EFFECT OF INDOLEBUTYRIC ACID ON ROOT REGENERATION AND SEEDLING SURVIVAL AFTER TRANSPLANTING OF THREE *Pistacia* SPECIES

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(Received March 17, 2008/Accepted September 15, 2008)

A B S T R A C T

The species of *Pistacia vera*, *P. mutica* and *P. khinjuk* can be used as rootstocks for pistachio cultivars due to their adaptability to severe environmental conditions and resistance to some pests and diseases. However, low percentage of seedling survival following transplanting has been a major problem. This study was conducted to test the effects of different concentrations of indolebutyric acid (IBA) on root regeneration of transplanted bare-rooted seedlings of these three species. The seedlings treated with IBA at all concentrations tested (1000, 1500, 2000, 2500 and 3000 mg l^{-1}) developed more roots, which were longer and had higher fresh and dry weight, and increased the survival rate of treated seedlings compared to their respective controls. The most effective concentrations in this respect were 2000 and 2500 mg l^{-1}. There was a significant interaction between the species and IBA concentration. Shoot height, root and shoot diameter, leaf number, shoot fresh and dry weight were not affected by IBA. Results also showed that survival rate of the seedlings was significantly correlated with root number, length and fresh and dry weight.

Key words: root regeneration, IBA, seedling survival, *Pistacia*

INTRODUCTION

The genus *Pistacia* is a member of the Anacardiaceae family and consists of eleven or more species (Zohary, 1952). Among these, *P. vera* L. is economically important due to its fruits (pistachio nuts). Seedlings of other species are used mainly as rootstocks for pistachio (Spiegel-

Roy, 1985). *P. mutica* F. and M. and *P. khinjuk* Stocks are two wild species of *Pistacia* naturally distributed in many parts of Iran at an area of 2.5-3 million hectares (Sheibani, 1996). Although their potential as a rootstock have been known for many decades due to resistance to some pests and diseases, the difficulties in both propagation and transplanting have limited the use of these two rootstocks (Rahemi and Baninasab, 2000). These limitations are mainly attributed to seed dormancy and very few lateral roots formed on seedlings.

In addition, transplanting often causes root damage, reducing the effective root area which in turn cause water stress (Kramer, 1995), decrease nutrient uptake (Bloom and Sukrapanna, 1990) and make plants more susceptible to diseases (Moss and Main, 1989). Rapid resumption of root initiation and growth are two of the principal processes responsible for seedling survival after transplanting (Burdett, 1987). Several studies have attempted to predict the quality of seedlings by assessing root regeneration capacity i.e., the ability of seedlings to initiate new roots upon planting (McCreary and Duryea, 1987). These studies concluded that the survival of a seedling after transplanting is a function of its ability to initiate new roots and that root regeneration capacity is not the only factor. Although root regeneration capacity may not be considered as ultimate predictor of planting performance, the ability to manipulate factors that regulate the quantity, quality, type and speed of root growth has the potential to play an important role in increasing the survival and growth of the planted trees (Scagel and Linderman, 2001).

Seedling root system can be manipulated to reduce the effects of transplanting shock by increasing the amount of their roots. This may be achieved by root pruning (Kozlowski and Davies, 1975), using such methods as wrenching (Van Dorsser, 1985), or under cutting (Aldhous and Mason, 1994) in the nursery to increase the amount of roots and improve root retention on lifting. Alternatively, root regeneration following transplanting in deciduous tree seedlings may be promoted by the prior application of plant growth regulators (Simpson, 1986; Davies et al., 2002).

Auxins are commonly used to stimulate root initiation in plants (Looney and McIntosh, 1968; Scagel et al., 2000). It has been reported that application of exogenous auxins to roots increased root regeneration of oak seedlings up to six fold (Struve and Arnold, 1986). Application of IBA and naphthalene acetic acid (NAA) to root system of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] by soil drench method increased lateral root production of the seedlings (Simpson, 1986). Also, application of IBA to the root system of *Pistacia chinensis* seedlings prior to transplanting greatly increased root regeneration potential (Lee and Hackett, 1976). However, in pea (*Pisum sativum* L.) and corn (*Zea mays* L.) application of auxins inhibited root elongation (Eliasson et al., 1989).

