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Original Research Article

Influence of Foliar Application of 5-Sulfosalicylic Acid, Malic Acid, Putrescine and Potassium Nitrate on Vegetative Growth and Reproductive Characteristics of Strawberry cv. 'Selva'

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ABSTRACT

In order to study the effect of foliar application of 5-sulfosalicylic acid, malic acid, putrescine and potassium nitrate on vegetative growth and reproductive characteristics of strawberry cv. Selva as a factorial experiment in a completely randomized design with 4 replications which the factors included 5-sulfosalicylic acid at three levels (0.5, 1.5 and 2.5 mM), malic acid at three levels (1.5, 2.5 and 5 mM), putrescine at two levels (1 and 2 mM) and potassium nitrate at two levels (1 and 2 %). As result has shown 5-sulfosalicylic acid at 2.5 mM and potassium nitrate at 2% concentration increased vegetative growth (dry weight, leaf area, length of roots), number of flowers, and weight of primary and secondary. 5-sulfosalicylic acid at 2.5 mM and malic acid at 5 mM of increased total phenolics, flavonoids, and non-flavonoids of strawberry but other treatments did not have a significant impact on this characteristic. The highest percentage of total soluble solids, titratable acidity and ascorbic acid was attained in fruits treated with 2.5 mM of foliar 5-sulfosalicylic acid and the lowest, was achieved in control

Key Words: Foliar application, strawberry, vegetative growth, reproductive characteristics

INTRODUCTION

During the last decade, strawberry production has spread throughout almost all parts of Iran and its cultivation is expanding in open field and greenhouse. Strawberry is one of the most delicious fruit of the world, which is a rich source of vitamin C, iron, potassium and fiber (Wang and Galletta 2002).

Micronutrient deficiency can greatly disturb plant yield and quality. The foliar application of macro and micro-nutrients have very important role in improving fruit set, productivity and quality of fruits. The foliar application has beneficial role in recovery of nutritional and physiological disorders in fruits (Shukla 2011). Foliar nutrition may play an important role in strawberry and application at proper time improved quality and quantity strawberry. Previous studies are indicative of a positive role of foliar nutrition in improved quality and quantity strawberry (Nasiri *et al.* 2010; Czuba 1993; Abdollahi *et al.* 2012).

Salicylic acid is the most readily accessible plant growth regulators which are effective in other forms of acetyl salicylic acid and methyl salicylate in plant as well (Raskin *et al.* 1992). Salicylic acid could induce the alternative oxidase enzyme activity in mitochondria which is involved in stress alleviation mechanism and enhancing or reduction in specific secondary metabolites of plants is reported (Raskin *et al.*1992; Kiddle *et al.* 1994; Vanlerberghe and McIntosh 1997; Kang *et al.* 2004; Prithiviraj *et al.* 2005; D'onofrio *et al.* 2009). Salicylic acid and its derivatives are widely in use to enhance fruits postharvest life by controlling their firmness (Kazemi *et al.* 2011a, b, c, d, e, f). Salicylic acid has been documented to enhance flesh firmness of harvested peaches during storage and banana fruits during ripening (Srivastava and Dwivedi 2000; Wang *et al*. 2007).

Potassium is known, not only to play an important role in fruits yield and quality but also in water use efficiency, it is easily absorbed and distributed through leaf tissues and plays an important role in growth of fruits (Arquero *et al.* 2006). Potassium is particularly well adapted to this form of fertilization because soon after foliar spraying takes place, it is rapidly translocated from the leaves (Mengel 2002). Foliar applications of potassium have a positive effect on the quality of table olives and improve the leaf potassium content (Dikmelik *et al.* 1999).

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Malic acid is the organic acid which plants could metabolize it by reaction of malic enzyme in mitochondria which could be considered as ability limited to plant. Malate is a common reserve anion acting as a counter ion for K and Ca in plant vacuoles, especially in nitrate dependent plants (Day 1977).

