Arginine Impact on Yield and Fruit Qualitative Characteristics of Strawberry

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Summary

Strawberries are considered as one of the most important crops grown in Iran for fresh local consumption and export. Using plant growth regulators to improve yield parameters is suggested. In this research influence of arginine at different levels and application time on qualitative and quantitative characteristics of strawberry 'Paros' were considered. Strawberry plants were foliar sprayed with arginine at concentration of 0, 250 and 500 µM in two stages (30 days after planting and at the start of bloom) with a factorial experiment in a completely randomized design with four replications in greenhouse conditions. Results showed that fruits of treated plants by arginine at concentrations of 250 and 500 µM had improved qualitative and quantitative characteristics compared to control treatment. Different levels of arginine application increased yield, weight of primary and secondary fruits and number of their achenes (quantitative characteristics), total soluble solid, inducing sugar, titratable acidity, anthocyanin, phenol, and vitamin C (qualitative characteristics). The best result was obtained by the arginine application at the rate of 500 µM at 30 days after planting.

Key words

arginine, qualitative and quantitative characteristics, strawberry

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Received: December 12, 2016 | Accepted: June 29, 2017

Introduction

Strawberry *(Fragaria × ananassa* Duch.) is a delicious fruit with high health value (Chung et al., 2002) and it is grown almost all over the world including Iran. Strawberries are considered as one of the most important crops grown in Iran for fresh local consumption and export especially during the period from December to April (Eshghi and Jamali, 2009). Strawberries are good sources of natural antioxidants including carotenoids, vitamins, phenols, flavonoids, dietary glutathionine and endogenous metabolites, and they exhibit high level of antioxidant capacity against free radical species: superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen (Wang and Jiao, 2000). The benefits of these fruits with high antioxidant activity include reduction of carcinogens in humans (Bickford et al., 2000; Chung et al., 2002: Kresty et al., 2001). Anthocyanins are typically present at high levels in strawberries and are thought to contribute significantly to the total antioxidative activity of this fruit (Wang et al., 1997). Total antioxidant activity, as well as total anthocyanin content, can vary among cultivars and cultivation techniques; these may affect overall protective benefits of human health and are worth further investigation (Chung et al., 2002).

The application of amino acids for foliar use is based on its requirement by plants in general and at critical stages of growth in particular. Plants absorb amino acids through stomas and it is proportional to environment temperature (Martin-Tanguy, 1997; Vasanth et al., 2006).

Arginine is one of the essential amino acids, considered as the main precursor of polyamines (PAs), agmatine and proline as well as the cell signaling molecules glutamine and nitric oxide (NO) (Chen et al,. 2004; Liu et al., 2006), which is produced by decarboxylation of arginine via arginine decarboxylase to form putrescine (Bocherueu, 1999; Evans and Malmberg, 1989). Polyamines and their precursor arginine have been implicated as vital modulators in a variety of growth, physiological and developmental processes in higher plants (Glastone and Kaur-Sawhny, 1990). Polyamines are involved in the control of cell cycle, cell division, morphogenesis in phytochrome and plant hormone mediated process and the control of plant senescence, as well as in plant response to various stress factors (Walters, 2000). The application of arginine significantly promoted the growth and increased the fresh and dry weights, certain endogenous plant growth regulators, chlorophylls a, b and carotenoids in bean (Nassar et al., 2003) and wheat (El-Bassiouny et al., 2008). Moreover, researchers reported positive role of arginine in alleviating the inhibition that occurs as the result of exposing plants to stress (Hassanein et al., 2008; Khalil et al., 2009). In higher plants, it has also been proposed that both endogenous and exogenous arginine have roles in plant growth responses (Nasibi et al., 2011; Song et al,. 1999; Zeid, 2009; Zhang et al., 2011). Positive effects of exogenous NO (Liu et al., 2011; Neill et al., 2003), PAs (He et al., 2002) and prolin (Gioseffi et al., 2012; Patton et al., 2007) on growth and development in stress conditions have been reported. However, not any data are available on the effect of exogenous arginine as a precursor of these compounds in growth and development of plants such as strawberry.

