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The Effect of Some Growth Regulators, Phenolic Acids and Time of Propagation on the Rhizogenesis of Olive Semi-hardwood Cuttings

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Abstract. Semi-hardwood cuttings 15-cm long of hard-to-root olive (*Olea europea cv.* Frantoio) were treated with 15 treatments including IBA at 3000 ppm and NAA at 1000 ppm both alone or in different combinations with catechol at 500 or 1000 ppm and cinnamic acid at 500 or 1000 ppm, and the control. The cuttings were collected in spring and fall during 1997 and 1998 seasons from the sub-terminal growth of the experimental trees. The cuttings were rooted in a growth chamber under 3000 lx in 16-h photoperiods at 20- 25 °C and 80-85% RH. Evaluations after 8 weeks revealed that, in general, the experimental treatments caused a significant increase in the percentage of rooted cuttings as compared with the control. Treating cuttings with IBA+ catechol at 1000 ppm yielded the highest rooting percentage (57.8%), followed by cuttings treated with IBA+ cinnamic acid at 1000 ppm (50.9%), while, the lowest percentage of rooted cuttings (17.3%) was obtained in the untreated cuttings. In addition, the rooting percentage of cuttings propagated in spring of both seasons was statistically higher than those collected in fall. This is may be due to the highest rate of cambial activity that enhances the root formation of cuttings in spring more than in fall. Generally, cuttings treated with NAA + cinnamic acid at 500 ppm followed by IBA + cinnamic acid at 1000 ppm produced the highest number of roots (28.8 and 23.9 roots per cutting, respectively) whereas the untreated cuttings gave 6.8 roots. Moreover, cuttings collected in spring of the second season produced significantly higher roots than those propagated in fall. It was also noted that cuttings treated with cinnamic acid at 1000 ppm either alone or in combination with NAA, and IBA + catechol at 1000 ppm gave the longest roots per cutting (14.6, 14.8 and 14.7 cm, respectively) while the control cuttings produced 9.1 cm root length. In addition, cuttings collected in spring of the second season and treated with the different experimental treatments produced significantly longer roots than those propagated in fall. Generally, cuttings treated with NAA + cinnamic acid at 1000 ppm gave markedly thicker roots (1.3 mm) than the most of the other treatments including the control that gave 0.767-mm root diameter. There were no obvious differences in the diameter of roots between cuttings propagated in spring and those collected in fall of both seasons. In conclusion, the primitive effect of IBA and NAA when used in combination with phenolic compounds on rooting of olive stem cuttings might be attributed to the synergistic influence.

Introduction

Olives are among the important tree fruits produced commercially in most of the Arab countries. Due to the nutritional value of the fruits and the ability of the tree to thrive well in many regions of the Arab world concentrate attention has been paid toward

increasing the cultivated area with different olive cultivars. During the last 40 years olive orchards have been established using mainly self- rooted cultivars [1]. Inspite of the high morphogenetic potential of the trees, the low rooting ability of difficult-to-root cultivars and the often unsatisfactory viability of cuttings of some easy-to-root cultivars are still limiting factors [2].

Propagation of clonal material can be by nucellar seed, by micro-propagation in tissue culture or by the use of "own-rooted trees" via cutting [3]. Cutting propagation preserves the characteristics of the parent plant, and true-to-type plants may be produced more quickly [4]. Hard wood cuttings have been used as the easiest propagation method for many tree fruits. However, cuttings of the different olive cultivars differ considerably in their ability to produce roots [5]. In some cases, certain fruit plants are very difficult to root even within cultivars of the same species, i.e. *Citrus sinensis* [6] and *Pyrus communis cv.* Bartlett [1], and improved methods are needed.