Polyamines (PAs) are known as a group of natural compounds with aliphatic nitrogen structure that are ubiquitous in plants. The major polyamines are found in every plant cell, such as spermidine (Spd), spermine (Spm) and putresine (Put) (Galston and Sawhney 1990). Polyamines are biologically active compounds involved in various physiological processes. They are cationic molecules, positively charged under intracellular pH, which are essential for plant growth and differentiation, related to aging and senescence, and usually involved in plant responses to stress (Friedman *et al.* 1989). They regulate growth, probably by binding to negatively charged macromolecules (Messiaen *et al.* 1997).

Salicylic acid, malic acid, potassium and polyamines treatments have the potential for commercial control of quality properties and increase shelf life of harvested fruit. Therefore, the aim of this research is to determine the effects of pre-harvest application of salicylic acid, malic acid, potassium and polyamines in strawberry cultivar ‘Selva’, specially the change in quality properties.

MATERIALS AND METHODS

Plant growth conditions and treatments

The experiment was conducted during 2011 and 2012 on strawberry plants at the experimental greenhouse, Ilam, Iran (Elevation 1339 m, Latitude East 33.638, Longitude North 46.431). Strawberry plants (*Fragaria* × *ananassa* Duch. cv. Selva) were grown under natural light conditions. The temperature conditions were 24 ± 5°C and 15 ± 4°C, during days and nights, respectively; with relative humidity of 70%. Induced and rooted daughter plants of Selva were potted in 3 plastic pots filled with 2:1 sandy loam soil and compost, after 2 weeks of establishment, in the beginning of November, the treatments, including: 5-SSA (5-sulfosalicylic acid) (0.5, 1.5 and 2.5 mM), malic acid (1.5, 2.5 and 5 mM) putrescine (1 and 2 mM) and potassium nitrate (1 and 2%) and distilled water as control. During the experimental period plants were fertilized with Hogland solution. Sprayed materials were used as follows: after plant establishment, at the beginning of flowering, and 15 days after the second time.

Measurements

In the end of experiment, plants were carefully taken out of their pots, roots were washed with distilled water, and the whole plants were oven dried for 72 hours at 70° c then. The following quality parameters of harvested fruits were determined: dry weight, number of runners, leaf area, number of flowers, length of the roots, length of flowering period, weight of primary and secondary fruits and number of their achenes, TP, MDA, TF, TNF, pH, TSS, TA, and vitamin C of ‘Selva’ strawberry. Weighed and their dry weights were expressed as gram. Length of roots was measure by using a ruler and was expressed as cm. Number of runners and flowers were counted throughout the experimental period. Leaf area was measured using a ∆T- leaf area meter and expressed as cm^2. Length of flowering period was calculated and expressed as days between the first appearing flowers till end of experiment. Primary and secondary fruits were weighed in order to measure their weight as gram and number of their achenes was counted afterwards.

Lipid peroxidation (malondialdehyde=MDA content)

Lipid peroxidation was estimated by determining the malondialdehyde (MDA) contents in the leaves according to method of Rajinder *et al.* (1981). A hundred milligram of leaf samples was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10 000 × *g* for 5 min at 4 °C. Aliquot of 0.3 ml supernatant was mixed with 1.2 ml of 0.5% thiobarbituric acid (TBA) prepared in TCA 20%, and incubated at 95 °C for 30 min. After stopping the reaction in an ice bath for 5 min, samples were centrifuged at 10 000 × *g* for 10 min at 25°C. The supernatant absorbance at 532 nm was then measured using a

Beckman UV-DU 520 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA). After subtracting the non-specific absorbance at 600 nm, MDA concentration was determined using the extinction coefficient 155 mM^{-1} cm^{-1}.

