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In Vitro Shoot Regeneration of Oregano (*Origanum minutiflorum* O. Schwarz & Davis)

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Abstract

In vitro shoot regeneration procedure for oregano (*O. minutiflorum* O. Schwarz & Davis), endemic species of Turkey, is described. Leaf segments and shoot explants (hypocotyl, single nodal segment and shoot tip) excised from 30-40 d old *in vitro* germinated seedlings and cultured on Murashige and Skoog (MS) and Gamborg (B5) media containing different combinations of benzyl amino purine (BAP) (0.0, 1.0, 2.0 or 3.0 mg L^{-1}) and naphtalene acetic acid (NAA) (0.0, 0.1 or 0.5 mg L^{-1}). The single nodal segments were the most succesful explant in all hormone combinations used. MS medium supplemented with 2.0 mg. L^{-1} BAP + 0.1 mg L^{-1} NAA was the most effective medium for shoot formation. However for all explants, B5 medium with all phytohormone combinations had no effective success and therefore were not suitable on *in vitro* shoot regeneration.

Key Words: *Origanum minutiflorum*, shoot regeneration, *in vitro*, micropropagation

Introduction

Endemic plants greatly contribute to the richness and diversity of the flora of Turkey. Bajaj et al. (1988) have been pointed out the growing interest worldwide in medicinal and aromatic plants. Conservation of endemic, endangerd, medicinal and araomatic plants is beyond regional scope and becomes of global signifigance (Ekim et al., 2000; IUCN, 2001). They should be protected by different methods including *in vitro* culture. *In vitro* propagation is a suitable method for plant regeneration, micropropagation and long-term storage of plant material.

There is a few information about *in vitro* culture of *Origanum* species in the current literature (Kumari and Saradhi, 1992; Crutis et al., 1996; Iyer and Pai, 2000; Tisserat and Silman, 2000; Minnas; 2001). Plants belonging to genus *Origanum* L. (Lamiaceae) are represented by 25 taxon in Turkey and 16 of them are endemic (Davis et al., 1982; Kitiki, 1996; Duman; 2000). Some species of these plants are well known in Anatolian folk medicine and widely used as spices and herbal tea (Baytop, 1983). They are of great economic importance which is not only related to their use as a spice. In fact, as recent studies have pointed out, oregano is used traditionally in many other ways as their essential oils have antimicrobial, antifungicidal, cytotoxic, antiviral, nematicidal and antioxidant properties (Lagouri et al., 1993; Sivropoulou et al., 1996). This work attempts to develop procedure for *in vitro* shoot regeneration of this species.

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Materials and Methods

Seeds of *O. minutiflorum* were collected from a natural habitat (C3 Antalya: Saklıkent Bakırlı tepesi etekleri, 1800 m) and were sterilized by immersion for 25 min in 10% sodium hypochlorite (NaOCl), then rinsed three times with sterile water. MS (Murashige and Skoog, 1962) and B5 (Gamborg et al., 1968) hormone-free media were used for seed germination *in vitro*. Seeds were germinated in a growth chamber at $25 \pm 2\,^{\circ}C$, continuously dark conditions, during 21 days. At the end of this period seedlings were incubated for 30-40 days under a 16-h light/ 8-h dark photoperiod (40 µmol. $m^{-2}.sn^{-1}$ light intensity) in the same media and conditions. Hypocotyl, shoot apex, leaf segments and single node explants excised from these seedlings were cultured on MS and B5 media supplemented with 6-benzyl amino purine (BAP) (0.0, 1.0, 2.0 or 3.0 mg L^{-1}) and naphtalene acetic acid (NAA) (0.0, 0.1 or 0.5 mg L^{-1}) combinations, 3% sucrose and 0.6% agar. Incubation conditions were the same as indicated for seedling development. The experiments were set up in a completely randomized design. Data were analyzed by analysis of variance (ANOVA) to detect signifigant diferences between means (Sokal and Rohlf, 1995). Means differing significantly were compared using Duncan's multiple ragne test (DMRT) at the 1% porbability level.

Results and Discussion

Seeds were cultured on MS and B5 hormone-free media for germination. At the end of 21 days, 100 % germination was observed from seeds cultured on MS medium and 20 % germination was observed from seeds cultured on B5 medium. Hypocotyl, shoot apex, leaf segments and single nodal segments were excised from 30-40 days old seedling (Figure 1) and were cultured on MS and B5 media supplemented with BAP (0.0, 1.0, 2.0 or 3.0 mg L^{-1}) and NAA (0.0, 0.1 or 0.5 mg L^{-1}) combinations. The influence of different BAP and NAA cominations of MS medium and various explant types on shoot differentiation of *O. minutiflorum* are given in Table 1. Single nodal segments are the best source for the highest shoot induction obtained on MS medium (Table 1, Figure 2) and no succees was obtained for other explants types. However for all explants, B5 medium has no effective success and therefore were not suitable on *in vitro* shoot regeneration.

Figure 1. 30-40 days old seedlings of *O. minutiflorum* cultured on MS medium.

