

Root Regeneration in Pistachio Rootstock is Affected by Auxin and Polyamines

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Abstract

Pistacia vera cv Badami-e-riz and *P. vera* cv Ghazvini are the most important and popular rootstocks in Iran, which tolerate salinity and phytophthora fungi. This study was conducted to evaluate the effects of various concentrations of polyamines and IBA on root regeneration of transplanted bare-rooted 'Badami-e-riz' and 'Ghazvini' pistachio rootstocks. The mean comparison between IBA and polyamines applications on root regeneration showed that 1000 mg l⁻¹ IBA significantly affected shoot length, leaf area, fresh and dry weight of shoot and root length. Root number, fresh and dry weight of root and root diameter mean in 'Badami-e-Riz' and 'Ghazvini' pistachio rootstocks. Though both chemicals increased lateral root formation and improved root regeneration, IBA treatments were more effective. However the percentage of seedling survival was higher value. Therefore, it can be used to help the survival of seedlings following transplantation.

Keywords: 'Badami-e-Riz', 'Ghazvini', IBA, Putrescine, Spermidine, Spermine

Introduction

The genus *Pistacia* is a member of Anacardiaceae family which consists of eleven species (Zohary, 1952). Among these, *Pistacia vera* L. is economically important due to its valuable nuts. Seedlings of pistachio cultivars, that produce small nuts, are used mainly as rootstocks for pistachio (Spiegel- Roy 1985). *P. vera* cv Badami-e-riz and *P. vera* cv Ghazvini are the most important and popular rootstocks in Iran. Although their potential as a rootstock have been known for many decades due to its resistance to salinity and drought, but their long tap roots and very few lateral roots have limited the use of these two rootstocks. 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 principal processes responsible for seedling survival after transplanting (Burdett, species. In addition, several studies have emphasized that polyamines(PAs) play a role in rooting (Davis et al.1988; Biondi et al. 1990). Tang (2005) demonstrated that PAs promoted root elongation and growth by increasing root cell division in regenerated Virginia pine plantlets. Shyr and Kao (1985) showed that application of PAs increased rooting in mungbean hypocotyl cuttings. Present study was carried out to assess the effect of different concentration of IBA and PAs such as Spermidine (Spd), Putrescine (Put) and Spermine (Spm) applied to root system on root regeneration, growth and seedling survival following transplanting seedling of two cultivars of pistachio rootstocks.

1987). Seedling root system can be improved to reduce the effects of transplanting shock by increasing the lateral roots. 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 increased root regeneration of oak seedlings up to six fold (Struve and Arnold, 1986). Application of IBA to the root system of *P. chinensis* seedlings prior to transplanting has also greatly increased root regeneration potential (Lee and Hackett 1976) Sawwan (1996) reported that treating roots of *P. vera* seedlings with IBA or NAA have induced root regeneration. Baninasab and Mobli, (2002) reported that root regeneration and seedling survival were enhanced by application of IBA at concentration of 2000 mg l⁻¹ in three *Pistacia*

Material and methods

Seeds of 'Ghazvini' and 'Badami-e-riz' (*P. vera*) were obtained from Rafsanjan, in the west of the Kerman province of Iran. Dehescence nuts of 'Ghazvini' and 'Badami-e-riz' dehulled and sound nuts were soaked in distilled water for 24h. Soaked nuts of two cultivars were sown on 15 March 2010 directly in a 5 kg black plastic bags filled with a 1:1 mixture of vermiculite and loamy soil. The bags were then transferred to the greenhouse with an average temperature of 24:17 day: night for the whole experiment period.

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IBA and PAs application

Forty five days after sowing the nuts, seedlings were removed from the containers and the media carefully washed from the root systems. The root systems of seedlings were dipped into solution of IBA at 0, 1000, 1500, 2000 mg l⁻¹ and PAs (Spd, Put and Spm) at 0, 0.5, 1, 1.5 mM. Treated seedlings were transplanted to new plastic bags of 35 cm diameter filled with a 1:1:1 mixture of vermiculite, loamy soil and peat moss on the same day. Six weeks after applying treatments, seedlings were removed from the containers and media was carefully washed from the root systems. The number, length and diameter of the roots were measured using the Delta-T SCAN image analysis system (Windias Software) Shoot length, leaf area 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.

