Pak. J. Bot., 40(3): 1135-1141, 2008.

ROOTING OF *OLEA EUROPAEA* 'DOMAT' CUTTINGS BY AUXIN AND SALICYLIC ACID TREATMENTS

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Abstract

The effects of various NAA concentrations and applications of SA with IBA in different ways were tested in leafy cuttings of very difficult to root 'Domat' olive. Cuttings were collected from bearing (mature) trees and rooted under mist. Cuttings had the highest rooting rate of 63.3% with routine 5 g l^{-1} IBA treatment. Primary root number (4.1), root length (32.10 mm), root thickness (1.10 mm), root fresh/dry weights and VR score were at the highest level with the same treatment. Untreated cuttings entirely failed to root. NAA treatments produced less rooted cuttings than those treated with IBA alone. Cuttings receiving 3 g l^{-1} NAA gave 36.6% rooting. Free applications of SA did not give rise to any root formation. No synergistic effect was detected caused by simultaneous applications of 5 g.l^{-1} IBA combined with SA concentrations ranged between 2.5-10 g.l^{-1}. In both pre applications and post applications of SA strongly inhibited the rooting of cuttings compared to simultaneous applications of both substances. Interval of 15 days between auxin and SA application might be too long to exhibit the promised phase dependent effect of SA on root formation was concluded.

Introduction

Olive trees can be propagated by different ways such as hardwood and semi-hardwood cuttings, suckers, ovuli and budding or grafting (Hartmann *et al.,* 2002). However, propagation by leafy cuttings under mist has become the most acceptable method throughout the world (Hartmann *et al.,* 2002). But some economically important olive cultivars show poor rooting in some olive producing countries (Fabbri *et al.,* 2004). So these cultivars are being propagated by budding or grafting in nurseries. For instance, 'Domat' olive is the predominant large fruiting table cultivar of Turkey and suitable for green consumption and canning industry, but it is very hard to propagate by leafy cuttings. Up to now, several investigations showed that the rooting of leafy 'Domat' cuttings ranged between 10-33% and root production was poor (Luma *et al.,* 1981; Özkaya & Çelik, 1993; İsfendiyaroğlu & Özeker, 2000). Indolebutyric acid (IBA) applications had been reported to be successful for rooting in olive cuttings (Loreti & Hartmann, 1964). But poor rooted cultivars may not respond well to exogenous IBA (Nahlawi *et al.,* 1975). Insensitivity to applied IBA in olive cuttings was explained by the differences in metabolism and transport of IBA (Wiesman & Epstein, 1987). IBA has long been used to promote the rooting in cuttings of a wide range of plant species (Hartmann *et al.,* 2002). However, naphthalene acetic acid (NAA) was found to be more effective than IBA in some plants which respond unsatisfactorily to IBA (Hartmann *et al.,* 2002). The effects of NAA particularly on difficult-to-root olive cuttings have not been well investigated so far (Fabbri *et al.,* 1994).

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There are ancillary compounds (growth retardants/inhibitors, polyamines, phenolics) that modify main hormone effects on rooting (Hartmann *et al.,* 2002). Salicylates, which are involved in phenolic compounds, have been considered as phytohormones (Raskin, 1992). In some woody and herbaceous plant species, salicylic acid (SA) highly promoted the *In vivo* rooting of cuttings when applied particularly with auxin (Bojarczuk & Jankiewicz, 1975; Kling & Meyer, 1983). But, SA inhibited IAA-induced rooting of apple stem slices *In Vitro* by enhancing oxidation of IAA during the auxin sensitive phase (24-96 h) (De Klerk *et al.,* 1997).

Adventitious root formation comprises three successive interdependent physiological phases (induction, initiation and expression) (Gaspar *et al.,* 1992). It was suggested that phenolic compounds which are known to inhibit root formation might actually enhance root formation if applied during the appropriate phase of rhizogenesis (Berthon *et al.,* 1993). Thus, applications of salicylic acid after IBA might be more effective on the auxin induced root formation than simultaneous treatments of both substances. However, it is very difficult to estimate the proper application time of SA *In vivo* cuttings due to the lack of uniformity in propagation material. Therefore, initial applications of SA to cuttings may also give useful results.

The objectives of this study were to determine the effects of (1) NAA applications and of (2) SA applied alone or after, before and in combination with IBA on the rooting of very difficult to root 'Domat' olive cuttings according to routine IBA treatment (5 g l^{-1}) which was predicted by former investigations.

