Role of endogenous polyamines in the alternate bearing phenomenon in pistachio

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1. Introduction

Pistacia vera L. is commonly cultivated for its nuts. Turkey has rich plant diversity and genetic resources owing to its diverse climatic and soil conditions. There are also wild Pistacia species that are distributed throughout Turkey (Kafkas, 2006). In particular, the Southeastern Anatolia Region is well suited for pistachio cultivation due to its suitable ecological conditions, accounting for 95% of the total pistachio growing area, covering 91.5% of pistachio production in Turkey (Yavuz, 2011). Moreover, Turkey is one of the main pistachio producers in the world after the United States and Iran (http://www.fao.org/faostat/).

One of the main problems in pistachio production is the alternate bearing (irregular crop load) phenomenon. Unlike other fruit tree species, pistachio produces floral buds regularly every year, but the abscission of buds occurs during summer. Alternate bearing is identified by a year of bumper nut load referred as ‘On-year’, followed by light, extremely light, or no yield referred to as ‘Off-year’ in pistachio. This phenomenon is a problem not only in pistachio, but also in a few other perennial fruit tree species, such as mango, olive, and pecan. The mechanism of alternate bearing in pistachio is different from that of other tree species. The inflorescence buds are formed one year before in pistachio, similar to that of other deciduous tree species. However, they abscise during summer in the ‘On-year’ trees, rather than the lack of flower bud formation. It is well known that significant abscission of pistachio floral buds starts in June and continues throughout July in the ‘On-year’ trees, causing a yield loss in subsequent ‘Off-year’ season (Crane and Nelson, 1971; Crane et al., 1973; Porlingis, 1974; Monselise and Goldschmidt, 1982; Pontikis, 1986; Crane and Iwakiri, 1987; Stevenson and Shaked, 1998; Vemmos, 1996b, 1999a; Lovatt and Ferguson, 2002; Köksal et al., 2004).

All pistachio cultivars used in production and in germplasm resources have the tendency of alternate bearing. In Turkey, Uzun, Kırmızı, and Siirt are the main cultivars used in pistachio production. Some of the cultivars have a strong tendency of alternate bearing, such as Uzun and Kırmızı, whereas some of them, such as Siirt, have a low yield after an ‘On-year’ season. Thus, alternate bearing is a significant economic problem adversely affecting the producers, consumers, and economy of a country.

In the present study, we aimed to investigate the physiological role of free polyamines (PAs) in fruit bud abscission of the Uzun pistachio variety, which has a strong alternate bearing tendency. The identification and quantification of PAs, such as putrescine (Put), spermidine (Spd), and spermine (Spm), at different physiological stages and in different organs, were carried out by high-performance liquid chromatography. Putrescine was the major PA in all the organs of pistachio trees. Furthermore, the concentrations of Put and Spd in the leaves and shoots sharply declined and reached the minimal levels during the fruit bud abscission period (June), and then increased during the period of kernel development. The concentration of Put and Spd in the panicles increased gradually until the beginning of kernel growth and then decreased until harvest. The concentration of Spm exhibited a negative correlation between shoots and leaves, especially in the ‘On-year’ trees. There was a significant decrease in PAs in the leaves during the period of bud abscission in the ‘Off-year’ trees. In the ‘On-year’ trees, the concentration of PAs exhibited a negative correlation with the panicles, especially when the concentration of Put and Spd increased during the kernel development period, and then decreased. The concentration of PAs in the panicles of the ‘On-year’ trees was higher than that in the nuts, and it exhibited a negative correlation with that in the nuts. The results suggest that PAs might lead to physiological changes during the growth and development stages of pistachios, and a strong relationship was observed.