The simulative effect of arginine as polyamines precursor on growth and yield component may act as protective agent in plants adapted to extreme environment (Miller et al., 2007). Paschalidis and Roubelakis-Angelakis (2005) reported that PAs, their precursor arginine and their biosynthetic enzymes are involved in stimulation of division, expansion and differentiation of cell and vascular development in tobacco plant. Hassanein et al. (2008) found that arginine at 2.5 mM was the most effective in improving growth and yield of wheat plant exposed to high temperature stress. Researchers concluded that foliar application of arginine on wheat exhibited significant increase in growth and all yield parameters compared to control treatment (Mostafa et al., 2009).

The objective of this research was to test the effectiveness of arginine on some qualitative and quantitative characteristics of 'Paros' strawberry *(Fragaria × ananassa* Duch.) plants.

Martial and methods

Treatments

This study was carried out at a commercial greenhouse (temperature range (15-20°C) system, planted on bed (plant and row distance 30*60cm) of Jiroft city, Iran. Induced and rooted daughter plants of 'Paros' were planted in the greenhouse. The treatments included arginine at 0, 250 and 500 μM concentrations. Plants were treated 30 days after planting (T1) and at the start of bloom (T2). Fruit characteristics such as yield, weight mean of primary and secondary fruits and number of their achenes (quantitative characteristics) total soluble solid, inducing sugar, titratable acidity, anthocyanin, phenol, dry weight, vitamin C (qualitative characteristics) were measured using following procedures.

Total soluble solids

Total soluble solids (TSS) were measured from five fruits per plant at harvest using a hand-held refractometer (American Optical Co., Keene, N.H.).

Titratable acidity and vitamin C

Titratable acidity and vitamin C measurement were determined by Basiouny and Woods (1992) method.

Assays of reducing sugars content

Reducing sugars were determined by extracting one gram fruit tissue sample with 20 ml of 50% ethanol and then incubated in the oven at 60°C for 2 h.Then one ml of the supernatant liquid was mixed with 0.5 ml of 0.1 N hydrochloric acid and boiled for 15 min. This mixture was then mixed with 0.5 ml of 0.1 N sodium hydroxide. One ml of the supernatant liquid was then taken for quantifying the reducing sugars using by Hodge-Hofreiter's method (Hodge and Hofreiter 1962).

Assays of anthocyanin content

An aliquot of extract was combined with ethanolic HCI solution (0.25 M) to give a dilution 1:10. The solution was mixed thoroughly, and the absorbance at 520 nm (A520) was read after 5 min, using the ethanolic HCI solution as blank. Total anthocyanin content was determined as cyanin (cyanidin 3-O-glucoside) equivalents (CyE) per 100 g of fresh tissue, using e = 26,900 and MW = 449.2 (Kim et al., 2003).

$$\%W/W = \frac{A}{sl} \times MW \times DF \times \frac{V}{WT} \times 100$$ (Kim et al., 2003).

Assays of phenol compound

Measurements were carried out according to a published protocol (Arnous et al., 2002), employing the Folin-Ciocalteu methodology. Gallic acid was used as the reference standard, and results were expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh tissue. An aliquot of extract was combined with ethanolic HCI solution (0.25 M) to give a dilution 1:10. The solution was mixed thoroughly, and the absorbance at 520 nm (A520) was read after 5 min, using the ethanolic HCI solution as blank.

Statistical analysis

The experimental design was a factorial experiment in a completely randomized design with single plant experimental units and four replications. Data were analyzed by analysis of variance (ANOVA) and the means were compared ($p \leq 0.05$) by Dancan's multiple rang test (DMRT). All analyses were performed by using SAS version (Ver. 9.1).