Material and Method

In this study, selected mature (bearing) olive trees were used as cutting sources. Trees were subjected to routine pruning in previous year. Cuttings were prepared from vigorous, one-year old wood about 6-7 mm in diameter and 20 cm in length. Sub-terminal cuttings with 4-5 leaves retained on the upper portion were used. They were collected on 10 March 2004, before the active growth period. All the cuttings were completely mixed and randomly grouped. Then they were dipped in for 5 sec in one of the solutions of rooting substances prepared by dissolving them in 50% isopropyl alcohol. Untreated control cuttings were only dipped in 50% isopropanol. Concentrations and application intervals of rooting substances are shown below:

- Untreated controls were only dipped in 50% isopropanol.
- 5 g l^{-1} IBA
- 1, 3, 5, 7 g l^{-1} NAA
- 2.5; 5; 7.5; 10 g l^{-1} SA
- 5 g l^{-1} IBA combined with 2,5; 5; 7,5; 10 g l^{-1} SA respectively.
- 5 g l^{-1} IBA and retreatment with 2,5; 5; 7,5; 10 g l^{-1} SA after 15 days.
- 2.5; 5; 7.5; 10 g l^{-1} SA and retreatment with 5 g l^{-1} IBA after 15 days.

Retreatments were made by pulling out the cuttings from rooting medium and basal wound was refreshed by taking a very thin slice (approx. 1-2 mm) from the cutting base. Subsequently, cuttings were dipped in proper solution. Cuttings were placed in 20 x 40 x 11 cm polystyrene flats filled with coarse perlite and placed in a low tunnel equipped with mist nozzles. Mist cycles were controlled by an electronic leaf, between 08:00h and 18:00h daily. Bazal heat at 25 (±2) ºC was provided by heating cables feeded with low voltage. Double layered shading cloth was suspended over the tunnel providing approximately 80% shading against the natural sunlight. Changes in daily ambient temperature and relative humidity were recorded.

Statistical design for experiments was completely randomized with 3 replications of 10 cuttings per treatment. Cuttings were evaluated 12 weeks after planting for percent rooted cuttings, primary root number, mean primary root length, fresh and dry weight of roots, mean primary root thickness and visual rating (VR) scale as follows: 0= dead; 1= alive, no callus or roots; 2= low callused; 3= medium callused; 4= heavy callused and 5= rooted.

Statistical analysis was carried out using SPSS. Data were analyzed by ANOVA. Duncan's Multiple range test was used to discern differences at the 5 % level of significance.

Results and Discussion

The analysis of variance indicated the significant ($p<0.01$) differences among treatments in relation to all rooting characteristics. Cuttings treated with 5 g l^{-1} IBA not only gave the highest number of rooted cuttings (63.3%), but also gave the highest number of primary roots (4.1), root length (32.1 mm), root thickness (1.1 mm), root fresh/dry weights (279.4/29.3 mg) and VR (3.8). Untreated controls did not produce roots at all (Table 1). Despite the relatively high rooting rates of IBA treated cuttings, the number and length of roots were too low as previously reported by several researchers (Luma *et al.,* 1981; Özkaya & Çelik, 1993; Özkaya, 1997; İsfendiyaroğlu & Özeker, 2000).

Rooting of the cuttings was significantly affected by NAA applications. Cuttings which received 3 g.l^{-1} NAA gave the highest rooting scores among different concentrations. But the increase in NAA concentration reduced the rooting (Table 1). Although cuttings did not respond well to NAA as in IBA. However, VR scores obtained at 3 g l^{-1} NAA (2.53) also pointed out good callusing which have been associated with potential rooting (Hartmann *et al*., 2002) (Table 1). The responsivitiy of 'Domat' cuttings to applied NAA have been differed from some olive cultivars. As a matter of fact, in basal cuttings of easily rooted 'Gemlik' olive, 2000 ppm quick dip of NAA was found to be more effective than equal concentration of IBA (Çelik *et al.,* 1993). Also, treatment with 5000 ppm liquid IBA was less effective as compared to 0.2% NAA powder in difficult to root 'Galega Vulgar' cuttings (Fernandes *et al.,* 2002). In this respect, combined applications of NAA with IBA would be more effective than using NAA alone to increase the rooting ability of 'Domat' olive cuttings.