Abstract: Alternate bearing is a significant economic problem adversely affecting the producers, consumers, and economy of a country. In the present study, we aimed to investigate the physiological role of free polyamines (PAs) in fruit bud abscission of the Uzun pistachio variety, which has a strong alternate bearing tendency. The identification and quantification of PAs, such as putrescine (Put), spermidine (Spd), and spermine (Spm), at different physiological stages and in different organs, were carried out by high-performance liquid chromatography. Putrescine was the major PA in all the organs of pistachio trees. Furthermore, the concentrations of Put and Spd in the leaves and shoots sharply declined and reached the minimal levels during the fruit bud abscission period (June), and then increased during the period of kernel development. The concentration of Put and Spd in the panicles increased gradually until the beginning of kernel growth and then decreased until harvest. The concentration of Spm exhibited a negative correlation between shoots and leaves, especially in the ‘On-year’ trees. There was a significant decrease in PAs in the leaves during the period of bud abscission in the ‘Off-year’ trees. In the ‘On-year’ trees, the concentration of PAs exhibited a negative correlation with the panicles, especially when the concentration of Put and Spd increased during the kernel development period, and then decreased. The concentration of PAs in the panicles of the ‘On-year’ trees was higher than that in the nuts, and it exhibited a negative correlation with that in the nuts. The results suggest that PAs might lead to physiological changes during the growth and development stages of pistachios, and a strong relationship was observed.

Key words: Alternate bearing, bud abscission, high-performance liquid chromatography, pistachio, polyamine
bearing has a negative effect on pistachio producers due to irregular yield and on consumers due to the high prices in the 'Off-year' seasons.

Due to this unique phenomenon of pistachio, efforts have been made to elucidate its cause. Studies have been carried out to improve horticultural practices, such as irrigation, pruning, and fertilization. It has been suggested that the competition for metabolites and nutrients between the nuts and floral buds causes the abscission in pistachio (Crane and Nelson, 1971; Porlingis, 1974; Uriu and Crane, 1977; Brown et al., 1995; Rosecrance et al., 1998; Vemmos, 1999b). However, it has also been reported that this nutrient imbalance is not the main cause of bud abscission and that role of internal physiological factors should be evaluated. Several studies have shown that they have a prominent role in alternate bearing (Porlingis, 1974; Nzima et al., 1997; Çetinkaya, 2004; Roussos et al., 2004; Okay et al., 2011). However, the significant genetic and physiological effects of internal factors on the abscission of floral buds in pistachio have not been fully understood.

The internal factors in plants have been found to be the most ideal molecules for binding to cell receptors in signal transduction required for the initiation of development and physiological events (Burg, 1968; Abeles and Leather, 1971; Adams and Yang, 1979; Smith, 1985; Galston and Kaur-Sawhney, 1987; Flores et al., 1989; Lavee, 1989; Weinbaum et al., 1994; Kakkar et al., 1998; Vemmos, 1999a; Lovatt and Ferguson, 2002; Ulger et al., 2004; Baninasa et al., 2007; Yamada et al., 2007). Polyamines (PAs), one of these internal factors, are ubiquitous polycationic nitrogenous bases of low molecular weight, which are regarded as multifunctional regulators of physiological processes (Konigshofer, 1989; Kakkar et al., 2000; Kuznetsov et al., 2006). They have been suggested to act as hormonal second messengers (Galston, 1986; Kakkar and Kaur-Sawhney, 2002; Kireççi, 2006; Lobata and Gomez-Jimenez, 2011). The most important PAs are putrescine (Put), spermidine (Spd), and spermine (Spm), and they exhibit hormone-like action (Smith, 1985; Pandey et al., 2000). They modulate several processes related to growth and development, i.e. cell division, rhizogenesis, senescence, flowering, and fruit ripening. They are also involved in stress responses and in almost every step of DNA, RNA, and protein synthesis (Galston and Kaur-Sawhney, 1987, 1995; Kaur-Sawhney et al., 1988; Sarjala and Savonen, 1994; Lelievre et al., 1997; Kushad, 1998; Kakkar et al., 2000; Pandey et al., 2000; Kaur-Sawhney et al., 2003).

To the best of our knowledge, there is no clear evidence regarding the possible role of PAs in the abscission of floral buds in pistachio. However, it has been previously reported that they play a significant role in the abscission of flower buds, fruitlets, and fruits in other plants (Aziz, 2003; Roussos et al., 2004; Gomez-Jimenez et al., 2010).

In the present study, we aimed to elucidate the effects of PAs on floral bud abscission by evaluating the concentration of PAs in several vegetative and generative organs of 'On-year' and 'Off-year' pistachio trees.

