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Rooting Response of Five Pomegranate Varieties to Indole Butyric Acid Concentration and Cuttings Age

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Abstract: The purpose of this study was to evaluate the effect of cutting age and Indole Butyric Acid (IBA) treatments on five pomegranate varieties propagation by stem cuttings. The experiment was carried out in a partially controlled glasshouse conditions at Mutah University, Jordan. The treatments comprised of two types of cuttings, i.e., hardwood and semi-hardwood; five concentrations of IBA, i.e., 3,000, 6,000, 9,000 and 12,000 ppm as quick dip (10 sec) as well as five Jordanian pomegranate varieties (Kdaree Hello, Hmadee Hmaree, Kdaree Sfaree, Zeklabi, Maleese). In this study, the percentage of cuttings that rooted, the number of roots produced per cutting, root length and diameter and root weight per cutting were recorded. It was obvious that the rootability of pomegranate is influenced by the interactive effect of cuttings age, IBA concentration and variety as well as by the single effect of either. The cuttings taken from hardwood stems had higher rooting percentage than those taken from semi-hardwood stems with a considerable differences in rootability between varieties under this study. The highest percentage of cuttings that rooted was observed in Hmadee Hmaree (70%), Zeklabee (69%) and Malesse (73%), while the lowest rooting percentage in Khdaree Hello (58%) and Kdaree Sfaree (49%) varieties. Zeklabee and Hmadee Hmaree varieties when compared with other varieties gave more favorable results at 6000 to 9000 ppm IBA in terms of the percentage of cuttings that rooted, the number of roots produced per cutting and root weight per cutting using both semi- and hard-wood cuttings. It was concluded that the increasing dose of IBA could be useful in increasing rooting potential and other root characteristics in pomegranate.

Key words: Pomegranate, cuttings age, hardwood cuttings, semi-hardwood cuttings, Indole Butyric Acid (IBA)

INTRODUCTION

Pomegranate (*Punica granatum* L.) of the family Punicaceae is native from Iran to the Himalayas in Northern India, cultivated and naturalized over the whole Mediterranean region since ancient times (Facciola, 1990). Pomegranate is a high value fruits and one of the most important endemic horticultural crops in Jordan growing in most of the regions through out the country and grows well in arid and semiarid regions due to its adaptation to adverse ecological conditions. Pomegranate has been known to considerable pharmacological properties with antimicrobial, antiviral, potent antioxidant and antimutagenic effects (Negi *et al.*, 2003; Seeram *et al.*, 2005) and has been in the market for preparation of tinctures, juice, cosmetics and therapeutic formulae (Kim *et al.*, 2002).

Pomegranate could be propagated either sexually by seeds or vegetatively using stem cuttings and sometimes as layers or suckers or by grafting (Hartmann *et al.*, 1997; Melgarejo *et al.*, 2008; Polat and Caliskan, 2009). Plants started from seed exhibit different characteristics

than the parent plants and desirable traits may be lost or undesirable ones acquired from parent to offspring, which seeds germinate easily without going through a rest period, but trees are not grown commercially from seed germination because seedlings do not come true to variety (Hartmann *et al.*, 1997). Grafting and layering of pomegranate trees is rarely done, because many different types of grafts have not been successful enough for use in commercial production. Tissue culture is another method of production that calls for the growth of the plant in a sterile environment using the tissue, seed, or cuttings (Abdelrahman and Al-Wasel, 1999). Propagation from cuttings (cloning) produces a plant with the same characteristics as the parent and thus maintains desirable fruiting traits. Cutting is undoubtedly, the main method used for the pomegranate tree propagation (Melgarejo *et al.*, 2008; Saroj *et al.*, 2008; Polat and Caliskan, 2009). The cuttings rooting is regulated by a number of factors, whose lack can limit the process. Cuttings are the easiest and most satisfactory method pomegranate propagation with 15-20 cm in length and pencil size or larger in diameter and use of semi-hardwood