The formation of roots on certain fruit cuttings is improved by treatment with root-induced hormones. Adventitious root initiation in olive cuttings can be stimulated by auxin but, in difficult-to-root cultivars, auxin either fails to promote rooting or promotes it only slightly [2]. The use of natural and synthetic auxins to induce root initiation in stem cuttings was the first practical application of plant hormones in agriculture [7]. In addition, there are several reports that indicated that phenolic compounds stimulate rooting and shoot development in tissue cultures of tree species including apple, pear and plum. The response to some phenolic compounds appears to be related both to genotype and the physiological state of the tissue [8].

Thus, the purpose of the present investigation was to study the influence of two growth regulators; indole-3- butyric acid and naphthaline acetic acid, both alone or in different combinations with two phenolic compounds; catechol and cinnamic acid at different concentrations on the rooting of difficult-to-root olive stem cuttings cv. Frantoio propagated in spring and fall. In addition, percentage of cuttings rooted, number of roots per cutting, and average of length and diameter of roots per cutting were also evaluated.

Materials and Methods

The present study was carried out during 1997 and 1998 growing seasons on stem cuttings taken from 16-year-old olive trees (*Olea europea cv. Frantoio*). The experimental trees grown at Dirab Experimental and Research Station, College of Agriculture, King Saud University in Ryiadh. Semi- hard wood cuttings 15-cm long were collected during the second week of March (spring) and September (fall) from the sub-terminal growth of the experimental trees. Basal leaves were removed and stem cuttings with 2-4 terminal leaves were prepared. The base of cuttings within a given group were dipped (5 cm from the cutting's base) for 12 h in one of the following solutions:

1. Distilled water as a control treatment.
2. Indole-3-butyric acid (IBA) at 3000 ppm.
3. Naphthalene acetic acid (NAA) at 1000 ppm.
4. Catechol at 500 ppm.
5. Catechol at 1000 ppm.
6. Cinnamic acid at 500 ppm.
7. Cinnamic acid at 1000 ppm.
8. IBA at 3000 ppm + catechol at 500 ppm.
9. IBA at 3000 ppm + catechol at 1000 ppm.
10. NAA at 1000 ppm + catechol at 500 ppm.
11. NAA at 1000 ppm + catechol at 1000 ppm.
12. IBA at 3000 ppm + cinnamic acid at 500 ppm.
13. IBA at 3000 ppm + cinnamic acid at 1000 ppm.
14. NAA at 1000 ppm + cinnamic acid at 500 ppm.
15. NAA at 1000 ppm + cinnamic acid at 1000 ppm.

Cuttings were then placed in a 15- cm deep pots containing perlite as a growing medium and kept in a growth chamber under 3000 lx in 16-h photoperiods at 20-25 ^{o}C and 80-85 % RH.

The 15 treatments were arranged in a completely randomized block design with 3 replications of 6 stem cuttings per replicate of each treatment. Thus, 540 stem cuttings were included in this investigation for each of the two times of propagation; spring and fall. Cuttings were harvested 8 weeks after planting and evaluated for percentage of cuttings rooted, number of roots per cutting, and average of length and diameter of roots per cutting.

Statistical analysis were performed using SAS computer package [9] with Duncan's multiple range test for means comparison [10, p.633].

Results and Discussion

The effect of IBA and NAA either alone or in a combination with catechol and cinnamic acid on the root production of olive stem cuttings presented in Tables (1-4). It was noted, generally, that the experimental treatments caused a significant increase in the percentage of the rooted cuttings as compared with the control. Treating cuttings with IBA + catechol at 1000 ppm yielded the highest rooting percentage (57.8%), followed by cuttings treated with IBA + cinnamic acid at 1000 ppm (50.9%), while, the lowest percentage of rooted cuttings (17.3%) was obtained in the control, (Table 1). These findings were, somewhat, in agreement with those reported by Basu *et al.* [11] who studied the synergistic effect between the phenolic compounds and IBA or NAA in rooting of cuttings. Epstein and Lavee [7], Marco *et al.* [12], Bartolini *et al.* [13], Lee [14], and Rkhis and Triqui [15] also obtained Similar results.

