Effects of IBA and Putrescine on Root Formation of Olive Cuttings

Elham ASLMOSHTAGHI [1] (✉)
Ali Reza SHAHSAVAR [1]
Mohammad Reza TASLIMPOUR [2]

Summary

Semi-hardwood cuttings of olive cv. 'Tokhmkabki' (low rooting ability) and cv. 'Roghani' (high rooting ability) were obtained from 1-year-old shoots. Cuttings were dipped in 2000, 4000, and 6000 mg·L^{-1} IBA, 150 or 300 mg·L^{-1} putrescine and their combination before rooting in greenhouse equipped with an automatic mist system. Rooting ability was evaluated four months after planting for each treatment. Satisfactory rooting occurred when IBA was applied with putrescine, whereas cuttings treated with IBA or putrescine alone showed a limited capacity of rooting in both cultivars. The greatest rooting percentage for cv. 'Roghani' was detected when IBA at 4000 mg·L^{-1} + putrescine 300 at mg·L^{-1}, IBA at 4000 mg·L^{-1} + putrescine at 150 mg·L^{-1} were applied. For cv. 'Tokhmkabki' the most successful treatments were IBA at 6000 mg·L^{-1} + putrescine at 150 mg·L^{-1} and IBA at 6000 mg·L^{-1} + putrescine at 300 mg•L^{-1}. The current findings confirm that putrescine can be a useful substance for increasing rooting percentage and root quality in cuttings of olive cultivars.

Key words

1,4- diaminobutane, indol-3-butyric acid, olive, polyamine, rooting ability, semi-hardwood cuttings

[1] Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran
✉ e-mail: emoshtaghi11@gmail.com
[2] Kazeroun Olive Research Station, Fars Agricultural and Natural Resources Research Center, Shiraz, Iran

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Introduction

The olive (*Olea europaea* L.) is a long-lived, evergreen tree native to the Mediterranean sea Basin (Avidan and Lavee, 1978). Traditionally; olive cultivars were vegetativly propagated by rooting of 0.4 to 0.6 m long hardwood cuttings. However, this method requires a lot of vegetative material, making genetic and sanitary control difficult. Formation pruning of trees propagated by hardwood cuttings is also expensive because the use of hardwood cuttings promotes a shrub-like growth (Centeno and Gomez-del-Campo, 2008). Hartmann (1946) developed a technique for olive tree propagation by leaf-stem cuttings. Although self-rooted cultivars can be very interesting in establishing new olive orchard, the low rooting ability of difficult-to-root cultivars and the often unsatisfactory viability of cuttings of some easy-to-root cultivars are limiting factors (Wiseman and Epstein, 1987; Wiseman and Lavee, 1995). Many plant and environmental factors, including genotype, nutritional status, phonological stage and environmental conditions determine seasonal variations in rooting ability of woody cuttings (Hartmann et al., 2002). Olive is one of the plants that have great variation between cultivars for rooting of cuttings. In almost every Mediterranean country, there are cultivars that are commercially important for certain country but which have low rooting ability (Ozkaya and Celik, 1993). The availability and mobilization of carbohydrates towards the base of cuttings appear to be a major factor related to rooting of olive cuttings (Aslmoshtaghi and Shahsavar, 2011). Mancuso (1998) showed a marked seasonal variation in rooting ability of olive cuttings, achieving the highest rooting (80%) in spring-summer and the lowest rooting ability (20-30%) in winter. Moreover high variability in olive rooting has been observed between cultivars, ranging from easy to difficult-to-root cultivars. 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). Some studies indicate a possible improvement of the auxin stimulation on adventitious root formation when it is conjugated with cofactors such as phenolics (Bartolini et al., 1986), including flavonoides (Curir et al., 1990) and hydrogen peroxide (Sebastiani and Tognetti, 2004; Sebastiani et al., 2002). Rugini et al. (1990) noticed that 1,4-diaminobutane (putrescine) in combination with IBA promoted early rooting and increased rooting percentage. Accordingly, the aim of the study was to evaluate the effect of IBA, putrescine and their combination on rooting of two olive cultivars with high (cv. 'Roghani') or low (cv. 'Tokhmkabki') rooting ability.

Materials and methods

Olive cuttings of the cv. 'Roghani' (easy-to-root) and cv. 'Tokhmkabki' (hard-to-root), which are important as black and green table olive cultivars in Iran, were taken in May and prepared to be 12-15 cm in length with 3-4 leaves. Indol-3-butyric acid (IBA) solution at 2000, 4000, 6000 mg·L^{-1} were freshly prepared by dissolving IBA powder (Sigma, St. Louis, Mo, USA) in an alcohol/water solution. Putrescine (1,4-diaminobutane, Sigma, St. Louis, Mo, USA) solutions at 150 and 300 mg·L^{-1} were prepared by dissolving the powder in distilled water. Semi-hardwood cuttings were prepared and divided into three groups. From each group and cultivar, 20 cuttings in four replications (total of 80 cuttings) were treated by dipping 2 cm of their basal ends in the IBA at 2000, 4000, 6000 mg·L^{-1}, putrescine at 150 and 300 mg·L^{-1}, and their combination. Untreated cuttings served as control. All of cuttings were placed in basal-heated benches that were filled with perlite and maintained at a constant temperature of (23±2°C). The benches were placed in to a polyethylene green house, the green house air temperature ranged between 16°C and 26°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 and shoots and roots fresh/dry weight and also leaf area of new leaves of cuttings. Data were subjected to analysis of variance (ANOVA). For comparison of means the Duncan's multiple range test was applied ($P<0.05$) using the SPSS 15.0 statistical package (SPSS, Inc., Chicago, USA).