or hardwood rooting hormone is possible (Melgarejo *et al.*, 2008; Saroj *et al.*, 2008; Polat and Caliskan, 2009). Polat and Caliskan (2009) revealed that the rooting of cuttings of pomegranate varied according to different dates of stem collections; the cuttings collected at the end of February had higher rooting potential than those taken at the beginning of October. This result could be attributed to the different concentrations of auxins and rooting cofactors (Polat and Caliskan, 2009) or could be due to the fact that carbohydrates content in late date may be more than early date of cuttings collection (Hambrick *et al.*, 1991; Polat and Caliskan, 2009). Cuttings should be maintained at a temperature between 21 and 27°C during the daytime and night-time temperatures of 15°C (Hartmann *et al.*, 1997). On the other hand, Amorós *et al.* (1997) stated that there has to be supporting heat for the cuttings to improve multiplication and that there should be a heat difference between the air and the soil and cuttings at 22°C presented a higher rooting percentage than those at 18°C and at normal temperatures; as also indicated by Hartmann *et al.* (1997), who fixed the ideal temperature between 21 and 27°C higher than the environment with the heat applied to the base of the cuttings, which helped to stimulate rooting.

Pomegranate cuttings often induced roots without exogenous auxin treatment (Melgarejo *et al.*, 2008; Saroj *et al.*, 2008; Polat and Caliskan, 2009). Few studies have investigated the effect of exogenous auxin on rooting capacity (Melgarejo *et al.*, 2008; Saroj *et al.*, 2008; Polat and Caliskan, 2009). It has been reported that application of exogenous auxins to roots increased the percentage of cuttings that rooted of pomegranate cuttings up to three fold (Melgarejo *et al.*, 2008; Saroj *et al.*, 2008; Polat and Caliskan, 2009).

Although, pomegranate cultivated area in Jordan is very limited, locally adapted varieties are still cultivated, harboring high diversity level diversity level in terms of morphological traits and at the DNA level (Awamleh *et al.*, 2009). Pomegranate varieties are available with different local Jordanian names; Kdaree Hello, Hmadee Hmaree, Kdaree Sfaree, Zeklabee and Malesse, they are either indigenous (landraces) or introduced from neighbor countries. In Jordan, pomegranate has been grown over the decades in the mountainous and valleys where irrigation is available. There is no reported study on the rooting potential of the pomegranate varieties grown in Jordan. The aim of this work was to study the rooting capacity of different pomegranate varieties, taking into account certain factors which influence root formation in cuttings of this species. In particular, tests were carried out with IBA application using both semi and hardwood cuttings, as factors influencing the rooting potential of

different pomegranate varieties. Moreover, this study will show propagation possibilities and output for some interesting varieties of clones and others that could be interesting as rootstocks to tolerate harsh environmental conditions.

MATERIALS AND METHODS

The experiment was conducted during the period of mid-February to April 2009 at Mutah University Agricultural Station, Karak, Jordan (31° 16' N, 35° 45' E and ca 920 m above sea level). This investigation was aimed at studying the effect of cutting age, different IBA concentrations and variety on rooting potential of five Jordanian pomegranate varieties from Jordan. Kdaree Hello, Hmadee Hmaree, Kdaree Sfaree, Zeklabee and Malesse varieties were selected for their widespread planting in orchards by local farmers in Jordan. From each variety, semi-hardwood stems (i.e., cuttings prepared from tender shoots and branches of the current years growth that are not too hard but show brown blotches on the green stem) and hardwood stems (i.e., the branches of the current years growth of plant with brown stems) were taken with a length of 20-35 cm at the morning and covered with moist burlap in mid-February 2009. Four IBA concentrations (3000, 6000, 9000 and 12000 ppm) in addition to the control were prepared by dissolving the hormone powder in ethanol. Few drops of NH₄OH were added to avoid precipitation of the hormone. The collected cuttings were wounded by making two opposite longitudinal incisions and dipped in solution of Benlate as a protective measure for fungal infection. Then, the bases of cuttings were treated for 10 sec with the prepared IBA concentrations and with water as a control treatment. The experiment was carried out in a partially controlled glasshouse conditions; the air temperature and relative humidity inside the greenhouse were registered by a thermo-hydrograph. The air temperature ranged from 20 to 22°C and relative humidity fluctuated between 60 and 70% at day/night. The temperature of the rooting medium was 24-26°C maintained by the use of the bottom heat as recommended by Hartmann *et al.* (1997). Mist was applied for 5 sec every ½ h during the experiment duration. The cuttings were inserted into raised benches containing perlite and peatmoss (1:1) as rooting medium. The rooting medium was 18 cm in depth and cuttings were placed on 8 cm depth with 3 cm space between cuttings.