2. Materials and methods

2.1. Plant materials

The study was conducted in the pistachio growing seasons of 2015 and 2016 at the Research and Experimental Area of the Pistachio Research Institute, Gaziantep Province, Turkey. Thirty-three-year-old Uzun trees grafted to Pistacia atlantica Desf. rootstock and planted at 10 m × 10 m intervals were used as plant materials. The shoots, leaves, panicles, and nuts were sampled from the 'On-year' and 'Off-year' trees to analyze the concentration of PAs during two consecutive years.

2.1.1. Sampling

In 2015, sampling was carried out at 35, 45, 55, 75, 85, 118, and 146 days after full blooming (DAFB), whereas in 2016 sampling was carried out at 36, 50, 64, 78, 92, 127, and 147 DAFB (Table). The plants were sampled in early morning and immediately transferred to the laboratory under cool conditions. The plant organs were rinsed several times with distilled water to remove dust, freeze-dried, ground, and stored at 4°C until further analysis.

2.1.2. Polyamine analysis

The samples (0.1 g) were extracted in 1 mL of cold 5% (v/v) HClO₄ for 1 h at 2–4°C in the dark in the presence of 1,6-hexanediame as an internal standard. The samples were then centrifuged at 37,000 × g for 15 min at 4°C and 0.2 mL of the supernatant was used for the derivatization of PAs using dansyl chloride. The determination and quantification of PAs were carried out according to the method described by Roussos et al. (2002) with some modifications.

The analysis of PAs was carried out using the Shimadzu SPD-20A high-performance liquid chromatography (HPLC) instrument equipped with an isocratic pump, with an Intersil ODS-5C-18 reverse phase column. The mobile phase consisted of 70% acetonitrile and 30% water, and the analysis was performed with a gradient flow rate of 1 mL min⁻¹ and column temperature of 30°C. The concentration of PAs was determined using a fluorescence detector at wavelengths of 256 and 360 nm, using 1,2-hexanediame as an internal standard. The concentration of PAs is expressed as µg g⁻¹ dry weight (d.w.).

2.1.3. Statistical analyses

The experiments were performed in a randomized complete block design with three replicates, including three trees per replicate. The statistical analyses were performed using the JMP statistical software from SAS (V7) (SAS Institute Inc. Cary, NC, USA). The differences
between the mean values were determined by the least significant differences (LSD) test at a P-value of 0.05.

3. Results
The concentrations of PAs in different tissues obtained from the ‘On-year’ and ‘Off-year’ pistachio trees during the growing seasons of 2015 and 2016 are presented in Figures 1–4.

3.1. Polyamine concentrations in the shoots
The concentration of PAs was significantly different between the ‘On-year’ and ‘Off-year’ trees (P < 0.05). The concentrations of Put and Spd were significantly higher in the shoots of ‘On-year’ trees than in the shoots of ‘Off-year’ trees (Figure 1). During the heavy bud abscission period (DAFB-75 in 2015 and DAFB-78 in 2016), the concentration of Put and Spd in the ‘On-year’ shoots decreased to the minimal levels, whereas their levels were relatively high in the shoots of the ‘Off-year’ trees. On the other hand, the PA ratio increased in the ‘On-year’ shoots during the first samplings and decreased at the last sampling, while staying almost constant in the ‘Off-year’ shoots. Marginal changes in the concentration of PAs were observed in the ‘Off-year’ trees. Spermine concentration was low during the early periods of flowering, followed by the harvesting period. In most of the samples, the concentration of Spm in the ‘On-year’ trees was higher than that in the ‘Off-year’ trees (Figure 1). Our results also showed that the PA ratio in the shoots of ‘Off-year’ trees, as well as the content of PAs, is not affected by the sampling time.

3.2. Polyamine concentrations in the leaves
The differences in the concentration of PAs in the leaf samples obtained from the ‘On-year’ and ‘Off-year’ Uzun trees at various periods were significant (P < 0.05). There were negative correlations between the ‘On-year’ and ‘Off-year’ trees with respect to the concentration of PAs in the leaf. The Put content in the leaves of the ‘Off-year’ trees was higher than that in the leaves of the ‘On-year’ trees during the two consecutive years. The content of PAs in the leaves of the ‘On-year’ trees was generally high on the early sampling dates, and then it decreased during the heavy bud abscission period (DAFB-75 in 2015 and DAFB-78 in 2016), and then increased until harvest. The concentration of PAs was low when the kernel started to develop together with the bud abscission. However, the leaf PA concentrations reached the maximal levels in the ‘Off-year’ trees during the kernel development and heavy bud abscission period. As can be concluded from Figure 2, the time of sampling had a significant effect on the PA ratios in the leaves of both ‘On-year’ and ‘Off-year’ trees (Figure 2).