Determination of total phenolics, flavonoids and nonflavonoids

Total phenolics (TP), flavonoids (TF), and non-flavonoids (TNF) were determined using the Folin-Ciocalteu colorimetric method described by Ough and Amerine (1988). Fruit phenolics were extracted from 10 g of fresh samples using 40 mL of 80 % (by volume) aqueous ethanol. The mixture was extracted (in water bath at 80 °C), kept for 20 min in inert atmosphere, and filtered through a whatman filter paper using a Büchner funnel. Extraction of the residue was repeated under the same conditions. The filtrates were combined and diluted to 100 mL in volumetric flask with 80 % aqueous ethanol, and the obtained extract was used for determination of TP, TF and TNF. The formaldehyde precipitation was used to determine flavonoids in fruit samples (Kramling and Singleton 1969). The content of TP and TNF was measured as follows: 0.5 mL of diluted extract or standard solutions of gallic acid (20–500 mg/L) was added to a 50-mL volumetric flask containing 30 mL of ddH_2O, then 2.5 mL of Folin-Ciocalteu reagent were added to the mixture and shaken. After 5 min, 7.5 mL of 7 % Na_2CO_3 solution were added with mixing and the solution was immediately diluted to 50 mL with ddH_2O. After incubation at room temperature for 2 h the absorbance of the solution was measured at 760 nm. The flavonoid mass fraction was calculated as the difference between total phenolic and non-flavonoid mass fraction. TP, TF and TNF were expressed as mg of gallic acid equivalents (GAE) per kg of fresh mass of edible part of fruits.

Ascorbic acid content (Vitamin C)

Ascorbic Acid (AA) content of strawberry was determined by the 2,6-dichlorophenolindophenol method (Tefera *et al.* 2007). An aliquot of 10 mL strawberry fruit juice extract was diluted to 50 mL with 3% metaphosphoric acid in a 50 mL volumetric flask. The aliquot was filtered and titrated with the standard dye to a pink endpoint (persisting for 15 sec). pH value was measured with a pH meter.

Total soluble solids and titratable acidity

To characterize the maturity and quality of the fruit total soluble solids (TSS), titratable acidity (TA) were determined. A sample of 15 strawberry was randomly harvested selected for quality measurements from each replicate of each treatment. TSS, expressed as Brix, was measured with a portable refract meter (Moraes Barros *et al.* 2012). Titratable acid (TA) was determined by diluting each 5 ml aliquot of strawberry juice to 100 ml with distilled water, then titrating to pH 8.2 using 0.1 N NaOH. Acidity was expressed as mg citric acid/100 ml juice.

Statistical analysis

The experiment was conducted as a factorial experiment in a completely randomized design with 4 replications, each consisting of 3 pots with each pot containing one plant. Data were analyzed by SPSS 16 software and and comparing averages was done by Duncan's test and a probability value of %5.

RESULTS

Table 1, indicates the effect of 5-SSA, malic acid, putrescine and potassium nitrate on dry weight, number of runners, leaf area, number of flowers and length of the roots. 5-SSA at 2.5 mM of increased dry weight of strawberry but other treatments did not have a significant impact on this characteristic. In general, application of 5-SSA produced significantly higher leaf area and dry weight of plant compared to control and other treatments. Number of runners decreased when strawberry treated with putrescine and on the other hand strawberry treated with 5 mM of malic acid produced the maximum number of runners. 5-SSA at 2.5 mM and potassium nitrate at 2% concentration increased the leaf area in comparison to control and other treatments. Length of roots was longer than control samples in all treated plants, and maximum of this parameter was obtained when 2.5 mM concentration of 5-SSA was applied.

Table 1. Effect of pre-harvest application of 5-SSA, malic acid, putrescine and potassium nitrate on dry weight, number of runners, leaf area, number of flowers and length of the roots.