Results

Results showed, fruits of treated plants by arginine, compared to control treatment improved qualitative characteristics. So, different levels of arginine application increased yield, weight of primary and secondary fruits and number of their achenes (quantitative characteristics). Effective treatment and best spraying time were 500 µM of arginine and 30 days after planting, respectively, for qualitative characteristics improvement (Fig 1-3).

The results showed that treatments applied 30 days after planting and at first blooming significantly increased yield of strawberry (Fig 1). Earlier treatments resulted in better improvement than the late application (Fig 1). Arginine at 500 µM increased yield in 'Paros' strawberry up to 2 kg/plant when applied 30 days after planting, but only up to 1.5 kg/plant when applied at first blooming stage (Fig 1).

The results showed that treatments applied 30 days after planting and at first blooming significantly increased fruit weight

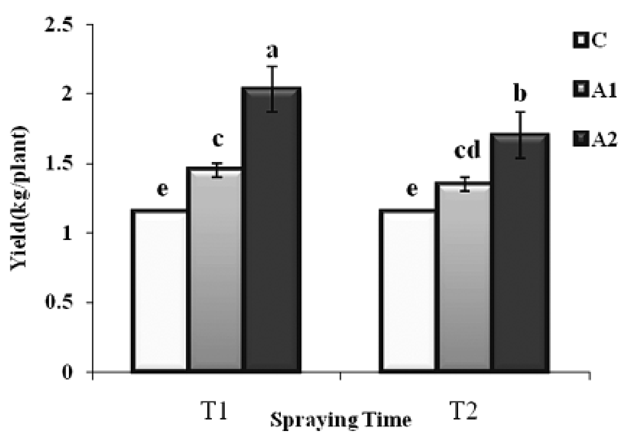

Figure 1. Effect of arginine on yield of 'Paros' strawberry. (C: control, A1: arginine 250 µM, A2: arginine 500 µM, T1: 30 days after planting, T2: start of bloom. Means followed by same letter are not significantly different at $P<0.05$ according to Duncan's multiple range test. Values are means ± standard deviation (bars) of four replications.)

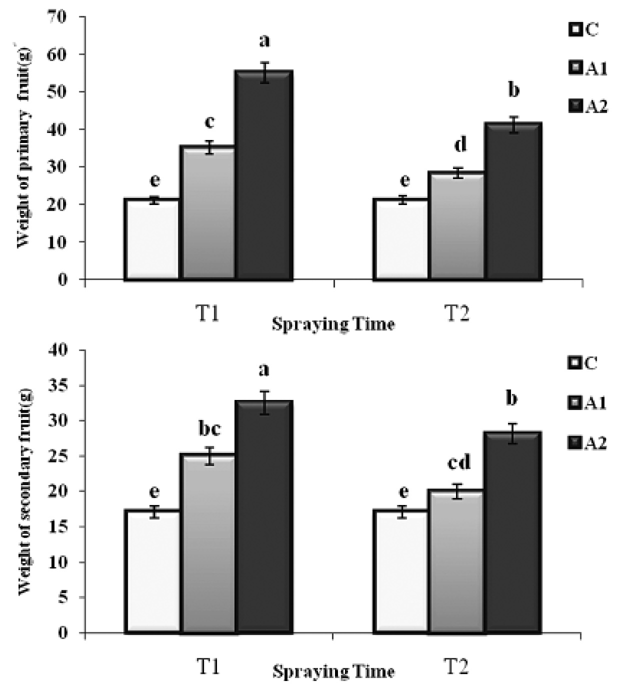

Figure 2. Effect of arginine on weight of 'Paros' strawberry fruit. (C: control, A1: arginine 250 µM, A2: arginine 500 µM, T1: 30 days after planting, T2: start of bloom. Means followed by same letter are not significantly different at $P<0.05$ according to Duncan's multiple range test. Values are means ± standard deviation (bars) of four replications.)

