A Comparison of Putrescine and Arginine Effects on Yield Components and Growth Parameters in Pistachio Trees

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Abstract

Introduction: Improving the growth characteristics of pistachio trees has always been of great importance. The present study aims to evaluate the effects of different levels of putrescine and its precursor arginine on some nut characteristics and physiological parameters of pistachio trees in ‘Owhadi’ cultivar.

Materials and Methods: Pistachio trees were treated with putrescine 0, 0.1, and 0.2 mM as well as arginine 0, 300, and 600µM at two stages (the green tip and the tight cluster of the bud) in a factorial experiment based on a randomized complete block design (RCBD) with 4 replications.

Results: According to the results, putrescine and its precursor arginine decreased the physiological disorders of pistachios and increased the crop yield. In addition, putrescine and arginine decreased the percentage of inflorescence bud abscission, fruit abscission, fruit deformation, as well as early splitting and indehiscent shells compared to the control treatment. In both ‘on’ and ‘off’ years, the effects of putrescine and arginine were greatly dependent on both the time of application and the concentrations used. According to the results, the exogenous application of these compounds, especially at the tight cluster stage of buds, increased indehiscent shells and the crop yield in both ‘on’ and ‘off’ years. The exogenous application of arginine at the tight cluster stage of buds decreased the abscission of inflorescence buds and other physiological disorders, but it increased indehiscent shells and the crop yield, especially arginine 600µM at the tight cluster stage of buds.

Conclusion: It seems that arginine plays a key role in the growth and development of vegetative and reproductive characteristics in pistachios.

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1. Introduction

Pistachios (*Pistacia vera L.*) are among the major horticultural products of Iran. They have been the most important exportable nut crop of Iran and have been cultivated since old times [1]. *Pistacia vera L.* has edible nuts and is commercially important. Pistachio trees are native to Western Asia and Asia Minor [2]. The yield and fruit characteristics of pistachio orchards in Kerman Province, Iran, are consistent in some growth seasons. The highest yields per tree have been recorded in some recent years, yet the lowest yields have been obtained in some growth seasons with favorable conditions and longer growth periods [1].

Various reports indicate fluctuations in pistachio yields [3, 4]. Pistachios are alternate bearing trees and are damaged by insufficient cultural management in terms of fertilization and irrigation [3]. Concerning fruit production, alternate bearing means the occurrence of a high 'on'-year yield followed by a low 'off'-year yield [5]. In some studies, it has been suggested that alternate bearing has been affected not only by genetic factors, but also by some other factors, such as cultural management [6, 7] as well as the rates of plant growth regulators, which have direct effects on plant physiology [6, 8]. Increasing pistachio yields and reducing flower bud abscission have attracted the attention of many researchers and studies on nutrient usage, fertilizers, and phytohormone applications. Several promising methods have been developed to improve the yield and growth potentials of pistachio trees. These methods have involved phytohormones [9- 13], pollen type and number [14- 16], fertilization management [17- 19], rootstock types (20, 21], irrigation management [22], etc.

PAs[1], such as Put[2], Spd[3], and Spm[4] are the other plant growth regulators and low-molecular-mass polycations existing in all living organisms [23]. Polyamines are involved in controlling the cell cycle, cell division, morphogenesis in phytochromes, and plant hormone-mediated processes. They are also involved in controlling plant senescence as well as in plant responses to various stress factors [24]. The role of endogenous polyamines in different stages of fruit development and in the reduction of fruit abscission has been reported in fruit trees [25- 28].