The influences of cuttings age, IBA concentration and variety were recorded in terms of the percentage of cuttings that rooted, the number of roots produced per cutting, root length and diameter and root dry and fresh

weight per cutting. Randomized Complete Block Design (RCBD) with a split-split plot arrangement was used with cutting age as main plots, IBA treatments as sub-plots and varieties as sub-sub-plots. Each treatment was replicated five times and each replicate was represented by 20 cuttings. Analysis of Variance (ANOVA) was used to test cutting age, IBA treatment and variety effects as well as their interactions. Data for recorded traits was analyzed using the following mathematical model:

$$Y_{hijk} = \mu + \rho_h + \alpha_i + \delta_{ij} + \beta_j + \alpha\beta_{ij} + \lambda_{hij} + \gamma_k + \alpha\gamma_{jk} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + \epsilon_{hijk}$$

where, Y_{hijk} is the observation of the i th cutting type for cutting age α in j th IBA concentration β within k th variety γ in the h th replication ρ ; μ is the general mean effect, $\alpha\beta$, $\alpha\gamma$, $\beta\gamma$, $\alpha\beta\gamma$ are the interactions. δ_{ij} , λ_{hij} and ϵ_{hijk} is the variation due to error a, error b and residual error, respectively.

Data was analyzed using three-way ANOVA using the statistical package MSAT-C and the differences between the means were compared using least significant differences at $p < 0.05$.

RESULTS

The percentage of cuttings that rooted, the number of roots produced per cutting, average root length and root diameter and root weight per cutting were significantly affected by cutting age, IBA concentration, variety effects and their interactions at $p < 0.01$ (Table 1). Hardwood cuttings gave higher percentage of rooting, greater number of roots, longer and thicker roots and

higher root fresh and dry weights than semi-hardwood cuttings at $p < 0.01$ (Table 1). Using hardwood cuttings, the percentage of cuttings that rooted, number of roots per cutting, root length and diameter and root fresh and dry weights increased by 21.3, 51.7, 63.3, 160, 109.3 and 136.4% compared with the semi-hardwood cuttings, respectively. The percentage of cuttings that rooted and other root characteristics were significantly influenced by increasing IBA concentration ($p < 0.01$). In general, rooting percentage, root length and other root-related traits were significantly increased by increasing IBA concentration up to 9000 ppm, except for average root length and root diameter and then sharply decreased by increasing IBA concentration to 12000 ppm although higher than the control treatment. For example, the percentage of cuttings that rooted increased by 30.7, 89.4, 96.6 and 56.5% at 3000, 6000 and 9000 and 12000 ppm IBA concentrations as compared with the control treatment, respectively. Varieties revealed significant differences for the percentage of cuttings that rooted and other root characteristics at $p < 0.01$ (Table 1). Cuttings obtained from Hmadee Hmaree and Zeklabee varieties gave higher rooting percentages, more number of roots per cutting and heavier root weights than other three varieties under study. The percentage of cuttings that rooted was about 70.7 and 69.2%, number of roots was about 18.0 and 14.8 per cut, root fresh weight was 0.33 and 0.86 g and root dry weight was 0.134 and 0.023 g for Hmadee Hmaree and Zeklabee varieties, respectively. The longest roots were observed in Hmadee Hmaree and Kdaree Sfaree and Zeklabee varieties, ranging from 21.16 to 23.01 cm, while the shortest roots were detected in Kdaree Hello and