Concerning the time of propagation, the data listed in Table (1) indicated that, the percentage of rooted cuttings propagated in spring was significantly higher than those of cuttings collected in fall. Likewise, Ibrahim *et al.* [16] obtained the best results of rooted cuttings in spring and summer. Whereas, Wiesman and Lavee [2] found the same results in spring and autumn. Such observations could be confirmed on the basis of the interpretation given by Hartmann and Kester [1] and Hartmann and Loreti [17]. They reported that, in certain plant species, the ability of stem cutting to root is greatly differed according to the time of propagation. In spring the cambial activity was at its highest rate which enhances the formation of callus tissue which is necessary for root formation. On the other hand, cambial activity decreases gradually during fall and almost stops at the wintertime.

Table 1. Effect of growth regulators and phenolic compounds on the percentage of rooted olive stem cuttings propagated in spring and fall of 1997 and 1998 seasons

Treatments	1997	1998	Mean
1 Control	19.3 h	15.2 j	17.3 j
2 IBA 3000 ppm	28.7 ef	24.5 hi	26.6 hi
3 NAA 1000 ppm	32.2 def	22.2 I	27.2 hi
4 Catechol 500 ppm	30.8 def	34.7 defg	32.8 efgh
5 Catechol 1000 ppm	29.0 ef	43.2 bc	36.1 def
6 Cinnamic acid 500 ppm	20.2 gh	30.2 gh	25.2 i
7 Cinnamic acid 1000 ppm	32.7 def	37.8 cde	35.3 defg
8 IBA + Catechol 500 ppm	34.5 de	40.8 bcd	37.7 cde
9 IBA + Catechol 1000 ppm	56.7 a	58.8 a	57.8 a
10 NAA + Catechol 500 ppm	27.5 efg	32.5 efg	30.0 fghi
11 NAA + Catechol 1000 ppm	42.7 bc	46.7 b	44.7 bc
12 IBA + Cinnamic acid 500 ppm	25.3 fgh	31.0 fgh	28.2 ghi
13 IBA + Cinnamic acid 1000 ppm	47.5 b	54.3 a	50.9 ab
14 NAA + Cinnamic acid 500 ppm	37.3 cd	37.7 cde	37.5 cde
5 NAA + Cinnamic acid 1000 ppm	45.8 b	36.8 cdef	41.3 cd
Seasons			
Spring	36.4 a	41.2 a	38.8
Fall	31.7 b	31.6 b	31.7

Means not sharing the same letter (s) within columns are significantly different ($P< 0.05$), Duncan 's multiple range test.

Regarding the effect of the experimental treatments on the number of roots per olive cuttings, data presented in Table (2) showed that, in general, IBA, NAA or the phenolic compounds when used singly produced a slight increase in the number of roots per cutting. Whereas, in combination with NAA cinnamic acid at 500 ppm followed by IBA + cinnamic acid at 1000 ppm caused a highly significant increase in the number of roots per cutting (28.8 and 23.9 roots, respectively) more than all of other treatments including the control that gave 6.8 roots per cutting. Likewise, Basu *et al.* [11], Bartolini *et al.* [13], Lee [14], Prasad *et al.* [18], and Singh *et al.* [19] noticed similar synergistic effect

of auxins when used in combination with phenolic compounds to promote initiation of roots in cuttings.

Table 2. Effect of growth regulators and phenolic compounds on the number of roots per olive stem cuttings propagated in spring and fall of 1997 and 1998 seasons