Arginine is one of the essential amino acids and is considered the main precursor of polyamines, agmatine, proline, and cell signaling molecules of glutamine and nitric oxide [29, 30], which is produced by the decarboxylation of arginine via arginine decarboxylase to form putrescine [31, 32]. Polyamines and their precursor arginine have been reported as vital modulators in a variety of growth, physiological, and developmental processes in higher plants [23]. According to some studies, the application of arginine significantly promoted growth and increased fresh and dry weights through certain endogenous plant growth regulators, including

[1] Polyamines (PAs)
[2] Putrescine (Put)
[3] Spermidine (Spd)
[4] Spermine (Spm)

chlorophylls a and b as well as carotenoids in beans [33] and wheats [34]. Moreover, researchers have reported the positive role of arginine in alleviating inhibitions, which occurs as the result of exposing plants to stress [35, 36]. In higher plants, it has also been reported that both endogenous arginine and exogenous arginine play a key role in plant growth responses [37- 40]. The positive effects of exogenous NO [30, 41], PAs [42], and prolin [43] on the growth and development of plants under stress conditions have been reported. However, no data is available on the effects of exogenous arginine as a precursor of these compounds on the growth and development of plants, including pistachios.The stimulating effects of arginine as a polyamine precursor on growth and crop yield in plants may act as a protective agent to make them adapt to extreme environments [42]. According to Paschalidis and Roubelakis-Angelakis (2005), PAS, their precursor arginine, and their biosynthetic enzymes are involved in stimulating cell division, expansion, and differentiation as well as vascular development in tobacco plant. Hassanein et al (2008) reported that arginine at 2.5 mM was most effective in improving the growth and yield of the wheat plant exposed to high temperature stress. Moreover, researchers concluded that the foliar application of arginine on wheats led to significant increments in growth and yield parameters in comparison to the control treatment [36]. Therefore, this study aims to compare the effects of putrescine and its precursor arginine on the yield and growth of pistachio trees in Owhadi cultivar.

2. Materials and Methods

2.1. Orchard management

This research was conducted in 2014 and 2015 on mature pistachio trees in 'Owhadi' cultivar, a commercial orchard located in Rafsanjan, Iran. This region has a hot and dry climate. The 28-year-old trees were grafted on 'Badami-e-Zarand' rootstock, at the spacing of 6×5 m using standard commercial practices of irrigation, soil fertilization, as well as pest and weed control scheduling during the experiment.

2.2. Treatments

The trees were sprayed at the green tip or the tight cluster with a putrescine solution at 0, 0.1, and 0.2 mM or argenine at 0, 300, and 600 µM. These treatments were applied separately at the two stages of the green tip of the buds and the tight cluster. The green tip or the tight cluster were defined as the date when 80% of the buds on each tree had a green tip or a tight cluster. This experiment was conducted in the two consecutive years of 2014 and 2015.

2.3. Measurements

2.3.1. Inflorescence bud abscission and fruit abscission

The number of initiated inflorescence buds and the total number of abscised buds on individual current-year shoots were counted six weeks after full bloom and at harvest time, respectively. The percentage of inflorescence bud abscission was calculated through dividing the number of abscised buds by the total number of buds initiated on each shoot.

The number of the fruit set and the total number of abscised fruits on each cluster were counted two weeks after full bloom and at harvest time, respectively. The percentage of fruit abscission was calculated through dividing the number of abscised fruits by the initial number of the fruit set on each shoot.

2.3.2. Fruit characteristics

The percentage of the early-split nuts was measured four weeks prior to harvest time. At harvest time, all clusters were detached from each shoot and sorted manually into blank, non-split, deformed, and split nuts.

2.3.3. Number of nuts per cluster and ounce as well as yield

After separating the nuts from each cluster, the total number of nuts per cluster was counted. To calculate the number of nuts per ounce, fruits without hulls were dried and weighted. The number of nuts per ounce is a marketing index indicating the size and weight of pistachio nuts. The lower the number of nuts per ounce is, the larger the nut size will be. In addition, the yield was calculated by weighting the total dried split nuts separated from each shoot.

2.3.4. Leaf area and shoot growth

The leaf area of fully-expanded leaves was measured by the Digital Leaf Area Meter (ADC, Hoddeston, UK). The length and diameter of the current-year shoots were measured at harvest time.

