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ROOT REGENERATION IN CUTTINGS OF *OLEA EUROPAEA* L.: EFFECT OF AUXIN AND AUXIN SENSITIVE PHASE

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Abstract

Olive is one of the oldest cultivated plants of Anatolia/Turkey. 'Domat' is the predominant large fruiting cultivar particularly suitable for green processing in Turkey. Leafy cuttings of 'Domat' olive are very difficult to rooting, so the nursery tree production is costly and time consuming. The effects of IBA and NAA mixtures were examined on the rooting of 'Domat' olive cuttings in two consecutive years. Moreover, the auxin sensitive phase of cuttings was also assessed. The mixing rate of 5 : 2 g/l (IBA : NAA) was found to be optimum for the highest rooting rates (up to 60%) and root quality in the two successive years. Application of IBA (5 g/l) at the 5th day of experiment initiation, significantly ($p = 0.001$) increased the per cent of rooting (63) compared to the cuttings treated at day 0 (46) and the figures of other rooting variables.

Introduction

The olive tree (*Olea europaea* L.) is probably one of the most ancient cultivated fruit species of the Mediterranean basin. Olive was propagated asexually by large parts (e.g. branches, ovules, suckers) in practice through the history of cultivation (Fabbri *et al.* 2004). But it has been mass propagated by rooting of semi-hardwood cuttings under mist since mid-1950s (Hartmann *et al.* 2002, Fabbri *et al.* 2004). However, either olive country has at least one or more cultivars known to be recalcitrant to root, so they have been propagated by grafting onto the seedling rootstocks in currency (Fabbri *et al.* 2004).

'Domat' is the predominant large fruiting table olive cultivar of Turkey and particularly suitable for green consumption or processed as snack by stuffing (Barranco *et al.* 2000, Can and İsfendiyaroğlu 2006). Despite some limited success was obtained in experimental conditions, leafy cuttings of 'Domat' were very recalcitrant to rooting in practice. So, the nursery trees have been produced by grafting onto seedling rootstocks and the nursery tree costs are consequently very high (Özkaya and Çelik 1993, Seyhan 1999, Gerrakakis and Özkaya 2005). IBA applications are useful for rooting of olive cuttings, but difficult to root cultivars do not respond well to exogenous IBA even at the supra optima concentrations (Fabbri *et al.* 2004).

In cuttings of some plant species, NAA was more effective than IBA in stimulating the adventitious roots. The mixtures of IBA and NAA are sometimes more efficient than either component alone (Hartmann *et al.* 2002). The effect of NAA in olive cuttings was not entirely cleared out yet. It is sometimes more effective than IBA depending on cultivar, concentration and formulation as well (Çelik *et al.* 1993, Fernandes *et al.* 2002, Khabu 2002). In 'Domat' olive NAA concentrations ranged between 1 and 7 g/l gave a constant dose responsive curve but none of the treatments induced roots as with IBA (İsfendiyaroğlu and Özeker 2008). For this reason, a wide range of NAA concentrations have to be combined with IBA for precise dose optimizations.

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On the other hand, adventitious root formation comprised successive interdependent physiological phases (e.g. induction, initiation, expression) was proved (Gaspar *et al.* 1997). However, De Klerk *et al.* (1995), pointed out a dedifferentiation phase before root induction during which the exogenous auxin was ineffective. As a matter of fact, maximal sensitivity to applied auxin occurred after a later time from cutting or explant collection was reported in several investigations (De Klerk *et al*. 1999). Initial auxin pulses may not match with the auxin sensitive or induction phase of rooting and therefore, it is less effective even at the high concentrations in difficult to root olives. In fact, significant changes in contents of some polyamines, which involved in root induction, occurred at the 3 - 7th days of *in vivo* rooting of 'Frangivento' olive (Rugini *et al.* 1991).

The objectives of the present study were (i) optimization of the NAA concentration combined with IBA and (ii) determination of the possible auxin sensitive phase of rooting in semi-hardwood cuttings of 'Domat' olive.

Materials and Methods

Cuttings were collected on 15 February 2013 and 2014 from selected bearing mature mother plants with care, at the Research Farm of the Ege University, Faculty of Agriculture, Menemen, İzmir, Turkey.

In the first year, cuttings were prepared from one-year-old vigorous shoots from the outer canopy about 5 - 7 mm in diameter. A shoot caliper was used in cutting collection. Subterminal cuttings of 20 - 25 cm in length, with 2 - 3 pairs of leaves were used. In the second year, more vigorous shoots grew on the scaffold limbs of the inner tree canopy (more juvenile), at the same thickness as those from previous year.