Treatments	1997	1998	Mean
1 Control	5.5 e	8.2 f	6.8 f
2 IBA 3000 ppm	12.0 de	17.2 bcde	14.6 e
3 NAA 1000 ppm	13.5 cd	12.8 ef	13.2 e
4 Catechol 500 ppm	12.3 de	14.5 cdef	13.4 e
5 Catechol 1000 ppm	11.8 de	13.3 def	12.6 ef
6 Cinnamic acid 500 ppm	11.7 de	11.2 ef	11.4 ef
7 Cinnamic acid 1000 ppm	15.0 cd	19.2 bcde	17.1 cde
8 IBA + Catechol 500 ppm	13.5 cd	19.2 bcde	16.3 cde
9 IBA + Catechol 1000 ppm	19.5 bc	22.5 abc	21.0 bcd
10 NAA + Catechol 500 ppm	13.7 cd	18.3 bcde	16.0 de
11 NAA + Catechol 1000 ppm	16.5 bcd	17.2 bcde	16.8 cde
12 IBA + Cinnamic acid 500 ppm	11.3 de	15.0 cdef	13.2 e
13 IBA + Cinnamic acid 1000 ppm	18.3 bcd	29.5 a	23.9 ab
14 NAA + Cinnamic acid 500 ppm	32.5 a	25.2 ab	28.8 a
15 NAA + Cinnamic acid 1000 ppm	22.8 b	21.3 abcd	22.1 bc
Seasons			
Spring	16.6 a	19.7 a	18.2
Fall	14.1 a	15.5 b	14.8

Means not sharing the same letter (s) within columns are significantly different ($P< 0.05$). Duncan's multiple range test.

Concerning the effect of the time of propagation on the number of roots produced per cutting, data presented in Table (2) revealed that, there were no obvious differences between spring and fall in the first season. However, in the second season, data revealed that cuttings propagated in spring gave significantly higher roots than those collected in fall. Hartmann and Kester [1], Ibrahim *et al.* [16] and Hartmann and Loreti [17] in accordance with those results obtained in the second season.

As for the effect of the different experimental treatments on the length of roots per cutting, data presented in Table (3). In general, cuttings treated with cinnamic acid at 1000 ppm either alone or in combination with NAA, and IBA + catechol at 1000 ppm produced the longest roots per cutting (14.6, 14.8 and 14.7 cm, respectively) while the control cuttings produced 9.1 cm root length. These findings were somewhat, in agreement with those obtained by many investigators, including Hammatt [8], Basu *et al.* [11], Bartolini *et al.* [13], and Ibrahim *et al.* [16].

Table 3. Effect of growth regulators and phenolic compounds on the length of roots (cm) per olive stem cuttings propagated in spring and fall of 1997 and 1998 seasons

Treatments	1997	1998	Mean
1 Control	8.3 e	9.8 a	9.1 c
2 IBA 3000 ppm	11.7 abcde	10.5 a	11.1 abc
3 NAA 1000 ppm	11.5 abcde	13.0 a	12.3 abc
4 Catechol 500 ppm	12.7 abcde	14.8 a	13.8 ab
5 Catechol 1000 ppm	14.2 abc	10.3 a	12.2 abc
6 Cinnamic acid 500 ppm	8.8 cde	11.7 a	10.3 bc
7 Cinnamic acid 1000 ppm	14.5 ab	14.7 a	14.6 a
8 IBA + Catechol 500 ppm	9.5 bcde	13.0 a	11.3 abc
9 IBA + Catechol 1000 ppm	15.5 a	13.8 a	14.7 a
10 NAA + Catechol 500 ppm	8.7 de	15.5 a	12.1 abc
11 NAA + Catechol 1000 ppm	12.5 abcde	11.5 a	12.0 abc
12 IBA + Cinnamic acid 500 ppm	14.2 abc	12.3 a	13.3 ab
13 IBA + Cinnamic acid 1000 ppm	15.8 a	10.8 a	13.3 ab
14 NAA + Cinnamic acid 500 ppm	11.0 abcde	13.8 a	13.4 abc
15 NAA + Cinnamic acid 1000 ppm	14.3 ab	15.3 a	14.8 a
Seasons			
Spring	12.6 a	14.5 a	13.6
Fall	11.8 a	11.0 b	11.4

Means not sharing the same letter(s) within columns are significantly different ($P< 0.05$), Duncan's multiple range test.