Present study was carried out to assess the effects of different concentrations of IBA applied to root system on root regeneration, growth and survival upon transplanting of seedlings of three *Pistacia* species.

MATERIAL AND METHODS

Plant Material

Seeds of *P. vera* and *P. mutica* were obtained from the Kerman Agriculture – Jahad Organization and *P. khinjuk* from the Research Centre of Natural Resources and Animal Science at Isfahan, Iran. Sound nuts of *P. khinjuk* and *P. mutica* were scarified by immersing them in concentrated H_2SO_4 for 20 and 90 min, respectively, and then washed for 24 h in running water. Naturally split *P. vera* hulled seeds were soaked in tap water for 24 h. The nuts of all species were then mixed with moist sphagnum peat (3:1, v/v) and stratified by keeping them at 5±1°C for 20 d. After stratification period, nuts were sown on 3 March 2002 directly into black plastic bags filled with 5 kg of a mixture of fine sand, leaf mould and loam soil (1 : 1 1, v/v). The bags were then kept in a greenhouse at 26.7°C (±4°C) under natural photoperiod for three months before being moved to an outdoor nursery area. Irrigation and weed control was maintained manually.

Experimental design

A 3 × 6 factorial experiment was used in a completely randomized design with five replications and four plants per a replication (plastic bag). For this purpose, seedlings of three *Pistacia* species: *P. vera, P. mutica* and *P. khinjuk* were treated with IBA at concentrations of 1000, 1500, 2000, 2500 and 3000 mg l^{-1}. Not treated seedlings (0 IBA) served as a control.

Auxin application

Dormant, one year old seedlings were removed from plastic bags on 13 March 2003. The root system was washed and dipped in IBA solutions for 30 s (Struve et al., 1983) and on the same day the seedling were replanted in new plastic bags filled with the same soil substrate. The root systems have been handled carefully so that no visible root damage occurred during transplanting. However, it is likely that some injury to the fragile, small root hairs have occurred. The IBA solutions was prepared by dissolving the required amounts in 50% ethanol and a few drops of ammonium hydroxide, to which eight drops per litre of Tween-20 were added as a surfactant. Three months after applying the treatments, the seedlings were removed from the containers and the soil substrate was carefully washed from the root system. The number, length and diameter of the roots were measured using the Delta-T SCAN image analysis system (Windias software). Shoot length, stem diameter, leaf number and fresh and dry weight of the roots and shoots were determined. Dry weight was determined after drying plant materials at 70°C for 72 h. Data were statistically analysed and the means compared using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

The analysis of variance showed significant differences among species, as well as IBA concentrations, for most of the traits measured. Also, there was a significant interaction between the species and IBA concentrations for some of the traits.

Irrespective of the species, IBA at all concentrations increased number of roots per a seedling compared to the control, although 2000 and 2500 mg l^{-1} IBA were more effective than the other concentrations (Tab. 1). There was a significant interaction ($p = 0.01$) between the species and IBA concentration; the increasing effect of 2000 and 2500 mg l^{-1} IBA was more pronounced in *P. khinjuk* but less effective in *P. mutica*. Previous studies have also shown that IBA increased the number of roots in Douglas-fir (Scagel et al., 2000), common oak and beech (Davies et al., 2002). On the other hand, it has been reported that IBA was not effective in improving root initiation of palms (Broschat and Donselman, 1990).

Total root length was also increased by IBA at all concentrations used compared to the control. In most cases, 2000 mg l^{-1} IBA was the most effective in promoting root elongation but, due to a significant interaction between the species and IBA concentration, in *P. vera* 2500 mg l^{-1} IBA produced the longest roots (1830 mm) while IBA applied at 1000 and 3000 mg l^{-1} on *P. khinjuk* and *P. mutica,* respectively, did not increase root length (Tab. 2). In general, the common increasing effects of IBA on *Pistacia* root elongation were consistent with the results found with *Acer saccharinum* (Richardson, 1958) and pear (Looney and Mc-Intosh, 1968), but not with the results obtained on herbaceous plants such as pea and corn (Eliasson et al., 1989).

