International Journal of Nuts and Related Sciences 2(3): 7-14, 2011
ISSN 2008-9937

Effect of Pre-soaking Seeds in Polyamines on Seed Germination and Seedling Growth of *Pistacia vera* L.cv. Ghazvini.

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Abstract: *Pistacia vera* L. is a decidious tree. To investigate the influence of soaking seeds in polyamines (PAs) spermine (Spm), spermidine (Spd) and putrescine (Put) on seed germination and enhancing lateral root development of 'Ghazvini' pistachio seedling rootstock, the experiment were carried out in petri dishes and plastic bags. Seeds were soaked in PAs(Spd, Spm, and Put) at three concentrations(0, 2 and 4 mMl^{-1} for 6 and 12 h and then seeds were put in petri dishes and sown in plastic bags to evaluate seed germination and lateral root formation in seedlings, respectively. The results showed that soaking seeds in PAs significantly increased hypocotyl- radicle length, mean germination time, (MGT) and germination rate (GR). The shoot length, leaf area, root number, fresh weight, dry weight, root diameter and root area of seedlings were significantly affected by soaking seeds in PAs. We concluded that pre-soaking seeds in PAs are a useful method to induce lateral root in *Pistacia vera* seedling rootstocks.

Keywords: Ghazvini; *Pistacia vera*; polyamines; spermidine; spermine; putrescine.

INTRODUCTION

Pistacia vera L. is a deciduous tree which belongs to *Anacardiaceae*. There are 11 different species in this genus (Khosh-Khoui *et al*. 2004). Wild *Pistacia vera* is indigenous to the northeast of Iran and the origin of cultivation is reached to 3-4 thousand years ago(Khosh-Khoui *et al*.2004). Because the root system of pistachio rootstocks do not have enough ability to produce lateral root and regenerate it, bare- root nursery seedlings should not be transplanted to their permanent planting site . Polyamines could play a major role in proliferation and growth of plant cell, root initiation and root development(Couee *et al*. 2004). They have been reported that spermidine and spermine contents show positive correlations with primary root growth whereas the putrescine level show neutral or negative effects on this trait. PAs can affect the growth seedlings via the pathways of altering root morphogenesis (Yao *et al*., 2010). A comparison of developmental effects and physiological concentrations suggested that agmatine and spermine in particular may play a significant role in the control of root development (Hummel *et al*., 2002).Tang and Newton (2005) demonstrated that PAs promote root elongation and growth by increasing root cell division in regenerated Virginia pine plantlets. Application of putrescine 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 spermidine on cotton plants significantly increased leaf area and enhanced photosynthesis per unit leaf area (Wahed, 2006). The purpose of this study was to evaluate the effect of pre-soaking seeds in various PAs on seed germination and improving lateral root of 'Ghazvini' pistachio seedling rootstock.

MATERIALS AND METHODS

This study was performed in two consecutive years (2009 and 2010). Seeds of pistachio were obtained from Rafsanjan, Kerman, Iran and were disinfected with a 0.2% benomyl solution for 60 min. Then they were dried on the paper tissue.

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PAs application

For each treatment,10 seeds were used. The seeds were soaked in 2,4 mM Put, Spd and Spm for 6 and 12 h and then placed in petri dishes on what man filter paper, moistened with double distilled water. Distilled water served as the control (0mM). The seeds were kept in lab condition for 20 days and the irrigation of them was carried out by double distilled water. Germination capacity, the mean germination time and germination rate were calculated from following equations

(GC)= $S_{NG}/S_{NO} \times 100$ (1). Where GC is Germination capacity, S_{NG} is the number of germinated seeds and S_{NO} is the number of experimental seeds with viability.

MGT= $\sum_{D.n} / \sum n$ (2). Where MGT is the mean of germination time (day), D is the time in days from the starting sowing day and n is the number of germinated seeds on a given day.

GR= $S_{Nm} / S_{NO} \times 100$ (3). Where GR is the germination rate and S_{Nm} is the number of germinated seeds at germination peak.