A factorial experiment was arranged in a completely randomized design with nine replications to compare the effect of these chemicals on root development of two pistachio rootstocks. Data were statistically analysed and means were compared using Duncan's multiple range test (DMRT). Results discussed in the text

section were significant at the 5% level of probability unless otherwise stated.

Results

Application of IBA at 1000 and 2000 mg l⁻¹ significantly increased root length of Badami-e-riz (Table 1). The highest root length of Badami-e-riz (44.55 cm) was obtained by the application of IBA at 1000 mg l⁻¹ (Table 1).

Although Spd at 1.5 mM increased root length up to 41.30 cm in Ghazvini rootstock cultivar, but PAs had no significant effect on root length (Table 1).

Root number was affected by IBA application. IBA at 1000 and 2000 mg l⁻¹ significantly increased root number per seedlings in both cultivars, with more pronounced effect on Ghazvini cultivar (Table 2). PAs also affected root number per seedlings. Spd at 0.5 and 1 mM significantly increased root number of Badami-e-riz, but Ghazvini was affected by 1 mM (Table 2). Application of Put at 1.5 mM also increased root number of seedlings of Badami-e-riz (Table 2). The results showed that Spd was more effective in increasing root number than Put and Spm in this study (Table 2).

Table 1. Effect of polyamines and IBA on root length of pistachio seedling rootstocks (cm)

Treatments	Badami-e-riz	'Ghazvini'	Mean
Control	31.00cd*	30.94ab	37.97D
IBA 1000mg/l	45.55a	38.44ab	42.00A
IBA1500mg/l	33.25bcd	39.38ab	36.32A-D
IBA2000mg/l	40.91ab	39.77ab	40.00AB
Spd0.5mM	30.55cd	39.35ab	34.95A-D
Spd1 mM	32.72bcd	39.50ab	36.11A-D
Spd1.5mM	28.55d	41.30a	34.92A-D
Put0.5mM	33.11bcd	30.08b	31.60CD
Put1mM	33.27bcd	34.78ab	34.03BCD
Put1.5mM	39.00abc	37.66ab	38.33ABC
Spm0.5mM	37.66a-d	37.88ab	37.77A-D
Spm1 mM	35.72bcd	34.80ab	35.26A-D
Spm1.5mM	34.84bcd	35.44ab	35.14A-D
Total Mean	35.76A	34.32A	

*Mean s followed by the same letters are not significantly (P=0.05) different according to DMRT.

Root diameter was also affected by IBA application in both cultivars. IBA at all concentrations used significantly increased root diameter in both cultivars (Table 3). Spd at all concentrations significantly increased root diameter in Badami-e-riz, but in Ghazvini only at 1mM increased root diameter of seedlings (Table 3). Put at 0.5 and 1.5 mM increased root diameter, in Badami-e-riz seedlings. Spm had no significant effect on root diameter in both cultivars. (Table 3).

Treatments with IBA at all concentrations increased both fresh and dry weight of roots in

both cultivars (Tables 4 and 5 respectively). Spd at 0.5 mM significantly increased root fresh and dry weight in seedlings of Badami-e-riz. On the other hand, all concentrations of Spd increased root fresh and dry weight of cv Ghazvini. Put at 1mM significantly increased root fresh weight of Badami-e-riz and Ghazvini respectively (Table 5) but had no effect on root dry weight in both cultivars (Table 5). Spm at 1 mM significantly increased root fresh weight in seedlings of Badami-e-riz.

Table 2. Effect of polyamines and IBA on the root number per pistachio seedling rootstocks.

Treatments	Badami-e-riz	'Ghazvini'	Mean
Control	6501.88c*	7709.55e	7105.72E
IBA 1000mg/l	24845.44ab	39578.44a	32211.94A
IBA1500mg/l	16363.66abc	19415.33bcd	17889.50BCD
IBA2000mg/l	23517.00ab	25364.11b	24540.55AB
Spd0.5mM	24344.66ab	13633.11de	18988.88BCD
Spd1 mM	24135.77ab	24191.66bc	24163.72AB
Spd1.5mM	19720.11abc	15752.77cde	17736.44BCD
Put0.5mM	13897.33abc	7356.33e	10626.83DE
Put1mM	10538.11bc	11313.22de	10925.66DE
Put1.5mM	24165.11ab	17263.33b-e	20714.22BC
Spm0.5mM	18526.66abc	15804.33cde	17165.50BCD
Spm1 mM	13730.88abc	14394.55de	14062.72CDE
Spm1.5mM	27724.00a	12542.66de	20133.33BC
Total Mean	16719.77A	15483.60B	

*Mean s followed by the same letters are not significantly (P=0.05) different according to DMRT.