Results and discussion

Significantly higher rooting was obtained on cv. 'Roghani' cuttings treated with IBA at 4000 mg·L^{-1} + putrescine 300 at mg·L^{-1} and IBA at 4000 mg·L^{-1} + putrescine at 150 mg·L^{-1} (71.25% and 70.0%, respectively) in comparison to the other treatments (Table 1). No differences were observed among the values of rooting obtained for cv. 'Tokhmkabki' cuttings treated with IBA at 6000 mg·L^{-1} + putrescine 150 mg·L^{-1} and IBA at 6000 mg·L^{-1} + putrescine 300 mg·L^{-1} (43.75% and 42.5%, respectively) (Table 2). Number of roots was significantly higher in cv. 'Roghani' cuttings treated with combination of IBA at 4000 mg·L^{-1} + putrescine at 300 mg·L^{-1} and IBA at 4000 mg·L^{-1} + putrescine 150 mg·L^{-1} (15.18 and 15.15, respectively) (Table 1). The highest number of roots on cv. 'Tokhmkabki' was obtained in cuttings treated with IBA at 6000 mg·L^{-1} + putrescine at 150 mg·L^{-1} and IBA 6000 +Put 300 mg·L^{-1} with a mean root numbers of 15.14 and 15.03, respectively (Table 2). Combined treatments of IBA at 4000 mg·L^{-1} + putrescine at 300 mg·L^{-1}, IBA at 6000 mg·L^{-1} + putrescine at 150 mg·L^{-1} and IBA at 6000 mg·L^{-1} + putrescine at 300 mg·L^{-1}, and IBA at 6000 mg·L^{-1} applied alone significantly increased root length in cv. 'Roghani' (20.52, 20.44, 20.33 and 20.31 cm, respectively) (Table 1). In cv. "Tokhmkabki", IBA at 6000 mg·L^{-1} + putrescine at 150 mg·L^{-1} significantly increased root length ($P<0.05$) (23.40 cm) compared to all other treatments. Generally, root elongation was induced better with combined treatments with IBA and putrescine than those receiving the IBA, except in those treated with the highest concentration of IBA (6000 mg·L^{-1}). The highest and similar values of root fresh weight was obtained in cv. 'Roghani' cuttings treated with IBA 4000 + putrescine 150 mg·L^{-1}, IBA 4000 + putrescine at 300 mg·L^{-1} and IBA 6000 + putrescine at 300 mg·L^{-1} (3.37 g, 3.35 g and 3.33 g, respectively), whereby these values were not significantly higher than those obtained in the treatments with IBA at 6000 + putrescine at 150 mg·L^{-1}, IBA at 4000 and IBA at 6000 mg·L^{-1} (3.26 g, 3.21 g and 2.86 g, respectively). Cv. 'Roghani' cuttings had the highest root dry weight of 1.74 g in the treatment with IBA at 4000 mg·L^{-1} + putrescine at 300 mg·L^{-1}, followed by 1.65 g obtained in the treatment with IBA at 4000 mg·L^{-1} + putrescine at 150 mg·L^{-1} (Table 1). The highest root fresh and dry

Table 1. Rooting, number of roots, length roots, roots fresh/dry weight in olive cv. 'Roghani' cuttings treated with IBA and putrescine (Put) alone and their combination

Treatments ($mg \cdot L^{-1}$)	Rooting (%)	Number of roots	Roots length (cm)	Roots fresh weight (g)	Roots dry weight (g)
Control	15.00 f	4.42 f	5.45 d	0.83 d	0.10 f
2000 IBA	23.75 ef	7.73 ef	11.28 c	1.27 cd	0.24 ef
4000 IBA	66.25 ab	14.06 ab	15.65 b	3.21 ab	0.47 df
6000 IBA	53.75 cd	11.25 cd	20.31 a	2.86 ac	0.43 df
150 Put	32.5 e	7.32 ef	6.65 d	0.94 d	0.51 de
300 Put	37.5 e	8.92 df	7.31 d	1.12 cd	0.51 de
150 Put + 2000 IBA	46.25 d	9.14 df	11.35 c	1.48 bd	1.04 c
150 Put + 4000 IBA	70.00 a	15.15 a	18.19 ab	3.37 a	1.65 ab
150 Put + 6000 IBA	67.5 ab	13.18 b	20.44 a	3.26 ab	1.10 c
300 Put + 2000 IBA	53.25 cd	11.29 cd	11.48 c	1.67 bd	1.38b
300 Put + 4000 IBA	71.25 a	15.18 a	20.52 a	3.35 a	1.74 a
300 Put + 6000 IBA	66.25 ab	13.78 b	20.33 a	3.33 a	1.36 b

In each column, means followed by different letters differ significantly at $P \leq 0.05$ according to Duncan's multiple range test.