2.4. Statistical analysis

The experimental design consisted of a factorial randomized complete block (with the two factors of time and treatment type) with four replicates. The replicate for each treatment was applied to each tree with five shoots. The analyses of variance were performed using SAS procedures (SAS Institute Inc., Cary, NC, USA). The means were separated by Duncan's multiple range test ($P< 0.05$).

3. Results

Putrescine and arginine treatments at the green tip or the tight cluster in the 'on' and 'off' years led to a significant decrease in the abscission percentage of inflorescence buds compared with the control. The untreated control shoots had the least rate of inflorescence bud retention at both stages of 'on' and 'off' years. The best treatment was arginine 600µM at the tight cluster stage, which had significant differences with the control and other treatments (Table 1).

The application of both putrescine and arginine treatments at the tight cluster stage in both years significantly decreased the percentage of fruit abscission (Table 1). The abscission of fruits on shoots treated at the secondary stage of both years differed significantly between the control and putrescine and arginine treatments. The best treatment was arginine 600µM at the tight cluster stage, which had significant differences with the control and other treatments (Table 1).

Table 1. Effects of putrescine and arginine treatments on inflorescence bud abscission and fruit abscission in Owhadi pistachio cultivar

Treatment		Inflorescence Bud Abscission (%)		Fruit Abscission (%)	
		Stage1	Stage2	Stage1	Stage2
On Year	Control	89.21a	89.10a	79.01a	78.21a
	A300 µM	82.21b	86.23ab	73.04ab	70.02b
	A600 µM	70.31cd	60.250e	67.21c	65.275cd
	P 0.1 mMol	85.14b	85.26b	75.24a	72.13ab
	P 0.2 mMol	72.23c	75.42c	70b	68.25bc
Off Year	Control	40.21a	39.41a	75.01a	73.2a
	A300 µM	31.27bc	30.28bc	70.01b	69.02b
	A600 µM	26.2d	20.2f	63.25c	57.32d
	P 0.1 mMol	33.28b	30c	71.02ab	70.2b
	P 0.2 mMol	25d	22.25e	72.09ab	61.386cd

Stage1= Spraying time at green tip stage of buds and Stage2= Spraying time at the tight cluster
Data in each column followed by the same letters are not significantly different (P< 0.05)

3.1. Effects of the exogenous application of putrescine and arginine on blankness, non-dehisced shells, dehisced shells, misshapen nuts, and early splitting

The exogenous application of putrescine and arginine significantly decreased blankness, non-dehisced shells, misshapen nuts, and early splitting, but it increased dehisced shells (Tables 2 and 3).

Arginine 600µM significantly decreased the disorders of pistachio nuts, such as blankness, non-dehisced shells, misshapen nuts, and early splitting at both stages of ‘on’ and ‘off’ years and was the best treatment among the control and other treatments. The putrescine treatment was not as efficient as arginine, especially at the tight cluster stage (Tables 2 and 3).

Table 2. Effects of putrescine and arginine treatments on blank nuts, non-dehisced shells, misshapen nuts, and early split of nuts in ‘Owhadi’ pistachio cultivar

Treatment		Blank Nuts (%)		Non-Dehisced Shells (%)		Misshapen Nuts (%)		Early Split Nuts (%)	
		Stage1	Stage2	Stage1	Stage2	Stage1	Stage2	Stage1	Stage2
On year	**Control**	14.28a	15.18a	24.13a	25.04a	12.03a	11.95a	2.21a	2.11a
	A300 µM	13.52ab	12.89bc	22.03b	21.14bc	10.87b	10.49bc	1.65b	1.74ab
	A600 µM	11.02c	9d	19.2cd	18d	9.18d	8.25e	1.02d	1.08d
	P 0.1 mMol	13.8ab	13.2ab	23.25ab	22.11b	11.710a	11.02ab	1.79ab	1.81ab
	P 0.2 mMol	11.74bc	11c	20.15c	19.02cd	10.08c	9.45cd	1.28c	1.32c
Off year	**Control**	20.04a	19.3a	20.12a	19.25a	9.51a	9.32a	3.12a	3.21a
	A300 µM	15.21b	14.11bc	16.98bc	16.12c	8.258b	8.02bc	2.01bc	2.52b
	A600 µM	12.69cd	10.25e	11.89de	10.254f	6.87d	6.18de	1.57e	1.74d
	P 0.1 mMol	15.87b	15.23b	18.35b	17.24bc	8.45b	8.18b	2.25b	2.71ab
	P 0.2 mMol	13.38cd	12.98cd	12.58d	12d	7.8c	7.54c	1.7d	1.89cd