Table 1: Effect of cutting ages, IBA concentrations, cultivars and their interactions on some root parameters in pomegranate

Treatments	Rooting (%)	Roots No. per cutting	Average root length (cm)	Average root diameter (cm)	Fresh root weight per cutting (g)	Average dry root weight per cutting (g)
Cuttings age (CA)						
Semi	58.13b	9.22b	11.95b	0.005b	0.193b	0.055b
Hard	70.47a	13.99a	19.52a	0.013a	0.404a	0.130a
IBA concentration (ppm)						
0	41.58e	6.84e	10.51e	0.006b	0.15d	0.049e
3000	54.34d	9.14d	12.71d	0.008ab	0.20c	0.063d
6000	78.74b	14.64b	17.81b	0.011ab	0.30b	0.109b
9000	81.76a	15.64a	21.82a	0.011a	0.52a	0.150a
12000	65.08c	11.78c	15.84c	0.009ab	0.30b	0.091c
Cultivar (C)						
Khdaree Hello	58.46c	7.48d	7.14c	0.007bc	0.042d	0.011d
Hmadee Hmaree	70.72b	18.00a	21.16b	0.006c	0.330b	0.134b
Kdaree Sfaree	49.74d	12.36c	21.47b	0.012ab	0.213c	0.083c
Zeklabee	69.24b	14.84b	23.01a	0.013a	0.864a	0.023a
Malesse	73.34a	5.36e	5.91c	0.007bc	0.042d	0.008d
Interactions						
CA×IBA	**	**	**	**	**	**
CA×C	**	**	**	**	**	**
IBA×C	**	**	**	**	**	**
CA×IBA×C	**	**	**	**	**	**

Means within column having different letter for each parameter are significantly different at 0.05 level of probability according to LSD test. **Significant at 0.01 probability level, respectively

Table 2: Interactive effects of cutting age, IBA concentration and cultivar on rooting percentage of pomegranate

Cutting age	IBA concentration (ppm)	Cultivars				
		Khdaree Hello	Hmadee Hmaree	Kdaree Sfaree	Zeklabee	Malesse
Semi-hard	0	32.0vw	38.6tu	21.6x	42.4r-t	61.8m-p
	3000	34.0uv	41.0st	43.6r-t	59.6o-q	75.8hi
	6000	48.0r	88.0c-e	74.0ij	90.4b-d	92.2a-c
	9000	67.2k-m	77.2g-i	32.4vw	95.6ab	82.2e-g
	12000	45.4rs	57.8pq	21.6x	76.0hi	54.8q
Hard	0	47.4r	64.0l-o	27.6w	34.4uv	46.0rs
	3000	61.0u-p	74.2ij	39.0tu	46.0rs	69.2j-l
	6000	88.4cd	84.8d-f	71.8i-k	69.2j-l	80.6f-h
	9000	95.0ab	96.8a	88.4cd	92.6a-c	90.2b-d
	12000	66.2k-n	84.8d-f	77.4g-i	86.2d-f	80.6f-h

Interactive means having different letter are significantly different at 0.05 level of probability according to LSD test

Table 3: Interactive effects of cutting age, IBA concentration and cultivar on root number of pomegranate

Cutting age	IBA concentration (ppm)	Cultivars				
		Khdaree Hello	Hmadee Hmaree	Kdaree Sfaree	Zeklabee	Malesse
Semi-hard	0	3.2tu	6.4o-q	4.4q-t	2.2u	2.2u
	3000	4.4q-t	9.6k-m	8.8l-n	4.6q-t	2.2u
	6000	5.6p-r	22.4b-d	17.8g	5.8p-r	3.4s-u
	9000	8.8l-n	24.4b	22.2c-e	14.0h-i	5.8p-r
	12000	4.4q-t	9.6k-m	23.4bc	10.4kl	4.6q-t
Hard	0	7.0u-p	18.8fg	5.4p-s	14.8hi	4.0r-u
	3000	8.8l-n	21.4c-e	5.4p-s	20.8d-f	5.4p-s
	6000	9.8k-m	28.4a	15.6h	26.8a	10.8kl
	9000	13.0ij	20.2ef	11.6jk	28.2a	8.2m-o
	12000	9.8k-m	18.8f-g	9.0l-n	20.8d-f	7.0n-p

Interactive means having different letter are significantly different at 0.05 level of probability according to LSD test

Malesse varieties with average root lengths of 7.14 and 5.91 cm, respectively.