After assuring of seed germination in the petri dishes , 112 seeds were soaked in PAs as the same previous method and 2 seeds were sown in 3 kg plastic bags instead of petri dishes, filled with 1:1(v/v) mixture of sand and soil. The plastic bags were kept in the glass house at a temperature of 26± 6 under natural photoperiode for 45 days. As soon as the seedlings were reached to 2 leaf stage, they were thinned into one seedling. After 45 days, the roots of each seedling were removed from container and washed with water. The number, area, mean diameter, fresh and dry weight of roots and leaf area were determined.

The number, area and mean diameter of roots were measured using the DELTA – T Scan Image Analysis System (Windia software). Dry weights were recorded after the plant materials had been oven dried at 70 °C for 48 h. The leaf area was measured using Leaf Area Meter (Delta- T Devices). The factorial experiments were arranged in a completely randomized design with four replications.

STATISTICAL ANALYSIS

The data were statistically analyzed using SPSS Inc- SPSS v 17.0 software and the means compared using Duncan's Multiple Range Test (DMRT).

RESULTS

Seed Germination

Hypocotyl- radicle length (cm)

The result showed that application of PAs significantly increased hypocotyls- radical length of germinated seeds. Seeds were soaked in Spd and Put at 4 mM for 6h was the most effective treatment on hypocotyls- radicle growth and control treatment showed the lowest. The soaking time had no significant effect on hypocotyls-radicle length(Table 1).

Germination capacity (GC%), germination rate (GR%) and mean germination time (MGT)

'Ghazvini' seeds were soaked in PAs for 12h were more effective compared to soaking for 6h on percentage of germination capacity. Soaking of seeds in 4mM Spd for 6h compared with control had the highest percentage of GC. Furthermore, soaking of seeds in Put at 2 and 4 mM for 12h had the highest percentage of GC(Table 1). Germination rate (%) was also affected by pre- soaking seeds in PAs. Seeds were soaked in PAs for 6h compared with control, significantly increased percentage of germination rate (Table 2). Put at concentration of 4 mM for 12h had the most and the control had the least germination rate. Mean germination time (MGT day) enhanced by soaking time of seeds in PAs(Table 2).

Soaking seeds in Spd, Spm and Put at both concentrations for 6h compared with control increased MGT day, which the control showed the least that of it (Table 2). Although Soaking seeds of Ghazvini cultivar in PAs for 12h were not showed significant difference, 4 mM Put obtained the most MGT(day).There was a significant difference between soaking time on MGT (day) (Table 2).

Fresh weight (g)and dry weight(g)

The fresh weight of seedlings of ' Ghazvini 'pistachio cultivar which their seeds were pre-soaked in Spd, Spm at 4mM and Put 2 and 4mM for 6h and 12h compared with control significantly increased. Soaking period of seeds before sowing in plastic bags had no significant effect on subsequent fresh weight of seedlings. Soaking seeds in PAs for 6h and 12h before sowing in plastic bags significantly increased dry weight of seedlings. Pre- soaking seeds in Spd, Spm and Put at 4 mM before sowing in the plastic bags significantly increased dry weight of seedlings as compared with none treated one (Table3).

Shoot length (cm) and leaf area (cm^2)

It was showed that pre- soaking seeds in PAs significantly affected shoot length of *P.vera* cv. Ghazvini seedlings. Pre-soaking seeds in Spd, Spm and Put for 6h compared to control significantly increased shoot length at P=0.05 level. When seeds were soaked in PAs for 12h, the average of shoot length was affected only by Spd and Spm at 4mM and Put at 2 and 4 mM and the control had the least shoot length (Table 4). Soaking of 'Ghazvini' seeds in PAs significantly affected leaf are (cm^2), and both pre- soaking periods increased leaf area, but 12h soaking were more effective. Soaking seeds in PAs for 12h significantly increased leaf area at all

concentrations, but Spm was the most effective (Table 4).