Stage1= Spraying time at the green tip stage of buds and Stage2= Spraying time at the tight cluster
Data in each column followed by the same letter are not significantly different (P< 0.05)

3.2. Effects of the exogenous application of putrescine and arginine on cluster characteristics and the yield

According to the results, putrescine and arginine significantly increased the number of nuts per cluster and the number of nuts per ounce when applied at the tight cluster stage of buds in the 'on' and 'off' years (Table 3). In contrast to other treatments, arginine 600 µM significantly increased the yield per shoot when applied at the secondary stage of spraying (Table 3). Putrescine 0.1 mM and argenine 300 µM were found out to have insignificant effects on the number of nuts per cluster, ounce, and yield (Table 3).

3.3. Effects of the exogenous application of putrescine and arginine on the leaf area and shoot growth

The exogenous application of putrescine and arginine significantly increased the leaf area (Table 4). According to the results of this search, putrescine and arginine had the least effects among the treatments on the length and diameter of current-year shoots during 'on' and 'off' years and at the stages of application (Table 4).

Table 3. Effects of putrescine and arginine treatments on dehisced shells, number of nuts per cluster, number of nuts per ounce, and yield of 'Owhadi' pistachio cultivar

Treatment		Yield Per Shoot (g)		Number of Nuts per Ounce		Number of Nuts Per Cluster		Dehisced Shell Nuts (%)a	
		Stage1	Stage2	Stage1	Stage2	Stage1	Stage2	Stage1	Stage2
On Year	Control	63.24e	61.28e	26.11a	26.02a	19e	19.25e	55de	54.38d
	A300 µM	75.38cd	74.32cd	25.21ab	25.43ab	21.03d	20.05d	58.21cd	57.18cd
	A600 µM	93.65a	80.25bc	24.01c	25ab	29.11a	26.35b	68.89a	65.32b
	P 0.1 mMol	70.23d	69.12d	25.24ab	25.56ab	21d	19.89de	57.87c	55.26d
	P 0.2 mMol	85.24b	78.32c	24.96c	25ab	25.32bc	24.11c	65.38b	60.35c
Off Year	Control	23.24f	23.28f	25.01a	25a	22.15e	22.01e	59.35de	60.02d
	A300 µM	25.38e	24.32ef	24.32ab	24.51ab	23.1d	22.95d	65.24c	62.35cd
	A600 µM	33.65a	32.25ab	23.11c	23.56bc	30a	27.45b	78.32a	75.4ab
	P 0.1 mMol	24.23ef	23.99ef	24.42ab	24.67ab	22.11e	22e	65c	61.28cd
	P 0.2 mMol	28.247c	27.32cd	23.96bc	24.01b	26.92bc	25.23c	70.25b	70b

Stage1= Spraying time at the green tip stage of buds and Stage2= Spraying time at the tight cluster
Data in each column followed by the same letter are not significantly different (P< 0.05)

Table 4. Effects of putrescine and arginine treatments on the leaf area, shoot length, and shoot diameter in 'Owhadi' pistachio cultivar