In addition, data presented in Table (3) show that, in general, cuttings collected in spring of the second season and treated with the different treatments gave significantly longer roots than that propagated in fall. These results were in accordance with those given by Hartmann and Kester [1], Ibrahim *et al.* [16] and Hartmann and Loreti [17].

Regarding the effect of the experimental treatments on the diameter of roots per cutting, data listed in Table (4). It was revealed that, in general, cuttings treated with NAA + cinnamic acid at 1000 ppm or catechol at 500 ppm, catechol at 500 ppm and IBA + catechol at 1000 ppm gave markedly thicker roots than the most of the other treatments including the control. Cuttings treated with NAA+ cinnamic acid at 1000 ppm gave markedly thicker roots (1.3 mm) than the control that gave 0.767-mm root diameter. Similarly, Sabbah *et al.* [4], Hammatt [8] and Bartolini *et al.* [13] concluded that both auxins IBA and NAA and some phenolic compounds can increase the percentage of cuttings that form roots and significantly increase the number and diameter of roots produced per cutting.

Concerning the effect of the time of propagation on the diameter of roots produced per cutting, data presented in Table (4) showed that, there were no obvious differences in the diameter of roots between spring and fall in both seasons. These results were in contrast with those given by Hartmann and Kester [1], Ibrahim *et al.* [16] and Hartmann and Loreti [17].

Table 4. Effect of growth regulators and phenolic compounds on the diameter of roots (mm) per olive stem cuttings propagated in spring and fall of 1997 and 1998 seasons

Treatments	1997	1998	Mean
1 Control	0.783 a	0.750 f	0.767 e
2 IBA 3000 ppm	0.967 a	0.983 cdef	0.975 bcde
3 NAA 1000 ppm	1.000 a	1.050 bcdef	1.025 bcde
4 Catechol 500 ppm	0.983 a	1.367 ab	1.175 ab
5 Catechol 1000 ppm	1.033 a	0.917 def	0.975 bcde
6 Cinnamic acid 500 ppm	0.800 a	0.850 ef	0.825 de
7 Cinnamic acid 1000 ppm	0.933 a	1.100 bcdef	1.017 bcde
8 IBA + Catechol 500 ppm	1.033 a	1.083 bcdef	1.058 abcd
9 IBA + Catechol 1000 ppm	1.017 a	1.333 abc	1.175 ab
10 NAA + Catechol 500 ppm	1.067 a	1.283 abcd	1.175 ab
11 NAA + Catechol 1000 ppm	0.917 a	0.900 ef	0.908 cde
12 IBA + Cinnamic acid 500 ppm	0.867 a	1.067 bcdef	0.967 bcde
13 IBA + Cinnamic acid 1000 ppm	0.933 a	1.317 abc	1.125 abc
14 NAA + Cinnamic acid 500 ppm	1.100 a	1.150 abcde	1.125 abc
15 NAA + Cinnamic acid 1000 ppm	1.100 a	1.500 a	1.300 a
Seasons			
Spring	0.924 a	1.051 a	0.988
Fall	1.013 a	1.169 a	1.091

Means not sharing the same letter(s) within columns are significantly different ($P < 0.05$), Duncan's multiple range test.

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تأثير استخدام بعض منظمات النمو والأحماض الفينولية وميعاد الإكثار على تجذير العقل الساقية للزيتون

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(قدم للنشر في ١٥/٨/ ١٤٢٠ وقبل للنشر في ٧/ ١/ ١٤٢١ هـ)

