ did not modify growth rate when comparing with the control. Whereas after 12 weeks, a decrease of ion levels reduced plantlet growth in the studied parameters: number of branches and knots, percentage of plantlets with roots.

Tables: Values are the average of determinations carried out in 24 **yemas** cultured for 4, 8 or 12 weeks under different conditions ± SD. Not-highlighted numbers are significantly different to the value of treatment 1. $p < 0.05$.

Table 1. Number of Branches

	time (weeks)		
Treatm.	**4**	**8**	**12**
1	2.7 ± 1.0	3.9 ± 2.2	3.9 ± 2.5
2	2.1 ± 0.3	2.3 ± 1.1	2.6 ± 1.5
3	2.2 ± 1.7	2.8 ± 1.6	2.7 ± 2.2
4	2.4 ± 1.7	2.4 ± 1.7	2.1± 0.5
5	2.2 ± 0.9	2.3 ± 0.9	2.4 ± 1.1
6	2.0 ± 0.0	2.0 ± 0.0	2.1 ± 0.3
7	1.9 ± 0.3	5.7 ± 3.2	5.3 ± 2.6
8	2.0 ± 0.0	3.3 ± 2.4	3.8 ± 3.1

Table 2. Number of Knots.

	time (weeks)		
Treatm.	**4**	**8**	**12**
1	4.8 ± 0.9	9.5 ± 1.8	12.8 ± 2.5
2	2.8 ± 0.5	3.9 ± 1.1	6.6 ± 2.2
3	2.4 ± 1.1	4.4 ± 1.2	5.7 ± 2.9
4	4.7 ± 1.2	4.7 ± 1.2	8.5 ± 2.4
5	5.3 ± 2.2	5.3 ± 2.2	9.2 ± 3.6
6	4.1 ± 1.7	4.1 ± 1.7	6.4 ± 3.4
7	3.1 ± 1.1	7.3 ± 4.5	7.0 ± 1.8
8	2.9 ± 0.7	4.7 ± 1.7	6.6 ± 3.3

Table 3. Main Axis Length

	time (weeks)		
Treatm.	**4**	**8**	**12**
1	2.7 ± 0.6	10.1 ± 1.3	14.2 ± 2.3
2	1.1 ± 0.1	2.6 ± 0.7	5.9 ± 2.9
3	1.4 ± 0.4	4.4 ± 2.4	6.1 ± 2.6
4	3.8 ± 0.9	3.8 ± 0.9	6.9 ± 2.5
5	4.3 ± 2.1	4.3 ± 2.1	10.5 ± 4.4
6	3.1 ± 1.8	3.1 ± 1.8	5.6 ± 3.1
7	1.9 ± 0.6	5.6 ± 2.3	9.1 ± 2.6
8	1.7 ± 0.4	4.2 ± 2.1	9.8 ± 5.1

Table 4. Root Occurrence (%)

	time (weeks)		
Treat.	**4**	**8**	**12**
1	86.9 ± 34.4	100	100
2	14.3 ± 35.8	75.0 ± 44.4	82.3 ± 39.2
3	52.2 ± 51.1	77.3 ± 42.8	80.0 ± 41.4
4	80.0 ± 41.0	80.0 ± 41.0	87.5 ± 34.1
5	100	100	100
6	28.6 ± 46.3	28.6 ± 46.3	57.8 ± 50.7
7	46.1 ± 51.8	100	100
8	73.3 ± 45.7	100	100

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

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