



# Changes in polyamine levels in relationship to the growth and development of parthenocarpic fruits (shotberries) of olive (*Olea europaea* L.)

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## ABSTRACT

The effects of applied polyamines (PAs), putrescine (Put) and spermidine (Spd) on parthenocarpic fruits (shotberries) and fruit set of olive were assessed. Factorial experiments were conducted in a randomized complete block design with three replications. Putrescine at 2.5 and 5 mM·l<sup>-1</sup> and spermidine at 1.25 and 2.5 mM·l<sup>-1</sup> were sprayed on branch units on olive trees (*Olea europaea* L. cv. Fishomi) at full bloom (FB) and two weeks after full bloom (2WAFB). The number of normal fruits and shotberries were evaluated. Endogenous PAs in the mesocarp and seeds were determined at both times of PAs application until fruit maturity. The results showed that Put was very effective in decreasing the percentage of the shotberries and increasing the percentage of normal fruits when PAs applied at FB. Mesocarp had the highest Put content in Put at 5 mM·l<sup>-1</sup> and Spd at 2.5 mM·l<sup>-1</sup> treatments respectively. In seed, the highest PAs content was found in Put treatment at 5 mM·l<sup>-1</sup> and the highest Spm and Spd content was observed in Spd treatment at 2.5 mM·l<sup>-1</sup>.

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## 1. Introduction

Fertility of a tree can be divided in three components such as number of flowers, the number of fruit set and the number of fruit at harvest time. Research on the optimal delivery yield of each of these components are necessary. In olive tree, number of flowers, especially the number of perfect flowers and fruit set are very important and other components of yield, number of parthenocarpic fruits or shotberries are very effective in the production and fertility (Ferguson et al., 1994). Olive shotberries are small, commonly seedless, fruit expressing quantitative parthenocarpy. They either abscise before harvest or, if harvested, had no commercial value. Consequently, increased incidence of shotberries has a negative impact on fruit and oil yield, and can seriously affect economic yield of olive orchards. The size of shotberries, compared to that of normal fruit, was related to reduced accumulation of assimilates while absence of the seed was proposed to play a key role in this developmental aberration (Marquez et al., 1990).

Shotberry formation seems to be affected by a series of environmental factors. A higher number of shotberries was reported in olive trees growing in areas where the air temperature reached to 41°C during the anthesis period than in areas with lower temperatures (Ayerza and Coates, 2004). Low pollen viability (Lavee et al., 2002), self-pollination (Sibbett et al., 1992) or poor pollination in general (Ayerza and Coates, 2004; Cuevas et al., 2001; Martin et al., 1994) have been proposed as factors promoting shotberry formation in olive trees. Ugrinovic and Stambar (1996) observed that shotberry formation was genotype-dependent, with cv. Leccino being more prone than other cvs studied. However, no significant impact on final fruit yield was reported. Increased incidence of shotberry appearance was also observed in repeated years of high fruit load. Existence of parthenocarpic fruits in olive is one of the major problems that most returns to the genetics of plant. In this case cultivars indicate many differences with each other. In addition to genetic factors, environmental factors and nutrition are an important influence in this case. The role of nutrition and polyamines are very important. Polyamines (PAs) have been reported as modulators of plant development (Galston, 1983), and stimulation of plant growth has been correlated with an increase in their biosynthesis in growing plant tissues (Smith, 1985; Bagni and Torrigiani,

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1992). Specifically, PAs have been associated with embryogenesis, root formation, pollen formation, floral initiation, early fruit development, leaf senescence and stress response (Tiburcio et al., 1990; Rastogi and Davies, 1991). Furthermore, plants showing altered levels of PAs display abnormal phenotypes that can be returned to wild type by the application of PAs (Kumar et al., 1996). Fruit development is induced by growth regulators (Gillaspy et al., 1993). In peas and tomato, induction of parthenocarpic fruit development by gibberellic acid (GA3) and other plant growth regulators results in changes in the levels of PAs, and in the expression of genes of PA biosynthesis (Carbonell and Navarro, 1989; Perez-Amador et al., 1995). There is evidence suggesting that PAs may have a role in early fruit development in several species (Costa and Bagni, 1983; Rugini and Mencuccini, 1985; Crisosto et al., 1988; Evans and Malmber, 1989; Egea-Cortines and Mizrahi, 1991). Changes in PAs content have been correlated with fruit growth during the cell division stage of several annual and woody crops, suggesting that PAs biosynthesis is associated with post-fertilization growth and development of ovary tissues (Slocum and Galston, 1985). In tissues of apple fruit (Biasi et al., 1988), avocado fruit (Kushad et al., 1988), peach fruit (Kushad, 1998) and grapeberry (Shiozaki et al., 2000), levels of free PAs are relatively high during the first weeks after full bloom, and decreasing gradually shortly afterwards. However, the role of these changes on fruit and seed growth and development still remains unclear in tomato, a transient increase in the amount of free PAs during early parthenocarpic fruit development was induced by GA3 applied one day before anthesis (Alabadi et al., 1989), and a transient increase of ornithine decarboxylase and spermidine synthase transcripts in GA3-treated ovaries has been also observed (Alabadi and Carbonell, 1998; Alabadi and Carbonell, 1999). The aim of the present study was to examine changes in polyamines in relationship to olive mesocarp and seed development.