Application of IBA at concentrations used significantly increased shoot fresh and dry weight of seedlings in both cultivars (Tables 6 and 7 respectively). Spd at 0.5 and 1mM also significantly increased both shoot fresh and dry weight of seedlings in both cultivars (Tables 6 and 7 respectively). In Badami-e-riz, Put at all concentrations significantly increased shoot fresh weight and at 1.5 mM significantly increased

shoot dry weight but in Ghazvini cultivar 0.5 mM of put was effective. (Tables 6 and 7 respectively). Spm at 1mM significantly increased fresh and dry weight of both cultivars (Tables 6 and 7 respectively).

The result showed that IBA at 2000 mg l⁻¹ significantly increased shoot length of seedlings in Badami-e-riz, but in Ghazvini cultivar, all concentrations of IBA were

Table 3. Effect of polyamines and IBA on root diameter of pistachio seedling rootstocks (mm).

Treatments	Badami-e-riz	'Ghazvini'	Mean
Control	1.34g*	1.41e	1.41E
IBA 1000mg/l	1.95a-e	2.75a	2.35A
IBA1500mg/l	1.83b-f	2.21abc	2.02ABC
IBA2000mg/l	2.33a	2.31ab	2.32A
Spd0.5mM	2.17abc	1.64cde	1.91BCD
Spd1 mM	2.11abc	2.33abc	2.17AB
Spd1.5mM	1.95a-e	1.95b-e	1.95BCD
Put0.5mM	2.00a-d	1.81b-e	1.91BCD
Put1mM	1.66d-g	1.61de	1.63DE
Put1.5mM	2.33ab	1.80b-e	2.01A-D
Spm0.5mM	1.66d-g	1.93b-e	1.80BCD
Spm1 mM	1.76c-g	1.71cde	1.73CDE
Spm1.5mM	1.51efg	2.17bcd	1.84BCD
Total Mean	1.79A	1.86A	

*Mean s followed by the same letters are not significantly (P=0.05) different according to DMRT.

effective in increasing shoot length of seedlings (Table 8). Shoot length was not affected by Spd in Badami-e-riz, but in Ghazvini shoot length was increased by Spd at 1 and 1.5 mM (Table 8). Put

had no effect on shoot length of both cultivars, but Spm at 1 mM increased shoot length of seedlings in both cultivars (Table 8).

Table 4. Effect of polyamines and IBA on fresh weight of root of pistachio seedling rootstocks (g).

Treatments	Badami-e-riz	'Ghazvini'	Mean
Control	1.35e*	0.76f	1.06E
IBA 1000mg/l	4.09 a	4.94a	4.50A
IBA1500mg/l	2.52bcd	2.99bc	2.76C
IBA2000mg/l	3.40ab	3.63b	3.52B
Spd0.5mM	3.22abc	2.01cde	2.61C
Spd1 mM	2.29cde	2.58bcd	2.43CD
Spd1.5mM	1.87de	1.58def	1.73DE
Put0.5mM	1.54de	1.23ef	1.38E
Put1mM	1.26de	1.42ef	1.34E
Put1.5mM	2.26cde	1.20ef	1.73DE
Spm0.5mM	1.49de	1.60def	1.54E
Spm1 mM	1.87de	1.26ef	1.56E
Spm1.5mM	1.14e	0.93ef	1.03E
Total Mean	2.02A	1.78A	

*Mean s followed by the same letters are not significantly (P=0.05) different according to DMRT.

Table 5. Effect of polyamines and IBA on root dry weight of pistachio seedling rootstocks (g).

Treatments	Badami-e-riz	'Ghazvini'	Mean
Control	0.17de*	0.10e	0.14F
IBA 1000mg/l	0.51a	0.56a	0.53A
IBA1500mg/l	0.29bcd	0.36b	0.33BC
IBA2000mg/l	0.38b	0.37b	0.37B
Spd0.5mM	0.38b	0.28bc	0.33BC
Spd1 mM	0.26bcd	0.29bc	0.28CD
Spd1.5mM	0.18de	0.24cd	0.21DEF
Put0.5mM	0.17de	0.11de	0.14F
Put1mM	0.17de	0.13de	0.15EF
Put1.5mM	0.34bc	0.15de	0.24CDE
Spm0.5mM	0.22cde	0.21cde	0.21DEF
Spm1 mM	0.15de	0.22cde	0.22DEF
Spm1.5mM	0.14e	0.15de	0.14F
Total Mean	0.25A	0.22A	

*Mean s followed by the same letters are not significantly (P=0.05) different according to DMRT

Table 6. Effect of polyamines and IBA on fresh weight of shoot of pistachio seedling rootstocks(g).