Table 2. Rooting, number of roots, roots length and roots fresh/dry weight in olive cuttings cv. 'Tokhmkabki' treated with IBA and putrescine (Put) alone and their combination

Treatments ($mg \cdot L^{-1}$)	Rooting (%)	Number of roots	Roots length (cm)	Roots fresh weight (g)	Roots dry weight (g)
Control	5.00 f	1.25 d	2.70 f	0.07 g	0.01 f
2000 IBA	11.25ef	2.87 d	4.10 f	0.2 g	0.1 def
4000 IBA	23.75d	8.43 bc	10.93 d	1.02 e	0.47 c
6000 IBA	37.5ab	11.97 ab	20.52 b	2.36 b	1.21 a
150 Put	10.00 ef	2.75 d	3.27 f	0.11 g	0.06 ef
300 Put	8.75 ef	2.87 d	3.38 f	0.17 g	0.08 def
150 Put+2000 IBA	15.00 e	5.08 cd	6.42 e	0.4 f	0.17 de
150 Put +4000 IBA	26.25 cd	10.75 ab	16.94 c	1.35 d	1.03 b
150 Put +6000 IBA	43.75 a	15.14 a	23.4 a	2.55 a	1.28 a
300 Put +2000 IBA	13.75 e	5.91 cd	6.42 e	0.51 f	0.21 d
300 Put +4000 IBA	31.25 bc	13.73 a	18.5 c	1.99 c	1.03 b
300 Put +6000 IBA	42.5 a	15.03 a	20.52 b	2.54 a	1.26 a

In each column, means followed by different letters differ significantly at $P \leq 0.05$ according to Duncan's multiple range test.

weight was obtained on cv. 'Tokhmkabki' cuttings treated with IBA at 6000 $mg \cdot L^{-1}$ + putrescine at 150 $mg \cdot L^{-1}$ and IBA at 6000 + putrescine at 300 $mg \cdot L^{-1}$, and also treated with IBA at 6000 $mg \cdot L^{-1}$ induced a very high root dry weight (Table 2). Auxin is well known to stimulate root formation of the cuttings (Hartmann et al., 2002). The most widely used auxin for commercial rooting is IBA, nevertheless, auxins have failed to promote root initiation or they had only a slight rooting effect in the case of hard-to root olive cultivars (Wiesman and Epstein, 1987; Rahman et al., 2002; Pio et al., 2005). There are great differences in the rooting potential among olive cultivars and these have been categorized in to three groups, easy, moderate and hard to-root cultivars (Avidan and Lavee, 1978; Wiesman and Markus, 2002; Ullah and Awan, 2004). Therefore, the selection of genotype with high rooting ability is of a prime importance. Most olive growers know that a rooting percentage lower than 20% in most cases means that vegetative propagation is not economically viable (Wiesman and Laree, 1995). 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. As shown in Tables 1 and 2, rooting in general was lower in control treatments than for any treatment involving a hormone. The results of Rugini et al. (1990) were nearly identical as to the results of our experiments, with the control and treatments involving putrescine alone producing a similar and lower level of rooting, treatments with IBA producing a second and greater level of rooting and treatments with a combination of putrescine and IBA producing the highest level of rooting in both cultivars (Tables 1 and 2). These findings are in agreement with previous reports on these two compounds (Rugini et al., 1990; Nag et al., 2001; Naija et al., 2009). The effectiveness of auxin to raise rooting percentage of the cuttings could be explained through increasing cambial activity and differentiation of root primordial tissue (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. Furthermore, exogenous application of arginine, a precursor of polyamine synthesis, was found to be effective in promoting root formation of some cuttings. Therefore, it seems that polyamines may be involved in the control of root formation in cuttings (Nag et al., 2001; Tang and Newton, 2005). Buritin et al. (1990) indicated that auxin-induced root formation was accompanied by increasing levels of putrescine. The application of putrescine during the root induction phase resulted in an increase of endogenous putrescine, endogenous auxin, and peroxidase activity. These observations provide evidence for the hypothesis that putrescine played a role in the rooting induction phase. This was a proof that auxin and polyamine were active factors in the rooting process (Rugini et al., 1990; Naija et al., 2009; Gemici et al., 2006). No significant differences were found in shoot fresh/dry weights and leaf area surfaces in both cultivars in all of the treatments.

Conclusion

The results of the present study suggest that IBA increases the rooting ability in the easy-to-root 'Roghani' olive cultivar but it was ineffective in stimulating rooting in the difficult-to-root 'Tokhmkabki'. Putrescine can be a useful substance in increasing olive rooting percentage and the quantity of roots in cuttings as obtained in both cultivars. Therefore, the idea of using putrescine with IBA also allows for obtaining satisfactory rooting from cuttings known as unfavorable to rooting.

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