The statistical analysis of obtained data revealed that the response to interactions between cutting age and IBA and variety, between IBA and cultivar and between cutting age, IBA and variety was significant (Table 1). It is clear from Table 1 and 2 that the percentage of cuttings that rooted in five pomegranate varieties responds differently to cutting age and IBA concentration. The rooting percentage of the five varieties increased as the concentration of IBA increased to 9000 ppm, however, considerable reduction in rooting percentage was occurred when IBA concentrations increased to 12000 ppm. Less rooting percentages were detected using semi-hardwood cuttings in the five pomegranate varieties at different IBA levels. The percentage of cuttings that rooted using hardwood cuttings was significantly increased using 6000 to 9000 ppm IBA, ranging from 80.6 to 88.4% and 88.4 to 96.8% at 6000 and 9000 ppm IBA, respectively (Table 2).

The data showed that increasing IBA concentration from 0 to 9000 ppm significantly improved roots number per cut in the five pomegranate varieties (Table 3) however, considerable reduction in root number per cut was detected when IBA concentration increased to 12000 ppm. In both cutting types, the control treatment

gave the least root number, while the highest number of roots per cutting was obtained when exogenous IBA increased to 9000 ppm in Zeklabee variety (28.2 roots/cutting) and at 6000 ppm IBA for Hmadee Hmaree variety (28.4 roots/cutting). On the contrary, the least root number per cutting was detected in control treatments in Khdaree Hello and Malesse varieties.

Significant effects of cutting age \times IBA concentration \times variety on root length and diameter were observed (Table 4, 5). Using the two cutting types, the increment in root length and diameter was more prominent with increasing IBA concentration to 9000 ppm for the five pomegranate varieties, however, significant reductions in root length and diameter were detected at 12000 ppm IBA in all pomegranate varieties except Kdaree Sfaree which showed significant increase in root length and root weight by increasing IBA concentration to 12000 ppm using semi-hardwood cuttings. Significant effects of cutting type \times IBA \times variety on root fresh and dry weight were also observed (Table 6, 7). The results clearly demonstrate that 6000 to 12000 ppm IBA treatments gave the best results under study using hardwood cuttings depending on the variety, however, significantly heavier roots cut were obtained in hardwood cuttings in comparison with semi-hard wood cuttings at different IBA levels for different pomegranate varieties.

Table 4: Interactive effects of cutting age, IBA concentration and cultivar on root length (cm) of pomegranate

Cutting age	IBA concentration (ppm)	Cultivars				
		Khdaree Hello	Hmadee Hmaree	Kdaree Sfaree	Zeklabee	Malesse
Semi-hard	0	2.21z	9.55r-w	5.84v-z	4.94x-z	2.84yz
	3000	2.32z	15.71n-p	9.55r-w	9.20s-w	4.37x-z
	6000	2.65yz	22.83g-k	17.30l-o	17.25l-o	5.75w-z
	9000	10.27q-u	27.60de	23.40f-k	22.19h-k	9.77r-v
	12000	2.65yz	16.46m-p	33.98b	13.80o-q	6.32u-z
Hard	0	4.74x-z	25.59d-h	20.73i-l	23.81e-g	4.81x-z
	3000	9.73r-w	20.22j-m	23.32f-k	26.78d-g	5.91v-z
	6000	10.82q-t	29.12cd	32.66bc	32.74bc	7.01t-x
	9000	13.52o-r	24.27e-i	28.51d	52.09a	6.56u-y
	12000	12.44p-s	20.23j-m	19.44k-n	27.29d-f	5.77v-z