Table 1. Effects of pre-soaking of seeds in PAs on hypocotyl-radicle length and germination capacity of Ghazviniseed .

Hypocotyl-Radicle length (cm)		GC%	
Soaking period (h)		Soaking period(h)	
6	12	6	12
6.25b*	12.87a	17.50b	
10.45ab	13.32a	52.50a	
14.52a	13.32a	70.00a	
11.27ab	10.55a	60.00a	
10.97ab	13.87a	55.00a	
8.42ab	14.07a	50.00a	
14.1a	16.70a	50.00a	
9.36A	13.3A	44.33A	

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

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Table 2. Effects of pre- soaking of seeds in PAs on mean germination time (MGT) and germination rate of Ghazvini seed

Treatment (mM)	MGT (Day)		GR	
	Soaking period (h)		Soaking period (h)	
	6	12	6	12
control	5.86b*	8.92a	11.25b	13.75c
Spd 2	8.92a	9.32a	27.50a	17.50bc
Spd 4	8.25a	8.32a	25.00a	27.50ab
Spm 2	8.35a	9.17a	22.50a	27.50ab
Spm 4	7.75a	8.25a	22.50a	22.50abc
Put 2	8.87a	8.15a	25.00a	22.50abc
Put 4	9.27a	8.00a	25.00a	30.00a
Mean	8.66 A	7.66B	20.14A	20.27A

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

Table 3. Effects of pre- soaking of seeds in PAs on fresh and dry weight of seedling rootstocks.

Treatment (mM)	Fresh weight g/plant		Dry weight g/plant	
	Soaking period (h)		Soaking period (h)	
	6	12	6	12
control	1.45b*	1.66b	0.35d	0.46c
Spd 2	1.59b	2.07a	0.53c	0.55bc
Spd 4	2.55a	2.97a	0.71a	0.82a
Spm 2	1.63b	1.87ab	0.66ab	0.74a
Spm 4	2.39a	2.73a	0.70a	0.73a
Put 2	2.53a	2.89a	0.55c	0.63b
Put 4	1.66b	2.67a	0.59bc	0.75a
Mean	1.86A	2.31A	0.53A	0.62A

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

Table 4. Effects of pre- soaking of seeds in PAs on shoot length and leaf area of Ghazvini seedlings of pistachio rootstock.

Treatment (mM)	Shoot length (cm)		Leaf area (cm^2)	
	Soaking period (h)		Soaking period (h)	
	6	12	6	12
control	10.75e*	21.12d	13.50b	15.31b
Spd 2	24.25cd	22.32cd	21.72ab	29.07a
Spd 4	32.62a	27.57ab	24.00a	31.67a
Spm 2	20.82d	22.50cd	25.42a	36.69a
Spm 4	25.75bc	25.37bc	31.65a	35.27a
Put 2	29.12ab	29.25a	28.05a	30.73a
Put 4	22.95cd	25.62bc	27.54a	29.46a
Mean	20.86B	24.00 A	22.10A	26.54 A

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

Table 5 .Effects of pre- soaking of seeds in PAs on fresh and dry weight of roots of Ghazvini seedlings of pistachio rootstock seed.

Treatment (mM)	Shoot length (cm)		Leaf area (cm^2)	
	Soaking period (h)		Soaking period (h)	
	6	12	6	12
control	1.40b*	1.24c	0.21d	0.14b
Spd 2	1.95b	1.56bc	0.43b	0.18b
Spd 4	1.59b	1.78bc	0.34bc	0.31a
Spm 2	2.93b	2.16abc	0.55a	0.29a
Spm 4	1.89b	2.20ab	0.41b	0.29a
Put 2	1.61b	1.68bc	0.25cd	0.31a
Put 4	1.38b	2.83a	0.42b	0.35a
Mean	1.73 A	1.77 A	0.34A	0.25A

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

Influence of pre-soaking seeds in PAs on root growth.