Treatments	Badami-e-riz	'Ghazvini'	Mean
Control	1.11f*	1.10d	1.10G
IBA 1000mg/l	2.19ab	2.24a	2.21A
IBA1500mg/l	1.72b-e	1.80ab	1.76B-E
IBA2000mg/l	2.16ab	1.80ab	1.98ABC
Spd0.5mM	2.35a	1.84ab	2.09AB
Spd1 mM	2.00abc	1.79ab	1.90A-D
Spd1.5mM	1.59c-f	1.50bcd	1.54DEF
Put0.5mM	1.83bcd	1.29cd	1.56DEF
Put1mM	1.65cde	1.61bc	1.63DEF
Put1.5mM	2.08abc	1.23cd	1.65C-F
Spm0.5mM	1.32ef	1.58bc	1.45EFG
Spm1 mM	1.97abc	1.61bc	1.79B-E
Spm1.5mM	1.36def	1.43bcd	1.60FG
Total Mean	1.66A	1.51A	

*Mean s followed by the same letters are not significantly (P=0.05) different according to DMRT.

IBA at all concentrations significantly increased leaf area of seedlings in both cultivars. The highest leaf area was obtained (38 cm²) by application of

IBA at 200 mg l⁻¹ in Badami-e-riz (Table 9). PAs at all concentrations increased leaf area of seedlings in both cultivars (Table 9).

Table 7. Effect of polyamines and IBA on dry weight of shoot of pistachio seedling rootstocks (g).

Treatments	Badami-e-riz	'Ghazvini'	Mean
Control	0.28e*	0.23f	0.25G
IBA 1000mg/l	0.66a	0.66a	0.66A
IBA1500mg/l	0.49bcd	0.51bc	0.50BCD
IBA2000mg/l	0.58abc	0.49bc	0.54BC
Spd0.5mM	0.63ab	0.54ab	0.58AB
Spd1 mM	0.58abc	0.53ab	0.56ABC
Spd1.5mM	0.43cde	0.43bcd	0.43DEF
Put0.5mM	0.44cde	0.26ef	0.35EFG
Put1mM	0.37de	0.42bcd	0.39DEF
Put1.5mM	0.58abc	0.34def	0.46CDE
Spm0.5mM	0.39de	0.44bcd	0.41DEF
Spm1 mM	0.51a-d	0.39cde	0.45CDE
Spm1.5mM	0.31e	0.32def	0.32FG
Total Mean	0.44A	0.39A	

*Mean s followed by the same letters are not significantly (P=0.05) different according to DMRT.

Table 8. Effect of polyamines and IBA on shoot length of pistachio seedling rootstocks(cm).

Treatments	Badami-e-riz	'Ghazvini'	Mean
Control	20.54cd*	17.35c	19.83C
IBA 1000mg/l	24.15abc	23.25a	23.70A
IBA1500mg/l	22.02abc	24.03a	23.02AB
IBA2000mg/l	24.50a	23.00a	23.75A
Spd0.5mM	23.11abc	20.51abc	21.81ABC
Spd1 mM	22.65abc	23.4a	22.90AB
Spd1.5mM	20.77bcd	23.31a	22.04ABC
Put0.5mM	22.44abc	19.26bc	20.85BCD
Put1mM	22.47abc	20.51abc	21.49A-D
Put1.5mM	22.47abc	18.24bc	20.36BCD
Spm0.5mM	18.16d	20.80abc	19.48CD
Spm1 mM	24.37ab	21.72ab	23.05AB
Spm1.5mM	21.30a-d	18.90bc	20.10CD
Total Mean	21.91A	20.35B	

*Mean s followed by the same letters are not significantly (P=0.05) different according to DMRT.

Table 9. Effect of polyamines and IBA on leaf area of pistachio seedling rootstocks (cm²).