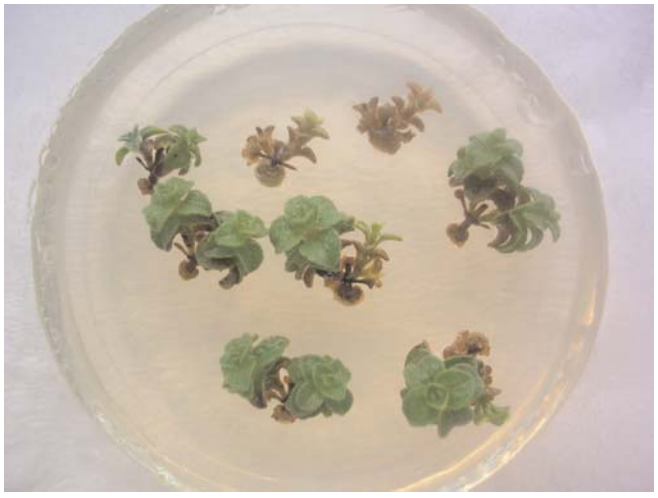

Figure 2. Shoot regeneration from single node explants of *O. minutiflorum*

The influence of NAA and BAP individually and combinations on shoot induction *in vitro*, seems to be general phenomenon (Celloreva, 1992; Kumari and Saradhi, 1992; Faria et al., 1998; Iyer and Pai, 2000; Minnas; 2001; Tepe et al., 2002). BAP was found to be the most efficient in promoting shoot regeneration as in Ayan et al. (2005) study's and micropropagation quantified by number of shoot/explant was higher in the presence of high BAP and low NAA concentrations therefore micropropagation was BAP-dependent. *In vitro* shoot formation may be subjected to change depending upon the explant types used (Zobayed and Saxena, 2003) Our results confirm those of Celloreva (1992), Iyer and Pai (1998, 2000) showing the achievement the shoot induction from nodal segments, that is the best source for the highest shoot induction. Similarly changeable response to shoot induction media from different explants was reported by different resaerchers (Gupta and Conger, 1998; Zobayed and Saxena, 2003; Ayan et al., 2005). In our study MS medium had more effective success than B5 medium. This shows that the importance of strength of the nutrient medium. Generally, low nutrient medium strength enhance *in vitro* regeneration (Kumari and Sardahi, 1992; Werbrouck and Debergh, 1994) and for this reason shoot regeneration of *O. minutiflorum* may increased.

The studies on optimization of *in vitro* propagation conditions of *O. minutiflorum*, endemic species of Turkey that has great economic importance, have been carried out.

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Table 1. Influence of different BAP and NAA combinations of MS medium and various explant types on shoot formation of *O. minutiflorum*.

Explant	BAP (mg L^{-1})	NAA (mg L^{-1})	Number of explant	Mean number of shoot/explant	Mean number of shoot/shooted explant	Shooted explant (%)
Hypocotyl	0.0	0.0	30	--	--	--
	1.0	0.0	30	--	--	--
	1.0	0.1	30	--	--	--
	1.0	0.5	30	--	--	--
	2.0	0.0	30	--	--	--
	2.0	0.1	30	--	--	--
	2.0	0.5	30	--	--	--
	3.0	0.0	30	--	--	--
	3.0	0.1	30	--	--	--
	3.0	0.5	30	--	--	--
Mean			30	--	--	--
Single node	0.0	0.0	30	--	--	--
	1.0	0.0	30	--	--	--
	1.0	0.1	30	0.4 (12) ± 0.14 a*	2 (12/6)	20
	1.0	0.5	30	0.3 (9) ± 0.085 a	1 (9/9)	30
	2.0	0.0	30	--	--	--
	2.0	0.1	30	0.8 (24) ± 0.31 b	4 (24/6)	20
	2.0	0.5	30	0.3 (9) ± 0.085 a	1 (9/9)	30
	3.0	0.0	30	--	--	--
	3.0	0.1	30	0.6 (18) ± 0.18 ab	2 (18/9)	30
	3.0	0.5	30	0.3 (9) ± 0.085 a	1 (9/9)	30
Mean			30	0.27	1	16
Shoot apex	0.0	0.0	30	--	--	--
	1.0	0.0	30	--	--	--
	1.0	0.1	30	--	--	--
	1.0	0.5	30	--	--	--
	2.0	0.0	30	--	--	--
	2.0	0.1	30	--	--	--
	2.0	0.5	30	--	--	--
	3.0	0.0	30	--	--	--
	3.0	0.1	30	--	--	--
	3.0	0.5	30	--	--	--
Mean			30	--	--	--
Leaf segments	0.0	0.0	30	--	--	--
	1.0	0.0	30	--	--	--
	1.0	0.1	30	--	--	--
	1.0	0.5	30	--	--	--
	2.0	0.0	30	--	--	--
	2.0	0.1	30	--	--	--
	2.0	0.5	30	--	--	--
	3.0	0.0	30	--	--	--
	3.0	0.1	30	--	--	--
	3.0	0.5	30	--	--	--
Mean			30	--	--	--

Numbers in the bracets () represent total shoot numbers.
*Means having different letters in same column are significantly different from each other ($p<0.01$) according to Duncan's multiple range test.
-- non observation

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