Cuttings were soaked in fungicide solution (0.1% prochlorase) before dipping for 5 sec in solution of 5 g/l IBA (dissolved in 50% isopropanol) or its combinations with NAA ranged between 1 - 5 g/l with respect to cutting year. Control cuttings were soaked in alcoholic solution.

In the second year, untreated cuttings were taken out of the rooting medium 5, 10 and 15 days, respectively after the experiment initiation for IBA applications. Basal portions of cuttings slightly washed and excessive water was taken with a paper towel before IBA applications. Basal cuts of cuttings were refreshed by cutting the 2 - 3 mm proximal ends of cuttings just under the bottom node and they were immediately soaked with 5 g/l IBA for 5 sec before re-planting.

Cuttings were planted in rooting trays (40 × 20 × 11 cm) filled with the mixture of perlite (horticultural grade) and vermiculite (No. 2) in equal volumes. Rooting trays were placed in a low polyethylene tunnel equipped with mist nozzles. Cycles of misting were controlled by an electronic leaf during the daylight hours (1 hr from dawn to 1 hr before dusk in bright days). A thermostatic device automatically closed the mist controller below 10°C ambient temperature during experiments. Bottom heat was adjusted to 25 ± 2°C. A shading cloth was suspended over the tunnel and natural sunlight was initially reduced more than 90% (İsfendiyaroğlu *et al.* 2009). Changes in climatic parameters in poly tunnel were recorded. The design of the experiment was a completely randomized block with three replicates of 20 cuttings for each treatment. Cuttings were examined ten weeks after planting to determine the rooting percentage, primary root number, root length, secondary root number, root fresh/dry weights and visual rating (VR) score as follows 0: dead; 1: alive, no callus or roots; 2: low callused; 3: medium callused; 4: heavy callused and 5: rooted.

Data were subjected to analysis of variance according to completely randomized design using SPSS (version 16.0, Inc., USA) statistical software. Significant differences between means were determined by Duncan’s multiple range tests. Results were interpreted according to the value of the probability (p).

Results and Discussion

Applications of IBA (5 g/l) combined with increasing concentrations of NAA significantly affected the entire rooting variables of olive cuttings ($p < 0.01$), except secondary root number ($p = 0.185$). Mixing IBA with 2 g/l NAA gave the highest percentage of rooting, mean root number, secondary root number, root fresh/dry weights and VR score, respectively (Table 1). Very few rooted cuttings obtained with IBA treatment alone. Untreated cuttings did not produce roots at all. Increase in NAA concentration after 2 g/l significantly decreased the figures of entire rooting variables (Table 1). Similar response was observed in VR scores of cuttings.

Table 1. Effects of IBA and IBA-NAA mixtures on the rooting of olive cuttings in the first year.

Auxin IBA (g/l)	NAA (g/l)	Rooting (%)	Root number	Root length (mm)	Secondary root number	Root fresh weight (mg)	Root dry weight (mg)	Visual rating
0	0	0.0 c	0.0 d	0.0 b	0.00	0.0 c	0.0 c	0.0 c
5	0	3.3 c	0.1 d	1.1 b	0.00	8.3 c	0.8 c	0.2 bc
5	1	33.3 a	1.6 b	14.5 a	0.03	223.3 a	18.6 a	1.7 b
5	2	40.0 a	2.9 a	14.7 a	0.17	251.2 a	23.0 a	2.2 a
5	3	20.0 b	0.9 c	4.3 b	0.00	92.2 b	7.6 b	1.0 ab
5	4	6.7 c	0.3 cd	2.2 b	0.00	47.1 b	4.3 b	0.4 bc
5	5	6.7 c	0.1 d	1.9 b	0.00	8.6 c	0.7 c	0.3 bc
	SEM*	3.490	0.236	1.372	0.199	24.723	2.208	0.188
	p**	<0.001	<0.001	<0.001	0.185	0.001	0.001	<0.001

SEM*: Standard error mean, p**: Probability, values are means of 3 replicates.

Combined applications of IBA (5 g/l) with three different concentrations of NAA significantly affected the rooting of olive cuttings ($p < 0.01$) apart from secondary root number ($p = 0.054$). Mixture of IBA with 2 g/l NAA gave the highest figures of entire rooting variables (Table 2).