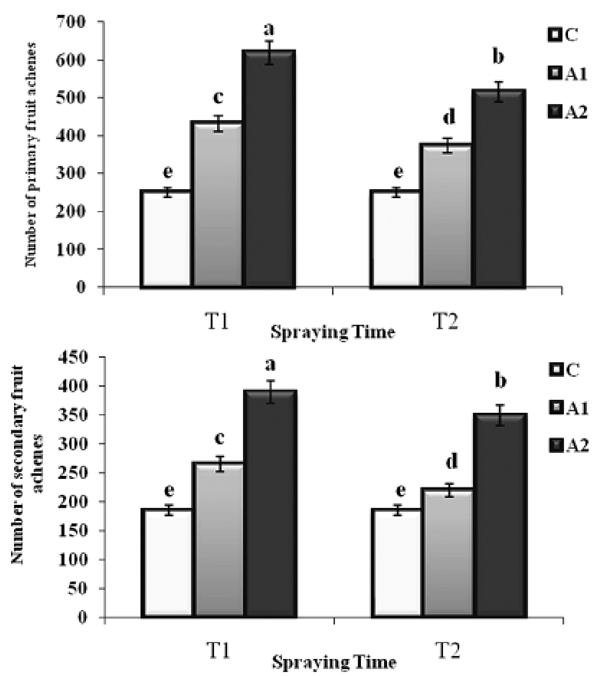

Figure 3. Effect of arginine on number of achens of 'Paros' strawberry fruit. (C: control, A1: arginine 250 µM, A2: arginine 500 µM, T1: 30 days after planting, T2: start of bloom. Means followed by same letter are not significantly different at $P<0.05$ according to Duncan's multiple range test. Values are means ± standard deviation (bars) of four replications.)

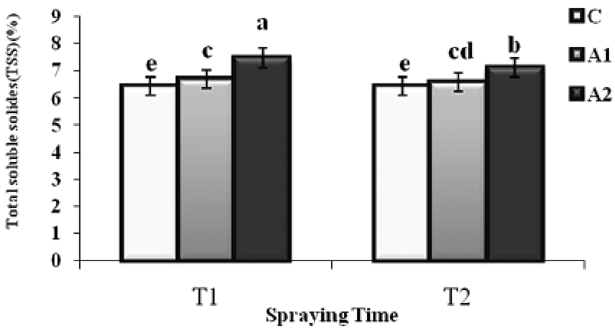

Figure 4. Effect of arginine on fruit total soluble solids (TSS) of 'Paros' strawberry. (C: control, A1: arginine 250 µM, A2: arginine 500 µM, T1: 30 days after planting, T2: start of bloom. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range test. Values are means ± standard deviation (bars) of four replications.)

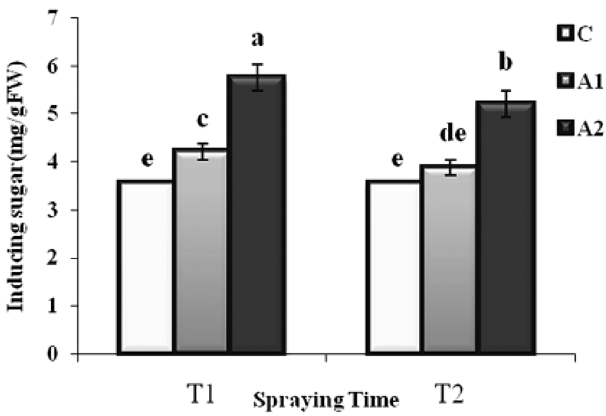

Figure 5. Effect of arginine on fruit inducing sugar of 'Paros' strawberry. (C: control, A1: arginine 250 µM, A2: arginine 500 µM, T1: 30 days after planting, T2: start of bloom. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range test. Values are means ± standard deviation (bars) of four replications.)

of strawberry (Fig 2). Earlier treatments had better effect than late application (Fig 2). Arginine at 500 µM increased weight of primary and secondary fruit of 'Paros' strawberry up to 50 and 30 g when applied 30 days after planting, but only up to 40 and 25 g when applied at first blooming stage, respectively (Fig 2).