## 2. Materials and methods

The present study was carried out on olive trees (cv. Fishomi) 25 years old, which had been propagated by cutting, at a commercial orchard in Shiraz region, Iran (2014 and 2015). All trees used were in an ON year. The trees were spaced 5 and 6 m between and along the row respectively. Trees received routine cultural practices for commercial fruit production including fertilization and irrigation. The lay out was a  $2 \times 3 \times 2$  factorial experiment in a randomized complete block design with three replications. Each block contained 12 trees. Two branches (north and south of each tree) with approximately uniform length, diameters and number of flowers and fruits were used for each treatment. Flowers or fruits on each branches were sprayed to the point of run-off. Treatments were 2 types of PAs, putrescine (0, 2.5 and 5 mM l<sup>-1</sup> Put) and spermidine (0, 1.25 and 2.5 mM l<sup>-1</sup> Spd). All treatments were applied at full bloom (FB) and two weeks after full bloom (2WAFB). Following that, the number of normal fruits, shotberries and fruit abscission were determined at 4, 8 weeks and at harvest time. Samples were taken from fruits (10 fruit per branch) on the treated branches for PAs analysis. All samples were kept in liquid nitrogen until brought to the laboratory and kept in freezer -80 °C. To determine the endogenous PAs in mesocarp and seeds samples, mesocarp and endocarp tissues were homogenized in chilled mortar in a solution of 0.2 N HClO<sub>4</sub>. The homogenates were centrifuged at 4 °C in a clinical centrifuge. The supernatants were analyzed for polyamines by high-performance liquid chromatography (HPLC) as described by Smith and Davies (1985). HPLC analysis was carried out on an Agilent 1200 series (Agilent Technologies, USA), equipped with a Zorbax Eclipse XDB-C18 column (4.6 × 5 μm i.d.; × 150 mm film thickness, RP), and a photodiode array detector (PDA). Elution was monitored at 280 and 230 nm. The column temperature was 30 °C.

The injection volume was 20 μL and it was done automatically using auto sampler. To determine the effect of PAs and time of application on fruit and pit weight, 50 fruits per each treated branches were use at harvest time (14 WAFB). Data were analyzed for significant differences using a factorial analysis of variance with type of PAs, time of application and various concentrations as main factors by least significant difference (LSD).

## 3. Result

### 3.1. Effect of polyamines on olive fruit growth

The data presented in this study are the average of two years experiment. The results showed that fruit growth was affected by the type of PAs applied (Table 1). All concentrations of PAs significantly reduced percentage of shotberries and increased percentage of normal fruits. Putrescine in comparison to Spd was more effective in reducing the percentage of shotberries in this study. Put at 5 mM l<sup>-1</sup> in both time of application (FB and 2WAFB) significantly reduced shotberries to 0.07% and 0.12% at harvest time respectively (Table 1). Put at higher concentration in comparison to control treatment significantly increased the percentage of normal fruits from 1.34 (control) to 9.37 at harvest time in treated branches at FB (Table 1). Although Put application at higher concentration again increased the percentage of normal fruits on treated branches at 2WAFB but its effect was lower than treated branches at FB (Table 1). Spd at both concentrations and in both time of applications significantly reduced the percentage of shotberries and increased the percentage of normal fruits but its effect was lower than Put in this study (Table 1).

We found that application of Put and Spd significantly increased fruit weight at harvest time. Time of application was very effective in this study. Application of polyamines at FB was more effective than when applied at 2WAFB in increasing fruit weight (Table 2). Compared to Spd, Put application tended to increase fruit weight at both times of applications (Table 2). Weight of 10 pits were decreased by application of PAs. Application of Put at 2.5 and 5 mM l<sup>-1</sup> at FB in comparison to control significantly decreased 10 pits weight at harvest time [8.37 (0), 7.5 and 7.1 g respectively] (Table 2). There was a significant difference between the different PAs, their concentrations and time of application, also there was the greatest decrease in the 10 pits weight at 5 mM l<sup>-1</sup> Put at FB application (Table 2).

### 3.2. Changes in free polyamines during flesh and seed development

Free Put in mesocarp tissue was affected by application of PAs at FB and 2WAFB. Put decreased rapidly during the first two weeks AFB, followed by an increase until 8 weeks AFB and then decreased there after (Fig. 1A and B). In the mesocarp tissue Put level increased by application of Put at 5 mM l<sup>-1</sup>. application of PAs at FB and 2WAFB had the same trend in increasing level of Put in the mesocarp tissue (Fig. 1A and B). Free Put in seeds was also affected by PAs types application at FB and 2WAFB. The level of Put in seeds rapidly dropped after 2WAFB, followed by an increase until pit hardening and then decreased there after (Fig. 2a and b). Put at both concentrations significantly increased Put free level in the seeds and the lowest level was belonged to Spd at 1.25 mM l<sup>-1</sup> (Fig. 2a and b).

Application of Spd at FB and 2WAFB increased endogenous level of Spd in mesocarp tissue. Spd at both concentrations when applied at FB sharply increased Spd after 8 weeks AFB and then decreased toward the harvest time. Put at both concentrations had no significant effect on increasing Spd in mesocarp tissue (Fig. 3A and B). In seed tissue application of Put and Spd at FB and 2WAFB signif-

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