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The Effects of IBA and H_2O_2 on Rooting of 2 Olive Cultivars

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Abstract: Two groups of olive cultivars were characterized as showing low (Tokhmkabki) and high (Roghani) rooting percentage after application of IBA treatment. Semi-hard wood cuttings were dipped in H_2O_2 (0-3.5% w/v) and IBA (4000 mg.l^{-1}) and also they were investigated in combination. H_2O_2 alone did not stimulate rooting of olive cuttings and there were no significant differences between this treatment and control in both cultivars. It was obvious that IBA treatments increased the rooting percentage, number of root cuttings, root length and root fresh\dry weight in 2 olive cultivars but the combination of IBA and H_2O_2 in some factors was more effective but there were no significant differences when IBA was used instead of in both cultivars.

Keywords: Olive, Rooting, Auxins, Hydrogen Peroxide

INTRODUCTION

The traditional methods used for olive multiplication since ancient times are the purely asexual method of propagation (using suckers or cuttings) and later on the method of grafting seedlings. The main techniques which are now used commercially for olive propagation are rooting of cuttings (Fontanazza, 1996; Cetintas gerakakis, 2005).Rooting hormones should be applied to the base of cuttings to increase overall rooting percentages, hasten root initiation, increase the number and quality of roots and encourage uniformity of rooting. The most widely used hormone is Indole butyric acid (IBA) (Wiesman and Epstein, 1987; Dauod et al., 1989; Fernandes Serrano, 2002; Cetintas gerakakis, 2005; Ozelbaykal and Gezerel, 2005; Bartolini et al., 2008).

In fact, Rugini and Fedeli (1990) reported that the biggest problem in vegetative propagation, in some species of olive is the low ability of regeneration leading to low percentage of rooting. All Mediterranean countries have one or two economically very important but difficult to root olive cultivars. In this research we used "Roghani"(high rooting ability) and "Tokhmkabki" (low rooting ability) cultivars. Various attempts have been made to enhance rooting ability of olive cultivars with different methods of IBA application.

The present study was carried out to investigate the rooting ability of two local olive cultivars (Roghani and Tokhmkabki) in response to H_2O_2 3.5% alone and combined with IBA 4000 mg.l^{-1}.

MATERIALS AND METHODS

Olive cuttings of the Roghani (easy-to-root) and Tokhmkabki (hard-to-root), which are important for black and green table olive cultivars in Iran, were prepared in 12-15 cm length and with 3-4 leaves, in May. Indol-3-butyric acid (IBA) solution at 4000 mg l^{-1}were freshly prepared dissolving IBA powder (Sigma, St. Louis, Mo, USA) in an alcohol/water solution. And hydrogen peroxide 3.5% (w/v) solution was prepared diluting a 35 %(w/v) H_2O_2 stock solution in distilled water. Semi-hardwood cuttings were immediately treated by dipping 2cm of their basal ends in the IBA without H_2O_2 (IBA 4000 mg l^{-1}, 0: control) and compared with cuttings dipped in H_2O_2 solution without IBA (H_2O_2 3.5 %, 0: control) and also treated with combination of IBA and H_2O_2. All of cuttings were placed in basal-heated benches that were filled with perlite and maintained at a constant temperature of (23±2^{o}C). The benches were placed in to a polyethylene green house, the

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green house air temperature ranged between 16 ^{o}C and 26 ^{o}C and the relative humidity was maintained at approximately 50%.
Sampling of semi-hard wood cuttings was performed approximately 120 days after the beginning of the rooting treatments and each cutting was scored for rooting percentage, number of roots, length of the roots, roots fresh/dry weight. Twenty cuttings per plot, replicated 4 times were used for each treatment. Data were statistically analyzed using MSTATC. Analysis of variance was performed to separate means and significant differences were determined with Duncan's multiple range tests at $P \leq 0.05$.

RESULTS AND DISCUSSION

Our results show that the application 4000 mg.l^{-1} IBA improved the rooting percentage in two olive cultivars in comparison with control. Olive rooting is apparently related to the genotype: in fact, the two genotypes used in this study have shown different rooting abilities. Combined application of IBA with H_2O_2 significantly promoted the rooting of cuttings according to untreated ones, but the rooting was low compared to the IBA alone. The highest rooting quality was obtained on "Roghani" cuttings treated with 4000 mg.l^{-1} IBA with a mean root number of 14.06. The mean number of root in "Roghani" was higher than that in "Tokhmkabki" (Table 1).