Treatment		Leaf area (cm^2)		Shoot Length (cm)		Shoot Diameter (mm)	
		Stage1	Stage2	Stage1	Stage2	Stage1	Stage2
On Year	Control	6a	6.03a	9.82a	9.63ab	75.21cd	74.01d
	A300 µM	5.89ab	5.62c	9.77a	9.51bc	76.32bc	75.02cd
	A600 µM	5.95a	5.63bc	9.69ab	9.58bc	82.21a	80.25a
	P 0.1 mMol	5.96a	5.55c	9.78a	9.6ab	76.017bc	74.58c
	P 0.2 mMol	6a	5.58c	9.65ab	9.48c	78.23ab	77.42ab
Off Year	Control	6.58c	6.63c	10.75b	10.74bc	85.11cd	85.09cd
	A300 µM	6.89ab	6.64c	10.89a	10.45d	86.4c	85.2cd
	A600 µM	6.96a	6.62c	10.753b	10.57c	92.021a	90.15ab
	P 0.1 mMol	6.98a	6.55cd	10.78b	10.45d	86.1c	84.28d
	P 0.2 mMol	7a	6.58c	10.87a	10.57c	88.24bc	87.35bc

Stage1= Spraying time at the green tip stage of buds and Stage2= Spraying time at the tight cluster
Data in each column followed by the same letter are not significantly different ($P < 0.05$).

4. Discussion

The present study showed that putrescine and arginine treatments decreased the percentage of inflorescence bud abscission. It was also found out that polyamines played a regulatory role in the process of inflorescence bud abscission [12]. The foliar application of arginine acts as a growth regulator due to the synthesis of polyamines and polyamines, being able to modulate plant metabolism and produce metabolites involved in stress tolerance [44, 45]. It has been reported that the exogenous application of polyamines has been effective in decreasing the endogenous abscission of inflorescence buds [12, 46]. This shows the key role of polyamines in pistachio buds and shoots, thereby exhibiting a negative correlation with inflorescence bud abscission [12]. The present study indicated that the exogenous application of putrescine 0.2 mM or argenine 600 µMol at the tight cluster stage of buds increased endogenous polyamine concentrations in the treated shoots and decreased the percentage of inflorescence bud abscission, being consistent with the studies by Khezri et al (2010) and Roussos et al (2004). However, the present study showed that the application of putrescine decreased the percentage of fruit abscission, which shows the inhibitory role of polyamines in fruit abscission [25- 27, 46]. The exogenous application of polyamines putrescine has been effective in decreasing fruit abscission in pistachios [46] and mangos [47], being consistent with the results of this study. However, internal and environmental factors, such as photo-assimilates and plant hormones are the probable causal factors of fruit abscission [48]. Since the fruitlet abscission of pistachios is assumed to be caused by unsuitable fertilization, fruit dominance in a cluster, and non-pollinated fruits [7], polyamines have been suggested to improve fertilization as well as subsequent embryo and fruit development [23]. Furthermore, researchers have reported that the application of ethephon increases fruit abscission in pistachios [49]. Polyamines reduce the abscission of inflorescence buds, flowers, and fruits, but ethylene increases them; in addition, polyamines have a negative correlation with ethylene because the precursors of both plant bio-regulators (SAM)[5] are unique [50]. It has been reported that the degeneration of ovary segments is the major cause of blanking in pistachios [51], and it has been shown that there is a correlation between kernel development and splitting [52].

Thus, a decrease in the percentage of blank and non-split nuts due to the application of putrescine or argenine could be attributed to the role of putrescine or argenine in improving the growth of reproductive organs and preventing ovary degeneration [23]; otherwise, it could be attributed to the involvement of PAS, their precursor arginine, and their biosynthetic enzymes in stimulating cell division, expansion, differentiation, and vascular development [53], improving the development of reproductive organs, as well as preventing ovary degeneration. The results showed that putrescine or argenine decreased the percentage of misshapen nuts. If the deformation of nuts in pistachios is the result of a disease, non-pollination, or other physical types of damage [12, 46, 54], polyamines will seemingly increase the resistance of pistachio hulls or shells against damaging factors [24, 46, 55]. The foliar application of arginine acts as a growth regulator due to the synthesis of polyamines and polyamines, which is able to modulate plant metabolism and produce metabolites involved in stress tolerance [44, 45]. Arginine is one of the most functionally diverse amino acids in living cells. In addition to serving as a constituent of proteins, it is a precursor of the biosynthesis of polyamines and proline, where molecules act as development and growth regulators [29, 30]. The role of amino acids in growth and differentiation processes is known to a considerable extent [56]. However, little research has been done on the precise causes of deformation in pistachio nuts.