None of the free applications of SA gave rise to root formation in cuttings. VR scores of applications did not significantly differ from untreated control cuttings (Table 1). Free applications of SA had more or less promoted the rooting of *Populus* cuttings depending on varietal differences rather than concentration (Bojarczuk & Jankiewicz, 1975). But they were ineffective in cuttings from *Tillia* clones (Morinsk & Smith, 1975). Irresponsibility of olive cuttings to SA treatments might be due to particular characteristics of this species.

Combined applications of IBA with various concentrations of SA significantly promoted the rooting of cuttings according to untreated ones. But the rooting was markedly low compared to the effect of IBA (5 g.l^{-1}) alone. Moreover, inconsistent results of rooting were observed in relation with the changes in SA level in combination. Cuttings had the highest rooting rate (26, 66%), longest and thickest roots with 7.5 g l^{-1} SA while 2.5 g.l^{-1} SA gave the largest number of roots (1.30), root fresh and dry weights besides VR score (Table 1). SA had synergistically acted with IAA and promoted the root formation in mung bean cuttings. But had no effect on *Acer* cuttings (Kling & Meyer, 1983). SA combined with NAA synergistically promoted the root number and root lengths of the cuttings of several *Populus* spp. Although this effect had seemed to be in relation with the clonal differences and cutting time rather than concentration and treatment method (Bojarczuk & Jankiewicz, 1975). In 'Domat' cuttings, regardless of the apparent overdose effect at 10 g l^{-1}, SA did not give rise to large differences on the rooting correlated with concentration in combined applications (Table 1) as formerly reported in clones of different *Populus* spp., (Bojarczuk & Jankiewicz, 1975). From this point of view, exogenously applied SA might have been ineffective or even suppressed the activity of IBA so that the particular phenolic metabolism of olive. Actually, some benzoic acid derivatives structurally related with SA did not significantly increase the number and length of adventitious roots of 'Frangivento' olive cuttings even in the presence of IBA (Bartolini *et al.,* 1988b). On the contrary, some of them were highly effective on grape cuttings (Bartolini *et al.,* 1988a).

SA applications 15 days after IBA were not as effective as the combined applications of SA with IBA on rooting. But 5 g l^{-1} SA remarkably augmented the rooting of cuttings compared to 2.5 g l^{-1} SA. Cuttings had the highest rooting rate, root number, length, weights, thickness and VR scores with 5 g.l^{-1} SA while they did not produce roots at all with higher SA concentrations (Table 1). The promotive effects of chlorogenic and ferulic acid on the formation of root meristemoids during the initiative phase which coincides with 4-6 days of rooting process of auxin induced hypocotyl cuttings of *Pinus radiate* as demonstrated by Smith & Thorpe (1977). On the other hand, IBA treated difficult to root mature leaf bud cuttings of *Ficus pumila* gave the highest rooting response to re-treatment of IBA at 9 days after experiment initiation (Davies & Joiner, 1980). In cuttings of 'Domat' olive, interval of 15 days between IBA and SA applications might have been too late to match with the early phases of rooting was thought. In fact, exceptionally high content changes of some endogenous polyamines, which are involved in root induction, occurred at 3-7 days of rooting in cuttings of 'Frangivento' olive (Ruggini *et al.,* 1991).

Pre-applications of SA gave rise to slight response of rooting in olive cuttings. However, no significant differences occurred among the treatments in relation with some rooting characteristics of cuttings (Table 1). SA found to be inhibitory on *In vitro* rooting of stem discs of apple when applied before auxin (Van Der Krieken *et al.,* 1997). This effect was attributed to enhanced oxidation of IAA during the auxin sensitive phase by SA (De Klerk *et al.,* 1997). In olive cuttings, SA may also inhibit the rooting by its promotory role in auxin oxidation. As a matter of fact, regardless of concentration and application interval, all the SA treatments did inhibit the rooting of 'Domat' olive cuttings recording free application of IBA.

Conclusions

This investigation showed that IBA was predictive on the root regeneration in 'Domat' olive cuttings. In stock plants under more proper physiological conditions, the rooting rate of 'Domat' olive cuttings would reach the level of intermediate-to-root cultivars even with the 5 g l^{-1} IBA is evident. But the poor root regeneration problem in 'Domat' cuttings does not seem to be overcome only with IBA applications. Relatively wide concentration range of NAA demonstrates that this auxin may not be sufficient for solitary applications. From this point of view, in quick dip applications, utilization of NAA by mixing with IBA in optimal proportions may be more beneficial for increasing the auxin mediated root formation in olive cuttings.