Treatment		Plant dry weight (g)	Number of runners	Leaf area (cm^2)	Length of roots (cm)	Number of flowers
Control	0	9.7 j	2.41fg	20 fg	13.77 g	7 bc
5-SSA (mM)	0.5	11.04 i	2.9de	28.9 de	15 f	6.2 c
	1.5	12.9 ef	3.55 c	35.7 c	28.7 b	6.7 bc
	2.5	16.01 a	4.3 a	45.21 a	30.14a	13.4 a
Malic Acid (mM)	1.5	13.07 e	3 f	22.5 f	18.7 e	11 b
	2.5	13.41 d	3.1 d	30.4 d	22 d	11.2 b
	5	15.7 b	5.3 a	40 b	29 b	13 a
Putrescine (mg -1)	50	11.8 h	2.41 fg	22 fg	15.14 f	11 b
	150	11.64 g	2.7 g	19.2 g	16.21 f	6 c
Potassium Nitrate (%)	1	12.63 f	3.14 ef	25.4 ef	17.9 e	7 bc
	2	15c	3.4cd	45.2 a	29.6 a	13.2 a

Means followed by same letter are not significantly different at 5% probability using Duncan's test.

Table 2, indicates the effect of 5-SSA, malic acid, putrescine and potassium nitrate on length of flowering period, weight of primary and secondary fruits and number of their achenes. 5-SSA at 2.5 mM and potassium nitrate at 2% concentration increased number of flowers, weight of primary fruit and weight of secondary fruit but other treatments did not cause any significant respond. Length of flowering period reached its maximum amount when 2.5 mM of 5-SSA was applied. The maximum number of achenes was obtained when potassium nitrate was used as treatment. 5-SSA treatments significantly increased number of achenes of primary and secondary fruits but other treatments did not have a significant effect on this characteristic. The highest percentage of TSS and TA and ascorbic acid content was attained in fruits treated with 2.5 mM of foliar 5-SSA concentration and the lowest was achieved in control (Table 3). Also highest of pH content was attained in fruits treated with 2.5 mM of foliar 5-SSA concentration. Table 4, indicates the effect of 5-SSA, malic acid, putrescine and potassium nitrate on total phenolics (TP), flavonoids (TF), and non-flavonoids (TNF) and lipid peroxidation (MDA). 5-SSA at 2.5 mM and malic acid at 5 mM of increased total phenolics (TP), flavonoids (TF), and non-flavonoids (TNF) of strawberry but other treatments did not have a significant impact on this characteristic. In general, application of 5-SSA produced significantly decreased lipid peroxidation (MDA) of fruits compared to control.

Table 2. Effect of pre-harvest application of 5-SSA, malic acid, putrescine and potassium nitrate on length of flowering period, weight of primary and secondary fruits and number of their achenes.

Treatment		**Length of flowering period (days)**	**Weight of primary fruit (g)**	**Weight of secondary fruit (g)**	**Number of achenes of primary fruit**	**Number of achenes of secondary fruit**
Control	0	15.04 c	8.33 c	6.14 c	123.14 c	105 c
5-SSA (mM)	0.5	15 c	11 b	8 b	149.8 bc	120 bc
	1.5	22.79 b	11.5 b	8.96 b	180 b	161 b
	2.5	33.11 a	17.33 a	14 a	225 a	208.69 a
Malic Acid (mM)	1.5	14.63 c	10.03 b	8.9 b	120 c	102.48 c
	2.5	20.7 b	11 b	8 b	186.4 b	165 b
	5	26.8 ab	17 a	13.8 a	223.1 a	205.5 a
Putrescine (mg -1)	50	21 b	10.11 b	8.14 b	150 bc	132.6 bc
	150	28 ab	13.7 ab	11ab	187 b	166.7 b
Potassium Nitrate (%)	1	21.36 b	14.11 ab	11ab	154 bc	131.11 bc
	2	32.5 a	16.8 a	13.66 a	220 a	200.14 a

Means followed by same letter are not significantly different at 5% probability using Duncan's test.

Table 3. Effect of pre-harvest application of 5-SSA, malic acid, putrescine and potassium nitrate on TP, MDA, TF and TNF of 'Selva' strawberry.