The application of putrescine or argenine in both years at the different stages of application increased the crop yield of pistachios, possibly due to the role of polyamines and their precursor arginine in improving reproductive growth [3, 44, 45]. However, polyamines are also known as polycationic nitrogenous and anti-senescence compounds [26, 32]. It is quite likely for these bio-regulators to be suitable for serving either as nitrogenous sources or as signal molecules regulating the abscission process as well as fruit growth and development, thereby affecting pistachio crop yields. The mechanism used by arginine in improving plant yields and developments is that it acts as a precursor of growth factors, such as polyamines and proline [29, 30]. Polyamines have been shown to be associated with cell division, so they could be utilized to regulate fruit development. The exogenous application of PAS has also been reported to promote reproductive development under normal growth conditions and protect reproductive structures against abiotic stress [47, 57- 60]. The overall positive effects observed in this study on the yield and fruit characteristics of pistachio fruits after applying arginine could be attributed to the positive effects of polyamine synthesis on floral development, which resulted in improving

[5] S-Adenosylmethionine (SAM)

both fruit set and fruit yield. In addition, a great body of evidence shows that endogenous polyamines are important for pollen germination and pollen tube growth [61]. Furthermore, putrescine enhances pollen tube ovule penetration and delays ovule senescence without affecting flower ethylene production [62, 63].

In addition, the effects of polyamines have been ascribed to the increase in ovule viability and the prolonged pollination period [64]. Bagni et al [65] reported that the biosynthesis of polyamines using arginine took place before pollen tube emergence. In addition, the effect of polyamines on improving fruit set has been observed in apples and pears [62, 63, 66- 68]. The same effects were reported by Biasi et al [69] on litchi and by Costa and Bagni [66] on olives. In terms of fruit characteristics, the results of the present study showed that the application of arginine exerted positive effects on quantitative and qualitative fruit characteristics, having been consistent with the study by Bregoli et al [70] on peaches and by Torrigiani et al [71] on nectarines. The application of putrescine or argenine in both 'on' and 'off' years increased the leaf area, with this probably leading to more photo-assimilate syntheses and accordingly greater carbohydrate availability so as to decrease inflorescence bud abscission and enhance nut splitting in both 'on' and 'off' years. The exogenous application of free polyamines significantly increased the endogenous contents of these compounds in the shoots and showed their absorbance capability via spray application; it also decreased physiological disorders [50, 72].

5. Conclusions

According to the results, putrescine and arginine treatments were very effective in improving the yield components and growth parameters of pistachio trees in Owhadi cultivar. The arginine treatment was more efficient than the putrescine treatment. Arginine could have been more absorbable into shoots via buds than putrescine, and it might have been more penetrable into inner compounds, such as polyamines, proline, and other compounds. This suggests that the effects of putrescine and arginine treatments on the decrease in the percentage of bud and fruit abscission could have been more due to their transfer into inner polyamines. It was also found out that the spray application of putrescine and arginine at the tight cluster stage was more efficient because during this stage, they were more absorbable into the shoots via buds than the first stage. Hence, the exogenous application of putrescine and arginine resulted in endogenous bio-regulator changes, including phytohormones, carbohydrates, and nutrients. It also significantly decreased physiological disorders and increased the pistachio yield.

Conflict of interest

The authors declare no conflicts of interest.

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