Application of IBA at all concentrations increased significantly both fresh and dry weight of the roots, with the largest increase at 2000 and 2500 mg l^{-1} (Tab. 3 and 4). A significant interaction was observed between species and IBA concentration; the increasing effect of IBA on both root fresh and dry weight was more pronounced on *P. mutica*. In *P. vera*, treatments with IBA up to 2500 mg l^{-1} and in *P. mutica* and *P. khinjuk* up to 2000 mg l^{-1} progressively increased both fresh and dry weight of the roots. Further increase in IBA concentration reversed this trend (Tab. 3 and 4). Davies et al. (2002) reported similar findings with *Fagus sylvatica* and *Quercus robur*, when they applied IBA at 3000 and 250-1000 mg l^{-1}, respectively.

Shoot height, root and shoot diameter, leaf number and fresh and dry weight of the shoots were not affected by IBA when compared to the control (data not presented). Previous studies have also shown that fresh and dry weight of the shoots was not affected by the auxin treatments (Looney and McIntosh, 1968). This is attributed to *Pistacia* bud break occurring before root regeneration (Lee and Hackett, 1976).

Table 1. Influence of IBA at different concentrations on root number of three species of *Pistacia* seedlings

IBA [mg l^{-1}]	Root number			Mean
	P. vera	*P. mutica*	*P. khinjuk*	
Control	72.8 d*	47.9 fgh	28.6 i	49.8 D
1000	88.2 c	59.4 ef	36.7 hi	61.5 C
1500	106.9 b	72.3 d	46.7 gh	75.3 B
2000	119.9 a	73.0 d	61.9 de	84.9 A
2500	114.0 ab	67.6 de	67.7 de	83.1 A
3000	89.3 c	55.5 efg	43.4 gh	62.7 C
Mean	98.5 A	62.6 B	47.5 C	

*Means followed by the same letters are not significantly ($p = 0.01$) different according to DMRT

Table 2. Influence of IBA at different concentrations on total root length of three species of *Pistacia* seedlings

IBA [mg l^{-1}]	Total root length [mm]			Mean
	P. vera	*P. mutica*	*P. khinjuk*	
Control	1265 c*	836 fg	544 h	881 E
1000	1538 b	1046 de	588 h	1057 D
1500	1550 b	1137 cd	812 fg	1166 C
2000	1773 a	1286 c	1134 cd	1398 A
2500	1830 a	1152 cd	926 ef	1303 B
3000	1488 b	827 fg	756 g	1039 D
Mean	1574 A	1055 B	793 C	

*Explanations, see Table 1

Table 3. Influence of IBA at different concentrations on root fresh weight of three species of *Pistacia* seedlings

IBA [mg l^{-1}]	Root fresh weight [g]			Mean
	P. vera	*P. mutica*	*P. khinjuk*	
Control	1.48 h*	0.78 k	0.30 o	0.84 E
1000	2.03 e	1.24 i	0.35 no	1.20 D
1500	2.54 c	1.75 g	0.49 m	1.59 B
2000	2.79 b	1.91 f	0.66 l	1.79 A
2500	3.01 a	1.67 g	0.61 l	1.76 A
3000	2.25 d	1.12 j	0.45 mn	1.27C
Mean	2.35 A	1.41 B	0.48 C	

*Explanations, see Table 1

Table 4. Influence of IBA at different concentrations on root dry weight of three species of *Pistacia* seedlings

IBA [mg l^{-1}]	Root dry weight [g]			Mean
	P. vera	*P. mutica*	*P. khinjuk*	
Control	0.57 f*	0.26 h	0.09 j	0.30 D
1000	0.73 d	0.43 g	0.10 j	0.42 C
1500	0.93 b	0.61 f	0.14 ij	0.56 B
2000	0.95 b	0.67 e	0.18 i	0.60 A
2500	1.03 a	0.59 f	0.17 i	0.60 A
3000	0.79 c	0.39 g	0.13 ij	0.44 C
Mean	0.84 A	0.49 B	0.14 C	

*Explanations, see Table 1

Table 5. Influence of IBA at different concentrations on survival rate of three species of *Pistacia* seedlings

IBA [mg l^{-1}]	Survival rate [%]			Mean
	P. vera	*P. mutica*	*P. khinjuk*	
Control	40.0 cd*,**	30.0 d	40.0 cd	36.7 C
1000	85.0 a	50.0 abcd	45.0 bcd	60.0 B
1500	65.0 abcd	70.0 abcd	70.0 abcd	68.3 AB
2000	90.0 a	75.0 abc	80.0 ab	81.7 A
2500	85.0 a	70.0 abcd	75.0 abc	76.7 AB
3000	70.0 abcd	60.0 abcd	60.0 abcd	63.3 B
Mean	72.5 A	59.2 A	61.7 A	