The results showed that treatments applied 30 days after planting and at first blooming significantly increased number of achenes of strawberry fruit (Fig 3). Earlier treatments had better effect than the late application (Fig 3). Arginine at 500 µM increased number of achenes of primary and secondary fruit of 'Paros' strawberry up to 600 and 400 when applied 30 days after planting, but only up to 500 and 300 when applied at first blooming stage, respectively (Fig 3).

The measured qualitative characteristics such as total soluble solid, inducing sugar, titratable acidity, anthocyanin, phenol, dry weight, and vitamin C were improved by arginine at different levels of application and by effective treatment; and the best

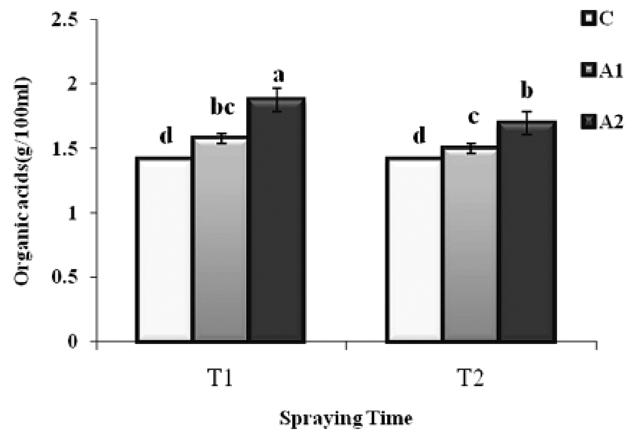

Figure 6. Effect of arginine on fruit organic acids of 'Paros' strawberry. (C: control, A1: arginine 250 µM, A2: arginine 500 µM, T1: 30 days after planting, T2: start of bloom. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range test. Values are means ± standard deviation (bars) of four replications.)

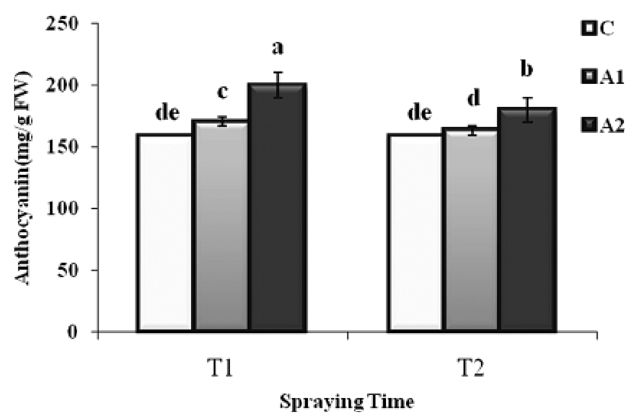

Figure 7. Effect of arginine on fruit anthocyanin of 'Paros' strawberry. (C: control, A1: arginine 250 µM, A2: arginine 500 µM, T1: 30 days after planting, T2: start of bloom. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range test. Values are means ± standard deviation (bars) of four replications.)

spraying time was arginine 500 µM 30 days after planting (Fig 4-9). The results showed that treatments applied 30 days after planting and at first blooming significantly increased qualitative characteristics of strawberry fruit (Fig 4-9). Earlier treatments had more effect than the late application (Fig 4-9). Arginine at 500 µM increased qualitative characteristics of strawberry fruit (Fig 4-9).

Discussion

The simulative effect of arginine as polyamine precursor on growth and yield components may act as protective agent in plants adapted to extreme environment (Nayyar et al., 2005; Khalil et al,. 2009). Paschalidis and Roubelakis-Angelakis (2005)