3.3. Polyamine concentrations in the panicles and nuts
The concentration of PAs in the panicles and nuts was determined only in the ‘On-year’ trees due to the bud abscissions in the ‘Off-year’ trees the previous year. The concentration of PAs in the panicles and nuts at different growing stages was significant (P < 0.05). The Put concentration in the panicles and nuts was almost stable on the initial sampling days, and then it gradually increased. The Put concentration reached its maximal level in the middle of July, and then gradually decreased until harvest. Maximal levels of Put were detected at the beginning of the kernel development or heavy bud abscission stage in the panicles and nuts (Figures 3 and 4).

In contrast to the Put levels, the Spm level was mostly stable or decreased slowly during the growing season, except at the beginning of the kernel development or heavy bud abscission stage (Figures 3 and 4). Among the

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<th>Plant parts</th>
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DAFB: Days after full blooming. Full flowering date in the study years: 10.04.2015 and 08.04.2016, respectively.
in the panicles and nuts, the content of Put was the highest, whereas the content of Spd was the lowest. The Spd content in the panicles decreased gradually during the growing season with a marginal increase during the heavy bud abscission period. The Spd concentration in the nuts exhibited a gradual increase during the growing season (Figures 3 and 4).

4. Discussion
PA concentrations in different periods and organs were determined in the Uzun cultivar (Figures 1–4). There were statistically significant differences between the organs and PA ratios in the ‘On-year’ and ‘Off-year’ trees for most of the period of the experiment (P < 0.05). Our results indicate that the level of PAs in different organs depends on the developmental stage of the fruit bud abscission and changes according to the organ. In most cases, the PA concentration was significantly lower in the organs of the ‘On-year’ branches (major bud abscission of stages) than in those of the ‘Off-year’ ones (Figures 1–4).

Several studies have reported that PAs play an important role in the growth and development of plants,
such as bud growth and flowering (Kaur-Sawhney et al., 1988; Evans and Malmberg, 1989; Wang and Faust, 1994; Kushad, 1998), generative organ development (Galston et al., 1997), fruit development and ripening (Kakkar and Rai, 1993; Galston et al., 1997; Aziz et al., 2001), and plant defense against biotic and abiotic stresses (Evans and Malmberg, 1989; Martin-Tanguy, 1997; Larher et al., 1998; Bouchereau et al., 1999; Walters, 2003; Alkazar et al., 2006; Kuznetsov et al., 2006; Kusano et al., 2007). Studies have also reported that PAs play an important primary role in abscission (Moss et al., 1972; Goldschmidt and Koch, 1996; Aziz, 2003; Roussos et al., 2004; Gomez-Jimenez et al., 2010). For this reason, PAs could play a very important role in the flower bud abscission of pistachio.

In the present study, we determined the levels of PAs in four different organs of pistachio during the growing season. Put was the dominant PA in all the organs. The panicles (318.64–810.49 mg/g d.w.) and nuts (60.16–330.78 mg/g d.w.) presented the highest level of Put among the four organs studied. The leaves exhibited the lowest level of Spm (0.90–10.03 mg/g d.w.). Roussos et al. (2004) studied the effects of PAs on bud abscission in pistachio and reported that Spm is the dominant PA in the Pontikis cultivar. The concentration of Put and Spd in the shoots