Table 1. The effects of different treatments on the rooting characteristics of 'Domat' cuttings.

Treatments	Rooting percentage (%)	Root number	Root length (mm)	Root thickness (mm)	Root fresh weight (mg)	Root dry weight (mg)	Visual rating
C	0.00g (0.00)[w]	0.00 f	0.00 e	0.00 g	0.00 d	0.00 e	1.06 h
B	63.33a (52.80)	4.10 a	32.17 a	1.12 a	279.46 a	29.33 a	3.83 a
N1	10.00de (18.44)	0.23 ef	6.69 c-e	0.16 e-g	35.16 d	4.63 de	1.66 e-g
N3	36.66b (37.24)	1.60 b	15.65 b	0.75 b	185.23 b	17.53 b	2.53 b
N5	26.66bc (31.01)	1.43 bc	10.95 bc	0.55 bc	145.60 bc	14.93 bc	2.20 b-d
N7	10.00de (18.44)	0.56 d-f	3.83 de	0.18 e-g	40.90 d	3.86 de	1.43 f-h
S2,5	0.00g (0.00)	0.00 f	0.00 e	0.00 g	0.00 d	0.00 e	1.00 h
S5	0.00g (0.00)	0.00 f	0.00 e	0.00 g	0.00 d	0.00 e	1.16 h
S7,5	0.00g (0.00)	0.00 f	0.00 e	0.00 g	0.00 d	0.00 e	1.16 h
S10	0.00g (0.00)	0.00 f	0.00 e	0.00 g	0.00 d	0.00 e	0.96 h
B+S2,5	23.33bc (28.79)	1.30 bc	10.99 bc	0.47 cd	149.300 bc	14.36 bc	2.26 bc
B+S5	20.00cd (26.57)	0.83 c-e	7.43 cd	0.37 c-e	59.50 d	15.93 b	1.93 c-e
B+S7,5	26.66bc (31.01)	1.06 b-d	14.15 b	0.57 bc	114.53 c	12.00 b-d	2.10 b-e
B+S10	13.33c-e (21.15)	0.26 ef	5.65 c-e	0.29 d-f	37.60 d	4.13 de	1.73 d-f
B→S2,5	3.33fg (6.14)	0.10 f	1.61 de	0.05 g	8.20 d	1.00 e	1.36 f-h
B→S5	16.66cd (23.86)	0.60 d-f	7.35 cd	0.32 c-e	59.63 d	6.50 c-e	1.96 c-e
B→S7,5	0.00g (0.00)	0.00 f	0.00 e	0.00 g	0.00 d	0.00 e	1.16 h
B→S10	0.00g (0.00)	0.00 f	0.00 e	0.00 g	0.00 d	0.00 e	1.23 gh
S2,5→B	6.66ef (12.29)	0.13 f	3.03 de	0.11 fg	12.66 d	1.40 e	1.83 c-f
S5→B	6.66ef (12.29)	0.30 ef	2.83 de	0.12 fg	26.20 d	3.03 e	1.73 d-f
S7,5→B	6.66ef (12.29)	0.10 f	2.20 de	0.15 e-g	11.90 d	1.33 e	1.73 d-f
S10→B	6.66ef (12.29)	0.06 f	1.63 de	0.17 e-g	4.60 d	0.53 e	2.00 c-e

[a,b,c,d,e,f,g,h] Mean separation, within columns, by Duncan's multiple range test, 5 %, [w] transformed values
B: IBA 5 gl^{-1}, C: Control, N: NAA, S: SA, +: Combined application, →: Arrow shows the retreated substance.

SA applications gave rise to inconsistent results on the rooting of cuttings, as formerly observed by some researchers in several woody species. In general, SA did negatively alter the IBA induced root formation in cuttings. In this investigation, SA applied before IBA did give the lowest rooting scores compared to combined and post-applications with IBA. This shows the importance of initial auxin pulses for root formation in olive cuttings. Furthermore, it is very difficult to predict the early phases (induction, initiation) of rooting precisely *In Vivo* material. Therefore, post-applications of relatively low concentrations of SA in shorter intervals (3-5 days) through a much longer period of time should be necessary to expose the probable phase dependent effect of SA was concluded. Our future investigations will be focused on obtaining more experimental data regarding the proper application time of SA on the rooting process.

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(Received for publication 10 February 2007)