Interactive means having different letter are significantly different at 0.05 level of probability according to LSD test

Table 5: Interactive effects of cutting age, IBA concentration and cultivar on root diameter (cm) of pomegranate

Cutting age	IBA concentration (ppm)	Cultivars				
		Khdaree Hello	Hmadee Hmaree	Kdaree Sfaree	Zeklabee	Malesse
Semi-hard	0	0.002b	0.002b	0.002b	0.002b	0.002b
	3000	0.003b	0.003b	0.004b	0.003b	0.003b
	6000	0.004b	0.005b	0.006b	0.005b	0.004b
	9000	0.009ab	0.006b	0.017ab	0.007ab	0.007ab
	12000	0.004b	0.003b	0.014ab	0.006b	0.005b
Hard	0	0.006b	0.003b	0.012ab	0.018ab	0.007ab
	3000	0.006b	0.013ab	0.014ab	0.019ab	0.010ab
	6000	0.009ab	0.020ab	0.021ab	0.021ab	0.012ab
	9000	0.012ab	0.003b	0.015ab	0.028a	0.010ab
	12000	0.010ab	0.003b	0.014ab	0.021ab	0.008ab

Interactive means having different letter are significantly different at 0.05 level of probability according to LSD test

Table 6: Interactive effects of cutting age, IBA concentration and cultivar on root fresh weight (g) of pomegranate

Cutting age	IBA concentration (ppm)	Cultivars				
		Khdaree Hello	Hmadee Hmaree	Kdaree Sfaree	Zeklabee	Malesse
Semi-hard	0	0.017o	0.209i-n	0.166i-o	0.911d	0.035no
	3000	0.022o	0.283h-k	0.181i-o	0.954cd	0.026o
	6000	0.033no	0.410f-h	0.337g-j	1.117bc	0.030no
	9000	0.045m-o	0.337g-j	0.233h-l	2.906a	0.024o
	12000	0.027o	0.292g-k	0.220i-m	1.256b	0.020o
Hard	0	0.054l-o	0.046m-o	0.056l-o	0.044m-o	0.006o
	3000	0.064l-o	0.217i-m	0.127k-o	0.115k-o	0.010o
	6000	0.067l-o	0.695e	0.157j-o	0.218i-m	0.014o
	9000	0.070l-o	0.578ef	0.339g-i	0.649e	0.094l-o
	12000	0.022o	0.231h-l	0.313g-j	0.467fg	0.158j-o

Interactive means having different letter are significantly different at 0.05 level of probability according to LSD test

Table 7: Interactive effects of cutting age, IBA concentration and cultivar on root dry weight (g) of pomegranate

Cutting age	IBA concentration (ppm)	Cultivars				
		Khdaree Hello	Hmadee Hmaree	Kdaree Sfaree	Zeklabee	Malesse
Semi-hard	0	0.005r	0.108j-l	0.065m-o	0.253cd	0.006r
	3000	0.005r	0.121i-l	0.0911-n	0.268c	0.008r
	6000	0.009r	0.243cd	0.144g-j	0.342b	0.006r
	9000	0.010r	0.189ef	0.104k-m	0.670a	0.007r
	12000	0.008r	0.148g-i	0.117i-l	0.313b	0.005r
Hard	0	0.009r	0.013r	0.022p-r	0.012r	0.002r
	3000	0.015r	0.060n-p	0.030o-r	0.031o-r	0.003r
	6000	0.020qr	0.223de	0.038o-r	0.064m-o	0.005r
	9000	0.021p-r	0.181fg	0.116i-l	0.172f-h	0.026o-r
	12000	0.005r	0.059n-q	0.101k-m	0.139h-k	0.015r

Interactive means having different letter are significantly different at 0.05 level of probability according to LSD test

DISCUSSION

Generally *Punica granatum* L. is propagated commercially by cuttings (Melgarejo *et al.*, 2008;