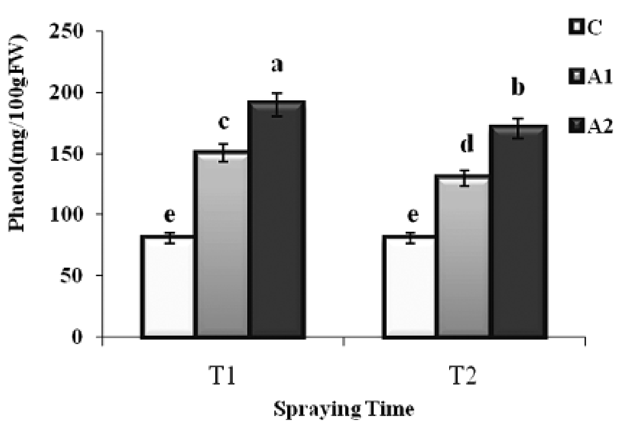

Figure 8. Effect of arginine on fruit phenol content of 'Paros' strawberry. (C: control, A1: arginine 250 µM, A2: arginine 500 µM, T1: 30 days after planting, T2: start of bloom. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range test. Values are means ± standard deviation (bars) of four replications.)

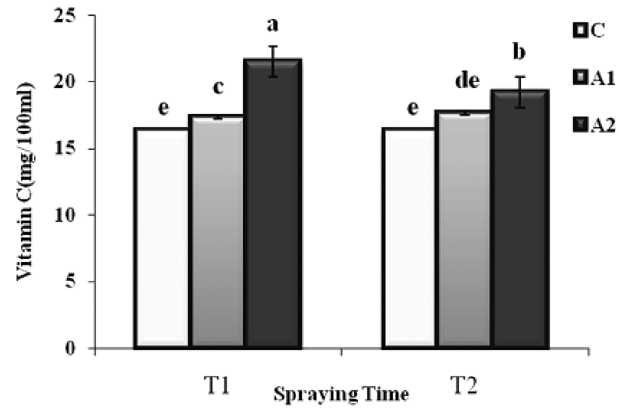

Figure 9. Effect of arginine on fruit vitamin C of 'Paros' strawberry. (C: control, A1: arginine 250 µM, A2: arginine 500 µM, T1: 30 days after planting, T2: start of bloom. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range test. Values are means ± standard deviation (bars) of four replications.)

reported that PAs, their precursor arginine and their biosynthetic enzymes are involved in stimulation of division, expansion and differentiation of cell and vascular development in tobacco plant. Hassanein et al. (2008) found that arginine was the most effective in improving growth and yield of wheat plant exposed to high temperature stress. Moreover, researchers concluded that foliar application of arginine on wheat exhibited significant increments in the growth and all yield parameters in comparison to control treatment (Mostafa et al., 2009).

Foliar applied arginine due to syntheses polyamines and polyamines act as growth regulator is able to modulate the plant metabolism and the production of metabolites involved in stress tolerance (Francisco et al., 2006; Tassoni et al., 2002). Aarginine is one of the most functionally diverse amino acids in living cells. In addition to serving as a constituent of proteins, arginine is a precursor for biosynthesis of polyamines and proline that act as growth regulators; so, it improves yield and development in plants (Chen et al., 2004; Liu et al., 2006). The role of amino acids in growth and differentiation is known to a considerable extent (Yagi and Al-Abdulkareem, 2006).