*Explanations, see Table 1

**Analysis were done using arcsin $\sqrt{X}$ transformed data

The survival of the seedlings was increased significantly by application of IBA (Tab. 5). Seedlings treated with 2000 mg l^{-1} IBA showed the greatest survival rate (81.7%). The analysis of variance showed that survival of the seedlings were significantly correlated with such traits as: root number, root length and root fresh and dry weight (Tab. 6). Therefore, it is extrapolated that increasing effect of IBA on survival rate of *Pistacia* seedlings is through enhancements of these traits.

In general, *P. vera* produced more and longer roots with higher fresh and dry weight than the other species analysed (Tab. 1 to 4, respectively). Shoot length, shoot diameter, leaf number, shoot fresh and dry weight were greatest in *P. vera* as well (data not presented). *P. vera* showed the highest rate of seedling survival (72.5%) (Tab. 5). These differences

Table 6. Correlation between 11 measured traits of *Pistacia* seedlings

Traits	1	2	3	4	5	6	7	8	9	10	11
1 – Root number	1										
2 – Root length	0.97*	1									
3 – Root diameter	0.67	0.69	1								
4 – Root fresh weight	0.94	0.95	0.74	1							
5 – Root dry weight	0.93	0.94	0.76	0.99	1						
6 – Survival	0.67	0.66	0.08	0.56	0.52	1					
7 – Shoot length	0.79	0.80	0.79	0.75	0.76	0.33	1				
8 – Shoot diameter	0.82	0.83	0.83	0.78	0.79	0.36	0.95	1			
9 – Leaf number	0.80	0.81	0.82	0.80	0.81	0.33	0.92	0.91	1		
10 – Shoot fresh weight	0.85	0.85	0.84	0.82	0.82	0.38	0.98	0.97	0.94	1	
11 – Shoot dry weight	0.83	0.84	0.84	0.81	0.81	0.35	0.98	0.98	0.94	0.99	1

*r > 0.47 at p = 0.05 and r > 0.59 at p = 0.01 are significant represent genetic diversity among the species studied.

The response of *Pistacia* seedlings to IBA treatments outlined in this study suggests that this technique has a potential application for increasing the ease by which this difficult–to-transplant species may be established successfully following transplanting. In summary, using IBA at 2000 mg l^{-1} is the most effective for root regeneration and growth.

Acknowledgement: We thank Mr B. Modarres for technical assistance, Mr P. Rastegari, Mr Modarres Hashemi and Mr Smaeili for providing seeds. This study was supported by Isfahan University of Technology Research Council, to whom thanks are due.

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WPŁYW KWASU INDOLILOMASŁOWEGO NA REGENERACJĘ KORZENI I PRZEŻYCIE SIEWEK PO SZCZEPIENIU TRZECH GATUNKÓW *Pistacia*

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S T R E S Z C Z E N I E

Gatunki *Pistacia vera*, *P. mutica* i *P. khinjuk* mogą być zastosowane jako podkładki dla odmian pistacji ze względu na ich przystosowanie do zróżnicowanych warunków środowiska i odporność na szkodniki i choroby. Jednakże głównym problemem jest niski stopień przeżycia po szczepieniu. W przeprowadzonych badaniach testowano wpływ różnego stężenia kwasu indolilomasłowego (IBA) na regenerację korzeni u szczepionych siewek tych trzech gatunków. Siewki traktowane różnymi stężeniami IBA (1000, 1500, 2000, 2500 i 3000 mg l^{-1}) wytwarzały więcej korzeni, były dłuższe, miały większą świeżą i suchą masę i wykazywały wyższą przeżywalność w porównaniu z siewkami kontrolnymi nietraktowanymi. Stężenia IBA 2000 i 2500 mg l^{-1} dawały najlepszy efekt. Otrzymane wyniki wykazują, że przeżywalność siewek była istotnie skorelowana z liczbą korzeni i ich długością, a także świeżą i suchą masą korzeni.

Słowa kluczowe: regeneracja korzeni, IBA, przeżywalność siewek, *Pistacia*