Figure 2. Changes in the concentrations of the free-form PAs (Put, Spd, and Spm) in the ‘On’ and ‘Off’ years of leaves at various physiological stages of 2015 and 2016 (mg/g d.w. (y-axis)). Other details are similar to Figure 1.
and leaves decreased after full flowering and fruit set. A similar decrease in the content of PAs has been reported in apple (Biasi et al., 1988), orange (Sagee and Lovatt, 1991), grape (Shiozaki et al., 2000), and olive (Pritsa and Voyiatzis, 2004). In the present study, the concentration of Spd increased in the shoots and leaves at the beginning of full flowering. The concentrations of Spd and Put were higher in the shoots of the ‘On-year’ trees than in the shoots of the ‘Off-year’ trees. This result showed that the degree of bud abscission was higher in the ‘Off-year’ trees than in the ‘On-year’ ones. Spm was found to be the only PA whose concentration increased in the buds of mature peach (*Prunus persica* L.) at the beginning of spring growth (Fraga et al., 2004). Rey et al. (1994) also reported that in hazel (*Corylus avellana* L.) buds, the high levels of free Spd and Spm positively correlated with bud burst during spring, and their levels decreased during autumn. Consistent with these reports, we also observed that the concentrations of Spd and Put in the shoots and leaves were low throughout the period of fruit bud abscission. On the contrary, Aziz (2003), Malik and Singh (2003), and Gomez-Jimenez et al. (2010) indicated a significant negative correlation between the concentration of PAs and the bud abscission in mango, grapevine, and olive.

PAs act as antisenescence agents, and they are known to be effective against abscisic acid and ethylene activity.
(Smith, 1985; Wang and Steffens, 1985; Crane and Iwakiri, 1987; Evans and Malmberg, 1989; Vemmos et al., 1994; Pandey et al., 2000; Chen et al., 2002). Although, ethephon (an ethylene producer), which is one of the most dominant hormones promoting senescence, has a role opposite to that of PAs, they share the same precursor, S-adenosylmethionine (SAM) (Smith, 1985; Wang and Steffens, 1985; Chen et al., 2002; Kaur-Sawhney et al., 2003). Several studies have indicated that abscission or ethylene biosynthesis can be delayed with low levels of SAM, where PAs and ethylene compete with each other; under this condition, PAs can become more dominant (Evans and Malmberg, 1989; Chen et al., 2002; Kuznetsov et al., 2002; Roussos et al., 2004). It is also known that low concentrations of PAs might trigger the mechanisms of senescence and therefore cause abscission (Srivastava et al., 1981; Smith and Davies, 1985; Evans and Malmberg, 1989; Aziz et al., 2001; Roussos et al., 2004; Gomez-Jimenez et al., 2010).

In the present study, we demonstrated that the concentration of PAs decreased in the shoots and leaves of the ‘On-year’ trees during the heavy bud abscission period, whereas their levels increased during the same period in the ‘Off-year’ trees. This suggests that there might be a physical stage in the trees.

Figure 4. Changes in the concentrations of the free-form PAs (Put, Spd, and Spm) in the ‘On’ years of nuts at various physiological stages of 2015 and 2016 (mg/g d.w. (y-axis)). Other details are similar to Figure 1.
translocation of PAs from the shoots/leaves to and from the pedicels to nuts. Antognoni et al. (1998) reported the translocation of PAs among different plant organs. In the present study, the concentration of PAs in the ‘On-year’ trees was lower than that in the ‘Off-year’ trees during the heavy floral bud abscission period, indicating an association between the floral bud abscission and the level of PAs in pistachio. The results of the present study also demonstrate that PAs have an important role in the bud abscission in pistachio. Further studies are necessary to understand the relationship between PAs and bud abscission.

In conclusion, the present study demonstrated that PAs play an important role in the bud abscission, causing alternate bearing in pistachio. Put was the major PAs in all the organs studied, followed by Spd and Spm, respectively. The results revealed a decrease in the concentration of PAs during the heavy bud abscission period in the ‘On-year’ trees. The concentration of most PAs in the organs of the ‘On-year’ trees was lower than that in the organs of the ‘Off-year’ trees. By measuring the concentration of Put and Spd in all the organs, it was possible to estimate the oncoming bud abscission. PAs generally exhibited a negative correlation with the floral bud abscission. Consequently, PAs might have an important physiological function in the abscission of flower bud in pistachio. The strong negative correlation between the PAs and bud abscission might be an early indication of the role of PAs in this unique phenomenon of Uzun. Thus, the exogenous application of PAs at different concentrations is suggested to mitigate alternate bearing in pistachio.

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