Table 1. Rooting, Number of roots, Root length and Root fresh/dry weight treated with IBA and H_2O_2 alone and combination of them in "Roghani" cultivar.

Treatments	Rooting (%)	Number of roots	Roots Length (cm)	Roots fresh weight (g)	Roots dry weight (g)
control	15b*	4.42b	5.5b	0.83b	0.14b
H_2O_2 3.5%	17.5b	4.32b	5.75b	0.78b	0.11b
IBA 4000 (mg.l^{-1})	68a	14.06a	15.5a	3.41a	1.62a
H_2O_2 3.5% + IBA 4000(mg.l^{-1})	66.25a	13.78a	16.11a	3.26a	1.51a

*Mean values within a column with same letter are not significantly different based on Duncan's multiple range test ($P < 0.05$).

The highest rooting quality was obtained on "Tokhmkabki" cuttings treated with IBA 4000 mg.l^{-1} + H_2O_2 3.5% with a mean root number of 9.53. Moreover, "Tokhmkabki" rooting quality could be significantly improved by IBA+ H_2O_2 in comparison to control. But this treatment did not differ from IBA alone (Table 2).

Table 2. Rooting, Number of roots, Root length, and Root fresh/dry weight treated with IBA and H_2O_2 alone and combination of them in "Tokhmkabki" cultivar.

Treatments	Rooting (%)	Number of roots	Roots Length (cm)	Roots fresh weight (g)	Roots dry weight (g)
control	5b*	1.27b	2.7b	0.13b	0.08b
H_2O_2 3.5%	6.5b	1.41b	3.01b	0.10b	0.06b
IBA 4000 (mg.l^{-1})	24.5a	9.32a	10.9a	2.02a	0.47a
H_2O_2 3.5% + IBA 4000(mg.l^{-1})	23.75a	9.53a	9.63a	2.18a	0.55a

*Mean values within a column with same letter are not significantly different based on Duncan's multiple range test ($P < 0.05$).

Auxin is well known to stimulate root formation of the cuttings (Hartmann and Kester, 1990; Khattak et al., 2001). Adventitious root initiation in olive cuttings can be stimulated by auxins, particularly indol-3-butyric acid (IBA) but in difficult-to-root cultivars, the auxin either fails to promote rooting or promotes it only slightly (Serrano et al., 2002). The majority of the cultivars showed a moderate or low rooting ability even in response to IBA treatment. The difficulty of rooting in some cultivars was partially attributed to the presence of continuous sheath of sclerenchyma cells (Centeno and Gomez-del-Campo, 2008) or to the increase in cortex thickness during rooting forming mechanical barrier to emergence of root initials. The maximum number of roots in IBA treated may be due to its effect on cell wall turgidity, which accelerates cell division (Rahman et al., 2002) The effectiveness of auxin to raise rooting percentage of the cuttings could be through increasing cambial activity and differentiation of root primordial (Davies and Joiner, 1980) or by stimulating redistribution and mobilization of some auxin cofactors towards base of the cuttings. Auxin-induced root formation requires cell division and appears to involve delaying or reversal of senescence process. These results confirm with the several finding like Wiesman and Markus, 2002; Serrano *et al*., 2002; Pio *et al.,* 2005 ; Rahman *et al*., 2002, and differ from Sebastiani and Tognetti, 2004 in which IBA+ H_2O_2 had significantly higher root number in comparison with those treated with IBA alone in "Frantoio" and "Gentile di Larino".

Root elongation was also induced with these treatments (IBA+ H_2O_2) and roots reached a length significantly higher than those of untreated cuttings in both cultivars. Successful rooting of cuttings is determined both by the number of roots formed and by root elongation and growth (Hartmann and Kester, 1990). No significant differences were found in roots fresh and dry weight when IBA+ H_2O_2 was applied in comparison with IBA alone in both cultivars. The lowest root fresh and dry weights were found in control cuttings. In the current study, treatments with 3.5 % H_2O_2 were not significantly higher than that of untreated cuttings in 2 cultivars in all parameters that measured. The combination of IBA and H_2O_2 in some factors was more effective than IBA alone but this treatment did not differ from IBA alone in 2 cultivars. Exogenously applied H_2O_2 might have been ineffective in rooting of these 2 cultivars (Table 1, 2).

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