Data in Table 5 and figures (1, 2 and 3) indicated that pre-soaking seeds in PAs significantly affected the root growth. Both pre- soaking time increased fresh weight of root, but the difference was not significant at P=0.05 level (Table5). Dry weight of root was also affected by pre- soaking time, Spd at 4mM, Spm at 2 and 4mM and Put at 4 mM in compared with control significantly increased dry weight of roots (Table 5). Pre-soaking seeds in PAs for 6h and 12h significantly increased root number (Figure1). Irrespective of time of soaking of seeds in PAs, Spm at both concentrations compared with control significantly increased root number at P=0.05 level (Figure1). The average root area (mm^2) was also significantly affected by pre-soaking seeds in PAs and the control showed the least root area (Figure2). Soaking seeds in Spm at 2 mM for 6h and in Spd at 4mM, Spm at 2 and 4 mM and Put at 2mM for 12h compared with control significantly increased root area at P=0.05 level(Figure2).

The average of root diameter significantly increased by soaking seeds in Put at 2mM for 6h (Figure3). There was no significantly difference between the average mean of time of soaking seeds in PAs (Figure3).

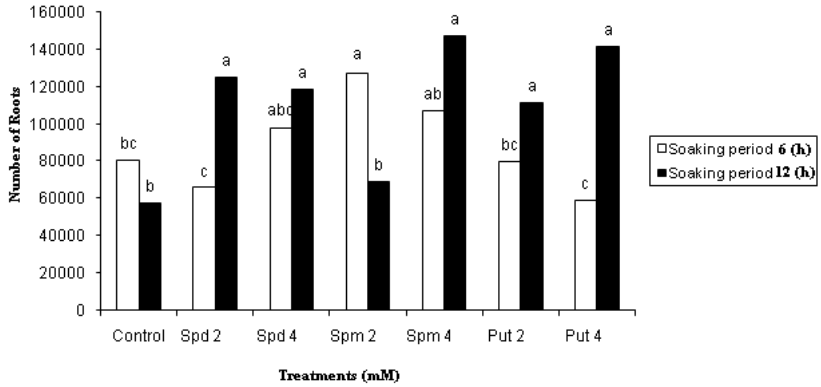

Figure. 1. Effects of pre- soaking of seeds in PAs on root number of Ghazvini seedlings of pistachio rootstock.

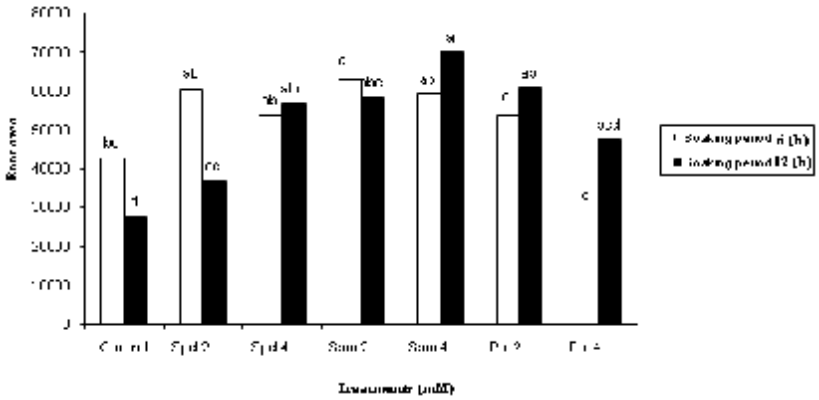

Figure.2. Effects of pre- soaking of seeds in PAs on root area of Ghazvini seedlings of pistachio rootstock.