Polyamines have been suggested to be associated with cell division. Therefore, they can be utilized to regulate fruit development. Exogenous application of PAs has also been reported to promote reproductive development under normal growth conditions and also to provide protection to reproductive structures against abiotic stress (Alburquerque et al., 2006; Bibi et al., 2010; Malik and Singh, 2006; Nayyar, 2005; Ndayiragije and Lutts, 2007; Zheng et al., 2009). The general positive effects observed in this study on yield and fruit characteristics of strawberry may be the result of applying arginine that contributed to synthesis of polyamines, which affected floral development and consequently fruit set and fruit yield. In addition, accumulating evidences have showed that endogenous polyamine is important for pollen germination and pollen tube growth (Wolukau et al., 2004). Furthermore, putrescine enhanced pollen tube ovule penetration and delayed ovule senescence without affecting flower ethylene production (Crisosto et al. 1988; Crisosto et al. 1992). Also, the effect of polyamines was ascribed to increase viability of ovule and prolonged pollination period (Kitashiba et al., 2005). Bagni et al. (1981) informed that biosynthesis of polyamines from arginine takes place before pollen tube emergence. On the other hand, the influence of polyamines in increasing fruit set was observed in apples and pears (Costa and Bagni, 1983; Costa et al., 1986; Crisosto et al., 1988; Crisosto et al., 1992; Franco-Mora et al., 2005). The same results were reported by Biasi et al. (1991) in litchi and by Costa and Bagni (1983) in olives. Our results cleared that application of arginine had a positive effect on fruit quantitative and chemical characteristics. This effect was in agreement with results obtained by Bregoli et al. (2002) in peach and Torrigiani et al. (2004) in nectarine. In the initial stage of fruit development, active cell division occurs that possibly needs sufficient polyamines (Bouchereau et al., 1999). At the later stage of fruit development, cell division gives way to cell enlargement, in which polyamine synthesis is reduced. On the other hand, decrease in polyamines at the late stage of fruit development has been regarded as a signal for fruit ripening. Moreover, since polyamines (especially Spd and Spm) share the same precursor (arginine), their function for improving apricot fruit was demonstrated (Paksasorn et al. 1995); also increasing physical and chemical characteristics in peaches (Bregoli et al., 2002), nectarine (Torrigiani et al., 2004) and pear (Franco-Mora et al., 2005). Bagni et al. (1981) reported that apple fruits treated with spermine and putrescine reached higher sugar content, while plum fruits treated with putrescine by Serrano et al. (2003) showed lower soluble solutions and titratable acids.

Arginine induces synthesis of flower and fruit related hormones (stimulates synthesis of phytohormones) (Tarenghi and Martin-Tanguy, 1995; Zeid, 2009), promotes uptake of macro and micro nutrients, chelating effect and rooting (Jones et al., 2002; Jones et al., 2005; Orlikowska 1992; Sarropoulou et al., 2014), promotes enzymatic activity by acting as natural stimulator of photosynthesis (argenine get readily absorbed through leaves and aid protein synthesis). It also leads to better synthesis of chlorophyll and helps to improve yield (Yagi and Abdulkareem, 2006), helps action of the stomas (El-Bassiouny et al., 2008; He et al., 2002), stimulates proteins and enzymes synthesis (Bibi et al. 2010), induces flowering and fruit set related hormones (Vasanth et al., 2006), increases stress resistance (Liu et al., 2006; Liu et al., 2011; Nasibi et al., 2011), improves pollination and fruit formation (Serrano et al., 2003; Singh and Singh, 1995; Mostafa et al., 2009). So, antioxidative properties of arginine-treated strawberry plants were improved by arginine. In this respect, both concentration of arginine used in the present investigation increased these properties in terms of stimulating the accumulation of some antioxidants as ascorbic acid, phenol compounds, titratable acidity, and anthocyanin (Fig 4-9). Therefore, arginine probably increases yield and improves qualitative characteristics of crop such as strawberry. This result is in agreement with the results obtained by El-Bassiouny et al. (2008), Hassanein et al. (2008), Kramer et al. (1991), Khalil et al. (2009), Liu et al. (2006), Mostafa et al. (2009), Nasibi et al. (2011), Sarropoulou et al. (2014), Vasanth et al. (2006), Yagi and A-Abdulkareem (2006) and Zeid (2009).

Therefore, the result of the present work may help to improve the yield and the quality of strawberry by foliar application of arginine, which may consequently increase endogenous antioxidant content in strawberry.

Conclusion

From the abovementioned results, it could be concluded that strawberry plants ‘Paros’ grown under greenhouse conditions enhanced production and improved fruit quality by foliar spraying of arginine. Treating strawberry ‘Paros’ two times, 30 days after planting and at first blooming, with arginine at 250 and 500 μM resulted in great promotion in yield quantitatively. and qualitatively and effective treatment and out of testes concentrations and times, Treatment of arginine at concentration of 500 μM 30 days after planting was the best for improvement of qualitative and quantitative fruit characteristics.

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