Saroj *et al.*, 2008; Polat and Caliskan, 2009) however, high genetic variation in rootability among varieties were existing with high interactive effect between varieties, IBA concentration and cutting age. No information

is available about rootability potential of pomegranate Jordanian varieties, therefore the current study were conduct to study the rootability of cuttings of five pomegranate varieties using different IBA concentration and two different cutting types (semi- and hard-wood cuttings). Propagation by cuttings is characterized by being easy to perform, cheap and the growth of the plant are usually fast. In addition, identical plants can be obtained and large number of plants could be obtained from one single mother plant (Howard, 1971). In our experiment, the majority of cuttings was rooted in a period of 8-10 weeks and the vegetative buds of the rooted cuttings began to grow 2-4 weeks after rooting. Propagation of the different kinds of fruit trees could be classified into easy-to-root and difficult-to-root species (Ashiru and Carlson, 1968), our results confirmed that pomegranate is easy-to- root species.

Previous studies revealed that pre-rooting treatment with synthetic auxin (IBA) and the temperature of the root medium had marked effects in promoting rooting potential in pomegranate (Amorós *et al.*, 1997; Hartmann *et al.*, 1997). It is stated that a heat difference between the air and rooting medium is of a prime importance to improve rooting capacity. In our experiment the bottom heat temperature was 2-4°C higher than the air temperature. Hartmann *et al.* (1997) stated that for most species, daytime temperatures of between 21 and 27°C and nighttime temperatures of 15°C, are satisfactory for pomegranate rooting. On the other hand, they indicated that there had to be a supporting heat for the cuttings in order to improve multiplication, with a heat difference between the atmosphere and the soil. Amorós *et al.* (1997) pointed out that hardwood cuttings of some pomegranate clones which have been subjected to base heat application during rooting improve their rooting percentage, with the positive effect being greater at 22°C than at 18°C or at normal air temperature (15.75%) and they conclude that the optimum temperature in this experiment was 22°C. In our experiment IBA treatment as a quick dip for 10 sec was effective in inducing rooting capacity in pomegranate semi-hard and hardwood cuttings. Similarly, the results obtained by Melgarejo *et al.* (2008) demonstrated that IBA generally increased the percentage of rooting (although not for all concentrations), with 12,000 ppm producing the best results were obtained in the clones studied. Moreover, wounding carried out at the base of the cutting further increased the percentage of cuttings that rooted (Melgarejo *et al.*, 2008).

The data statistical analysis revealed highly significant differences among varieties for the percentage of cuttings that rooted, root length and diameter and root

fresh and dry weight. The genetic variation was also reflected in the differences observed among the varieties in their response to IBA concentration. Similarly, a wide genotypic variation for rooting capacity was detected in pomegranate (Melgarejo *et al.*, 2008; Saroj *et al.*, 2008; Polat and Caliskan, 2009), cherry (Yazici *et al.*, 2009), apple (El-Shazly *et al.*, 1999) and fig (Antunes *et al.*, 2003; Zare and Hassan-pour, 2008). From this perspective, it is clear that Hmadee Hmaree (70.72%), Zeklabee (69.24%) and Malesse (73.34%) with high rooting capacity percentages would have more advantages than the other two pomegranate varieties under study (Khdaree Hello and Kdaree Sfaree) which significantly had lower rooting potential. The number of roots was highest in the cutting of the Zeklabee and Hmadee Hmaree varieties and lowest in Khdaree Hello and Malesse varieties. Therefore, selection genotypes with high rooting capacity with vigorous roots are of prime importance for pomegranate commercial seedling production. The differences in carbohydrate contents in the cuttings was considered as an important factor that determines the variability in rooting capacity among genotypes (Hambrick *et al.*, 1991; Hartmann *et al.*, 1997); where relatively high carbohydrate content was associated with easy rooting cutting. The relatively higher response of some genotypes may be also attributed to high availability of endogenous natural auxin in plant tissue (Salisbury and Ross, 1969). Certain level of endogenous auxin is already presenting in the cuttings, therefore treating cuttings with IBA could optimize the auxin level in the cutting and consequently improves the percentage of cuttings that rooted (Melgarejo *et al.*, 2008; Saroj *et al.*, 2008; Polat and Caliskan, 2009). The effect of auxin in vascular differentiation is well established. Auxin is known to stimulate both cambial activity and xylem development in many woody species (Zakrzewski, 1983) and it is required for formation of the primordium initial cell (Davis and Hassig, 1990).