Treatment		**TP w (gallic acid)/ (mg/kg)**	**MDA (nmol gfw^{-1})**	**TF w (gallic acid)/ (mg/kg)**	**TNF w (gallic acid)/ (mg/kg)**
Control	0	81.642 e	27.28 a	30.57 g	36.11 h
5-SSA (mM)	0.5	85.37 d	23.87 b	33.13 e	48.89 e
	1.5	90.28 c	19.60 de	37.39 c	54.87 d
	2.5	125.77 a	16.25 g	45.29 a	75.15 a
Malic Acid (mM)	1.5	86.55 d	20.36 d	31.52 f	49.80 e
	2.5	91.85 c	20.07 d	36.15 d	55.10 d
	5	110.59 b	18.40 f	44.89 a	74.12 a
Putrescine (mg -1)	50	76.43 f	22.80 c	21.06 i	43.162 f
	150	70.14 g	22.35 c	21.94 h	40.27 g
Potassium Nitrate (%)	1	75.01 f	20.147 d	31.37 fg	57.35 c
	2	81.91 e	19.125 ef	40.46 b	64.74 b

Means followed by same letter are not significantly different at 5% probability using Duncan's test.

Table 4. Effect of pre-harvest application of 5-SSA, malic acid, putrescine and potassium nitrate on pH, TSS, TA, and vitamin C of 'Selva' strawberry.

Treatment		**pH**	**TSS**	**TA g (citric cid)/ (g/L)**	**Vitamin C mg/100 g**
Control	0	3.40 abc	5.69 fg	4.07 g	23.29 j
5-SSA (mM)	0.5	3.39 bc	6.64 de	5.46 f	30.92 i
	1.5	3.36 bcd	7.55 c	8.53 c	51.55 c
	2.5	3.49 a	9.54 a	9.13 a	64.50 a
Malic Acid (mM)	1.5	3.315 cd	5.93 f	6.56 e	45.20 e
	2.5	3.40 abc	6.95 d	7.21 d	47.58 d
	5	3.43 ab	8.26 b	8.61 b	55.21 b
Putrescine (mg -1)	50	3.00 e	5.75 fg	5.05 f	33.51 h
	150	3.05 e	5.28 g	5.29 f	37.96 g
Potassium Nitrate (%)	1	3.29 d	6.25 ef	6.71 e	42.47 f
	2	3.49 a	9.51a	9.1 a	63.17 a

Means followed by same letter are not significantly different at 5% probability using Duncan's test.

DISCUSSION

As it has been indicated in Table 1, 5-SSA and potassium nitrate had increased effects on leaf area, length of roots and number of flowers. This result was in agreement with Duo and Danfeng (2003) who demonstrated that adding potassium, increased leaf surface, vegetative growth, net photosynthetic rate and chlorophyll content of plants. Generally the essential element potassium has a great regulatory role within plant cells and organs such as activating more than 50 enzymes, osmosis regulation and photosynthesis and loading and unloading of sugars in phloem (Mengel 2007). The role of potassium in ionic balance is reflected in nitrate metabolism. Spray with potassium result an increase in leaf potassium content which was accompanied by increasing rates of photosynthesis, photorespiration and RuBP carboxylase activity. In this respect, Pettigrew (2008) reported that potassium deficiency can lead to a reduction in both number of leaves and area of individual leaves. Coupling this reduced amount of photosynthetic source material with a reduction in the photosynthetic rate per unit leaf area, the result is an overall reduction in the amount of photosynthetic assimilates available for growth. These results are in agreement with those reported, Ahmed *et al.* (2006), Zakaria *et al.* (2011), Dewdar and Rady (2013). Potassium had not significant change in number of runners and dry weight, whereas 5-SSA caused an increase in these parameters. Results indicated a positive influence of 5-SSA on leaf area which was in agreement with Amin *et al*. (2007) on onion and Yildirim *et al.* (2008) on cucumber. It is suggested that because SA has anti-senescence influence on plant organs, vegetative growth may be prolonged following its application consequently leading to higher leaf area. Martin-Mex *et al.* (2005) also reported that SA treatment was able to increase root fresh weight ofAfrican violet. SA caused an augmentation of these three parameters which was according to Shetti *et al.* (1992) and Yakito (2001) findings. SA has been reported to close stomata which results in suppressed respiration rate and minimized weight loss of fruits (Zheng and Zhang 2004).