Exogenously applied IBA increased the root number of ‘Chalkidikis’ olive from basal shoots (juvenile), but did not affect their rooting percentage which was essentially high. Adult cuttings responded well to IBA by increasing the percent of rooting and number of roots produced (Porlingis and Therios 1976). In ‘Domat’ olive, regardless of possible multi factorial (climate, mother plant, cutting material) year differences, IBA (5 g/l) with NAA (2 g/l) increased the percent of rooting by 50% and increased the root number and root length of cuttings from water sprouts by 90 and 120%, compared to previous years cuttings (Tables 1, 2). Results of two years showed that the mixing ratio of 5:2 for IBA-NAA might be a proper concentration of rooting. Several investigations also pointed out the similar ratios of both auxins for *in vitro* root regeneration of olive cultivars (Fabbri 2004).

Effects of IBA (5 g/l) applications of the three intervals of five days after insertion, found to be significant on rooting percentage, root number, root fresh weight ($p = 0.001$), root length and root dry weight ($p = 0.002$). But, secondary root number did not affect by day intervals ($p = 0.236$). VR scores were also significantly influenced by day intervals ($p < 0.001$). Application at the 5th day of experiment initiation significantly increased the figures of entire rooting variables and VR score. Cuttings gradually lost their ability to rooting in application of auxin after 10 days and onward (Table 3).

Table 2. Effects of IBA and IBA-NAA mixtures on the rooting of olive cuttings in the second year.

Auxin IBA (g/l)	NAA	Rooting (%)	Root number	Root length (mm)	Secondary root number	Root rresh weight (mg)	Root dry weight (mg)	Visual rating
0	0	0.0 c	0.0 c	0.0 d	0.0	0.0 c	0.0 c	0.5 b
5	1.5	43.3 b	2.9 b	21.7 c	0.2	243.9 b	24.9 b	2.5 a
5	2	60.0 a	5.5 a	32.3 a	2.4	451.9 a	47.2 a	3.0 a
5	2.5	50.0 ab	4.6 a	26.9 b	2.1	302.7 ab	30.8 ab	2.3 a
	SEM*	5.690	0.526	3.111	0.334	43.015	4.500	0.252
	P**	<0.001	<0.001	<0.001	0.054	0.001	0.001	<0.001

SEM*: Standard error mean, p**: Probability, values are means of 3 replicates.

Table 3. Rooting of juvenile cuttings with delayed IBA applications (5 g/L) after experiment initiation in the second year.

Day	Rooting (%)	Root number	Root length (mm)	Secondary root number	Root fresh weight (mg)	Root dry weight (mg)	Visual rating
0	46.7 b	3.1 a	22.6 ab	0.0	214.8 a	21.8 a	2.9 b
5	63.3 a	3.4 a	33.8 a	1.3	228.5 a	24.4 a	3.9 a
10	30.0 c	1.2 b	12.6 bc	0.5	121.3 ab	14.2 a	2.9 b
15	10.0 d	0.3 b	4.9 c	0.0	26.4 b	2.4 b	1.6 c
SEM*	8.012	0.434	4.630	0.124	31.410	3.450	0.348
p**	0.001	0.001	0.002	0.236	0.001	0.002	<0.001

SEM*: Standard error mean, p**: Probability, values are means of 3 replicates.

Similar results were formerly reported in mature *Ficus pumila* cuttings in which the IBA applied at the 9th day of rooting significantly increased the per cent of rooting, but decreased the number of roots formed compared to application at the day 3 (Davies and Joiner 1980). However, in hypocotil cuttings of *Pinus radiata*, IBA had to be supplied over the first 4 days for root formation, thus it was necessary for both pre- and post-initiative stages of rooting was concluded (Smith and Thorpe 1977).

In 'Domat' olive cuttings, delayed (5 days) application of IBA gave rise to a very slight decrease in root production whereas the per cent of rooting remarkably increased (Table 3) as reported in mature *Ficus pumila* cuttings (Davies and Joiner 1980).

In fact, maximal sensitivity to applied auxin occurs not directly after taking the cutting but a later time in plant species tested (De Klerk *et al.* 1995, 1999). The obtained results showed that *in vivo* rooting of 'Domat' cuttings need a relatively long time as in plants show indirect root formation from basal callus tissues (Hartmann *et al.* 2002), but the time course occurrence of the first biochemical event may not very different from other plants in which the direct root regeneration occurred.

In conclusion, delayed (ca. 5 days) pulses of 5 g/l IBA to partially juvenile semi hardwood cuttings of 'Domat' olive could be a proper approach to improve the root regeneration and probably useful in other difficult to root olive cultivars.

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