ملخص البحث. عوملت عقل ساقية نصف خشبية من الزيتون صنف فرانتويو بخمس عشرة معاملة تضمنت منظمات النمو ، اندول حمض البيوتريك بتركيز ٣٠٠٠ جزء في المليون و نفثالين حمض الخليك بتركيز ١٠٠٠جزء في المليون و بعض المواد الفينولية كالكاتيكول بتركيز ٥٠٠ و١٠٠٠ جزء في المليون و حمض السينماميك بتركيز ٥٠٠ و١٠٠٠ جزء في المليون سواء بصورة فردية أو في مخاليط مختلفة من منظمات النمو مع هذه المواد الفينولية. وتم تجذير العقل في غرفة النمو تحت قوة إضاءة ٣٠٠٠ شمعة / قدم لمدة ١٦ ساعة إضاءة يوميا و كانت درجة الحرارة ٢٥ °م نهاراً و ٢٠ °م وتراوحت الرطوبة النسبية بين ٨٠- ٨٥ ٪. وأظهرت النتائج بوجه عام بعد ٨ أسابيع من الزراعة أن معاملة عقل الزيتون بكل المعاملات المستخدمة في التجربة تؤدي إلى زيادة النسبة المئوية للتجذير بمقارنتها بالمعاملة الضابطة. كما أوضحت النتائج أن معاملة عقل الزيتون بإندول حمض البيوتريك + الكاتيكول بتركيز ١٠٠٠جزء في المليون يعطي أعلى نسبة مئوية للتجذير (٥٧.٨٪) يلي ذلك معاملة العقل بإندول حمض البيوتريك + حمض السينماميك بتركيز ١٠٠٠جزء في المليون (٥٠.٩٪) بينما كانت أقل نسبة مئوية لتجذير العقل في المعاملة الضابطة (١٧.٣٪). وبالإضافة إلى ذلك أعطت العقل التي تم إكثارها في الربيع خلال موسمي التجربة أعلى نسبة مئوية للتجذير بمقارنتها بتلك التي تم إكثارها في الخريف ، وربما يرجع ذلك إلى النشاط العالي لنسيج الكامبيوم المسؤول عن تكوين الجذور العرضية على العقل وذلك في الربيع عما هو في الخريف. وبوجه عام وجد أن العقل التي عوملت بنفثالين حمض الخليك في مخلوط مع حمض السينماميك بتركيز ٥٠٠ جزء

في المليون وبإندول حمض البيوتريك + حمض السينامك بتركيز ١٠٠٠ جزء في المليون أعطت أكبر عدد من الجذور (٢٨.٨ و٢٣.٩ جذور لكل عقلة ، على التوالي) بينما أعطت العقل الغير معاملة ٦.٨ جذور فقط. كما أعطت العقل التي تم إكثارها في ربيع الموسم الثاني عدداً أكبر من الجذور بالمقارنة بتلك التي تم اختيارها في الخريف. ونتجت أطول الجذور على العقل المعاملة بحمض السينامك بتركيز ١٠٠٠ جزء في المليون سواء بمفرده أو مخلوط مع نفثالين حمض الخليك وكذلك بمعاملة العقل بإندول حمض البيوتريك + الكاتيكول بتركيز ١٠٠٠ جزء في المليون (١٤.٦ ، ١٤.٨ ؟ ١٤.٧ سم ، على التوالي) بينما أعطت عقل المقارنة جذوراً طولها ٩.١ سم. وبالإضافة إلى ذلك نتجت أطول الجذور على عقل الزيتون التي تم إكثارها في الربيع في الموسم الثاني بمقارنتها بتلك التي تم اختيارها في الخريف. وعلى العموم، وجد أن معاملة العقل بنفثالين حمض الخليك + حمض السينامك بتركيز ١٠٠٠ جزء في المليون أعطى جذور أكثر سمكا (١.٣ مم) بالمقارنة بتلك الناشئة على معظم عقل المعاملات الأخرى بما فيها عقل المقارنة والتي أعطت جذوراً سمكها ٠.٧٦٧ مم. ولم يكن هناك فرق في سمك الجذور على العقل التي تم إكثارها في الربيع أو الخريف خلال موسمي التجربة. عموماً أظهرت النتائج أن التفاعل بين الأوكسينات والمواد الفينولية المستخدمة في التجربة قد أدى إلى زيادة تأثير تلك المواد على التجذير في العقل الساقية للزيتون مقارنة باستعمالها بصورة فردية.