As it has been indicated in table 2, 5-SSA and potassium nitrate had increasing effects on length of flowering period, weight of primary and secondary fruit, number of achenes of primary and secondary fruit. Length of flowering period, weight of primary and secondary fruit, number of achenes of primary and secondary fruit increased when plants were treated with potassium nitrate, Khayat *et al.* (2010) reported similar results for 'Selva' strawberries, and also our findings were in accordance with previous works (Rahemi and Asghari 2004, eshghi *et al.* 2012). Similar results were obtained by Pettigrew *et al.* (2008) and Kumar *et al.* (2011). They found that the treatments of potassium fertilizer applied in two forms (soil addition plus foliar spray) in the appropriate time lead to an increase in boll number and boll weight, and consequently increaseing in cotton yield. Kaya (2002) reported by increasing the concentration of K_2SO_4 (from 5 to 10 mM), fruit number and yield were increased. Our findings showed that length of flowering period and weight of fruit were increased by 5-SSA treatment. Similar results were obtained by Jamali *et al.* (2011), and Yakito (2001). They found that the treatments of SA applied in the appropriate time lead to an increase in length of flowering period and weight of fruit.

According to the results (Table 3 and 4), potassium did not affect the TP, MDA, TF, TNF, but pH, TSS, TA and vitamin C of fruits were increased by potassium treatment. The pH of the juice was significantly affected by potassium applications, increased potassium levels resulted in higher titratable acidity content, total soluble solids, vitamin C, average fruit weight and pH of fruit juice (Filiz and Sahriye 2010; Majid *et al.* 2010). It is well known that potassium plays a key role in carbohydrate metabolism and photosynthesis (Marschner 1995) and, as a consequence, an optimum potassium supply determines better sugar content into sink organs. 5-SSA did affect the TP, MDA, TF, TNF, pH, TSS, TA and vitamin C of fruits. Vitamin C, pH, TSS and titratable acidity (TA) of fruits treated with higher 5-SSA concentrations was higher than those of control fruits. It has been suggested that TA decreases in fruits in result of breakup of acids to sugars during respiration (Ball 1997). Han and Li (1997) have also reported that apple fruits treated with SA had increased TA content at the end of storage. Our results showed that SA had significant effect on maintaining higher content of vitamin C in peach fruits. Kazemi *et al*. (2011 a, b, c, d, e, f) have also reported that fruits treated with SA were observed with maximum vitamin C content. The application of 2 mM SA effectively increased antioxidant compounds, ascorbic acid content and total suspended solids (TSS) and prevented fungal infection of strawberries(Amborabe *et al.* 2012). Ghasemnezhad *et al.* (2010) reported that the decrease of total phenolic levels might be due to breakdown of cell structure in order to senescence phenomena during the storage period. The effect of salicylic acid treatments on maintain of total phenolics content plausibly may be attributed to delay in senescence process. Our results are in agreement with those of Kazemi *et al.* (2011 a, b, c, d, e, f), who reported treatment with salicylic acid significantly reduced the membrane permeability and MDA content. In conclusions the present study revealed that sequential pre-harvest foliar application of 5-SSA and potassium is quite useful in ‘Selva’ strawberry for increased number of flowers, weight of primary fruit and weight of secondary fruit, total phenolics (TP), flavonoids (TF), and non-flavonoids (TNF) and total soluble solids and titratable acidity and ascorbic acid content, which helps in getting higher marketable fruit yield with better firmness and other quality parameters.

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