In the current study, it is clear that vegetative propagation by cuttings is effective, simple and cheap method of the multiplication of pomegranate rootstocks, which has been used with great success worldwide (Melgarejo *et al.*, 2008; Saroj *et al.*, 2008; Polat and Caliskan, 2009). The percentage of cuttings that rooted in the control treatment was more than 40% rooting rate, however, considerable improvement in rooting percentages have been achieved by IBA treatment. The physiological processes leading to induction of root initiation are not yet fully elucidated. An increase of free auxins was found in the bases of the cuttings before the beginning of root initiation (Nordstrom *et al.*, 1991). It was been postulated that easily rooting species, as opposed to those which were difficult to root, have an ability to

hydrolyze auxin conjugates at the appropriate time to release free auxins which may promote root initiation (Epstein and Ludwig-Muller, 1993).

The age of rooting cuttings can play an important role in improving rooting capacity (Ghosh *et al.*, 1988; Sandhu *et al.*, 1991; Singh, 1994; Melgarejo *et al.*, 2008). The interactive effect of this factor with IBA concentration is very clear. Meantime, an obvious increase in rooting percentage and number of roots per cutting was attained by hardwood cuttings. The obtained results are confirmed with those listed by earlier investigators on numerous pomegranate genotypes (Melgarejo *et al.*, 2008; Saroj *et al.*, 2008; Polat and Caliskan, 2009). The root formation process on the cuttings is a complicated one which regulated by many different internal factors like the concentration of endogenous auxin, the rooting cofactors, carbohydrate substances stored in the cuttings as well as the nitrogen content, these factors may interact to influence the rooting percentage, root length and diameter and root weight.

Significant interactions occurred between stem age, IBA level and variety in terms of the percentage of cuttings that rooted, the number of roots produced per cutting and root weight per cutting. High overall levels of rooting were obtained for some of the treatment combinations (Table 2). The percentages of rooted cuttings were significantly greater for hardwood cuttings than for semi-wood cuttings when 6000 to 9000 exogenous IBA was used in the five pomegranate varieties. The same patterns occurred with root weight per cutting and the number of roots produced per cutting (Table 3, 6). The IBA stimulated greater root production in both hard and semi-hardwood cuttings, however, root production in hardwood cuttings treated with IBA was statistically greater than in semi-hardwood cuttings (Table 1). Saroj *et al.* (2008) found that 2500 ppm as quick dip (5 min) gave the highest percentage of rooting compared to the control and those treated with 5,000 ppm. This treatment (IBA 2,500 ppm) also induced fibrous root system, which is essential for better establishment of plants under field conditions. Melgarejo *et al.* (2008) showed that pomegranate rooting potential can be increased by IBA applications in high concentrations, 12,000 ppm IBA gave the best results, while when wounding was carried out at the base of the cutting, further increment in the percentage of cuttings that rooted occurred in most of the clones using low IBA application concentration (3000 ppm). Moreover, Polat and Cliskan (2009) found that low IBA concentration (1000 ppm) positively increased the rooting percentage of cuttings and other root characteristics although it was not to satisfactory level.

In conclusion, pomegranate can generally be defined as a species which root easily, which can also be observed from this investigation. However, the IBA treatments have been essential for the obtaining of high rooting percentages. In fact, this experiment that carried out on 5 pomegranate varieties, all have rootability higher than 80% at 9000 ppm IBA treatment. Although, the ability of generating roots by IBA treatment were enhanced by IBA treatment, but it seems to be that considerable variability was related to the variety. Hardwood cuttings of pomegranate varieties seem to have a clearly higher rootability than those of semi-hardwood cuttings. Some varieties used in the current study could be interesting as rootstocks to tolerate the harsh environments prevailing under arid and semi-arid conditions.

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