Treatments	Badami-e-riz	'Ghazvini'	Mean
Control	15.02g*	11.16h	13.10F
IBA 1000mg/l	37.81ab	37.23a	37.52A
IBA1500mg/l	34.93abc	36.34ab	35.63AB
IBA2000mg/l	38.04a	35.95abc	37.60A
Spd0.5mM	33.52cd	33.11bcd	33.31BC
Spd1 mM	33.83cd	32.32cde	33.07BC
Spd1.5mM	34.41cd	32.26cde	33.33BC
Put0.5mM	31.24de	27.43fg	29.33E
Put1mM	30.06ef	28.36efg	29.21E
Put1.5mM	29.32ef	26.47g	27.90E
Spm0.5mM	28.82.ef	31.32def	30.70DE
Spm1 mM	34.53bcd	30.44def	32.48CD
Spm1.5mM	27.50f	28.72efg	28.11E
Total Mean	28.38A	26.54A	

*Mean s followed by the same letters are not significantly (P=0.05) different according to DMRT.

Discussion

The results of this study showed that dipping root system of pistachio seedlings at 5- leaves stage in IBA solution caused significantly increase root length. Maximum root length was obtained at 1000 mg l⁻¹ in Badami-e-riz . The presented here were agreement with previous report on pistachio species by Baninasab and Mobli, 2002; Mobli and Baninasab,2009 who found that IBA at 1000 mg l⁻¹ significantly increased root length. In this study it was showed that IBA at lower concentrations were more effective in root elongation and it was in support of previous report that higher concentrations of auxin may provoke ethylene production and inhibit root growth (Taize and Zeiger 2002). PAs had no significant effect on root length, but Tang and Newton (2005) reported that PAs application could increase root elongation. PAs may have different effects on root elongation in woody and herbaceous plants such pea, tomato and maize (Shen and Galaston 1985).

The results also showed that IBA and PAs increased root number in both seedling rootstocks. IBA was more effective in increasing root number in seedling rootstocks than PAs. Application of IBA can increase endogenous level of IBA and decreases IAA-oxidase activity in plant (Nag et al. 2001; Husen and Pal 2007). PAs application also induces the endogenous synthesis of PA in plant tissues (Lee 1997)

Hummel et al . (2002) reported that the increase in endogenous PAs were accompanied with the enhancement of mitotic cell division and lateral root formation. The same results were reported in *Citrus limonia* (Yao et al. 2010).

In the present study root diameter was affected by IBA and PAs. IBA at 1000 and 2000 mg l⁻¹ significantly increased root diameter. Our data were consistent with the report of other researchers (Noor Elahi et al. 2003) who found that IBA at 2500 mg l⁻¹ significantly increased root diameter in litchi. PAs in compare with control significantly decreased root diameter in both seedling rootstocks.

The presented data also indicated that application of IBA and PAs significantly exerted differential influence on root fresh and dry weight of seedling rootstocks. IBA at 1000 mg l⁻¹ strongly increased both root fresh and dry weight of seedling rootstocks. This data was consistent with previous report (Mobli and Baninasab 2009) who found IBA at 1000 to 2500 mg l⁻¹ significantly increased both root fresh and dry weight of pistachio species. Some concentrations of Spd and Put increased both root fresh and dry weight in seedling rootstocks. The increase in fresh and dry weight of root was related to the increase in cell division, cell differentiation (He- Lixiong et al. 2002; El-Quensi et al. 2010).

In this study IBA at all concentrations significantly increased shoot fresh and dry weight of both cultivars. Spd and Spm at 1mM significantly increased both fresh and dry weight. Application of Put on *Syngonium* plants significantly increased both fresh and dry weight of leaves and leaf area (El- Quensi et al. 2010). It was reported that application of Spd on cotton significantly increased leaf area and enhanced photosynthesis per unit leaf area (Wahed, 2006).

In this study shoot length was increased by application of IBA at 1000 to 2000 mg l⁻¹ in both seedling rootstocks. The result of this study was in agreement with Noor, et al.(2002) who found that maximum shoot length in cuttings of olive treated with 3000 mg l⁻¹ IBA might be due to the absorbed more nutrient and ultimately it produced more growth and lengthen the shoot. The present study showed that application of Spd and Spm at 1mM significantly increased shoot length in both seedling rootstocks. Abdel Aziz et al. (2009) showed that application of Put at 200 mg l⁻¹ significantly increased shoot elongation in gladiolus. The enhance effect of PAs on shoot elongation could be due to the stimulatory effect on cell division (Bias and Ravishanker 2002).

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