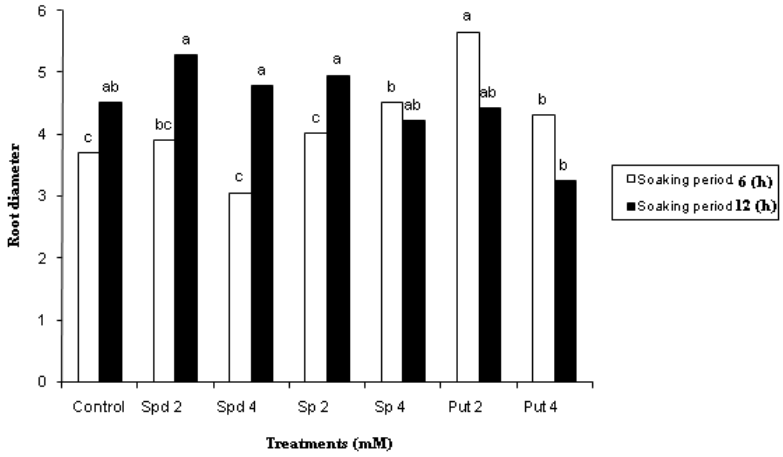

Figure. 3. Effects of pre- soaking of seeds in PAs on root diameter of Ghazvini seedlings of pistachio rootstock

DISCUSSION

Seeds pretreatments are physical or chemical aids to secure rapid and uniform seed germination. Our study showed that pre-soaking seeds of pistachio in PAs can increase hypocotyls- radicle length. Both Spd and Put at higher concentration significantly increased hypocotyls- radicle length. Application of PAs could hinder the inhibitory effects of inhibitors on growth of the embryonic axis during pistachio seed germination. The use of difluoro methyl ornitine (DFMO) biosynthesis inhibitor of PAs inhibit growth of the embryonic axis during seed germination in chick- pea and the effect is partially reversed by the addition of polyamines (Bagni, 1970). Hypocotyle length was increased in cotton plant by application of Spd (Wahed,2006). Quick germination of pistachio seed can be due to the increase of endogenous IAA and decrease of ABA content. Zaghlool (2002) reported that exogenous Spd increased IAA and decreased ABA content during seedling stage of *Vigna radiate* L. PAs application on seed pretreatment significantly increased average leaf area in pistachio seedlings. Pre-soaking seeds in Spm for 12h was the most effective treatment in increasing average leaf area. It is postulated that polyamines may translocated from root to the shoot and activating endogenous plant hormones (IAA, GA). Bagni, 1970 reported that synthesis and translocation of spm and spd during germination of bean (*Phaseolus vulgaris*). The greater part of the PAs were translocated from cotyledons to embryo and afterward into the different part of the growing seedlings.

Our study indicated that PAs significantly exerted differential influence on fresh and dry weight of seedlings at different concentrations (Table 3). Higher concentration of Spd, Spm and lower concentration of Put increased fresh and dry weight of the seedlings. The effect of these chemicals on increasing fresh and dry weight of seedlings were similar to the results obtained by Nassar *et al*., (2003) in *Phaseolus vulgaris* L. and in cotton plant (Wahed, 2006).

Our data showed that pre-soaking seeds of pistachio in PAs improved the seedling height (Table 4).

To date, there is no direct evidence indicating the promotive effect of PAs on pistachio seedlings. However some reports provide indirect evidence. The efficient uptake of nutrients and water by fine roots has been well accepted (Strand *et al*., 2008). In our study, the increased seedling length by pre-soaking seeds in PAs is probably due to the uptake of nutrients and water by the root system. Polyamines can also act as source of nitrogen, which stimulates growth (Smith, 1982).

The data of the study demonstrates that pre-soaking seeds in PAs affect the root system architecture of *Pistacia vera* seedlings. The most changes include the root number, root area, fresh and dry weight of roots. The increase in root number is resulted from the promoted mitosis of root tip cells and more fine roots (Yao *et al*., 2010).

CONCLUSION

As a result of the study, we concluded that pre-soaking seeds of pistachio rootstocks in PAs improve seed germination without chilling requirement. Pre-soaking of pistachio seeds in PAs reduced the effects of transplanting shock by increasing the number of roots of seedlings.

ACKNOWLEDGMENT

We thanked Mr B. Baninasab for the valuable help with this study and providing facilities to measure the characteristic of root by DELTA – T Scan image analysis system (windia software). This work was financially

supported by Shiraz University of Research Council.

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