INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 12, p. 204-211, 2014

RESEARCH PAPER OPEN ACCESS

Change in chlorophylls composition and some morphological attributes of strawberry (*Fragaria* × *ananassa* Duch cv. Camarosa) in response to salicylic acid spray

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Key words: Leaf fresh weight, strawberry, thrice spray, total chlorophyll.

http://dx.doi.org/10.12692/ijb/5.12.204-211 Article published on December 15, 2014

Abstract

In order to study the effect of salicylic (SA) acid on the strawberry (*Fragaria* × *ananassa* Duch cv. Camarosa) plants, a two-factorial field experiment was carried out according to completely randomized design with three replications in 2011 and 2012. The factors included different concentrations of SA (0, 0.5 and 1 mM) and number of SA application (1, 2 and 3 times). Chlorophylls content and fresh and dry weights of leaf and root were performed at the harvest time. The results showed that chlorophyll a, chlorophyll b and total chlorophyll content significantly increased with increasing the SA concentration and the number of SA application as the highest amount was found in 1% SA and thrice sprays. The numbers of SA application significantly enhanced leaf fresh and dry weights, whereas different SA concentration had no significantly effect. Moreover, root fresh and dry weights had not significantly affected by SA sprays. Overall, the results suggested that thrice spays of SA in 1 mM concentration, which showed the better growth characters, could be more appropriate to use in strawberry spraying programs.

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Introduction

Strawberry (Fragaria × ananassa Duch.) is a non-climacteric fruit characterized by unique and highly desirable taste and flavor and is an important fruit produced in commercial scale in Iran (Babalar *et al.,* 2007). Strawberries are a relevant source of bioactive compounds due to high levels of vitamin C, vitamin E, β-carotene and phenolic com-pounds such as anthocyanins, substances related to health benefits (Van De Velde *et al.,* 2013). Strawberries possess a high level of antioxidant activity and therefore have a consequent beneficial effect on the maintenance of consumer health (Van De Velde *et al.,* 2013).

Quality of strawberry fruits depends mainly on their appearance (colour and biometrical characteristics), fruit size, firmness, and chemical composition (Gunness *et al.,* 2009). Values of these attributes determine crop value and fruit acceptance by consumer. Colour is probably the most important appearance attribute in strawberry fruit (Silva *et al.,* 2007).

All quality attributes of strawberries are highly influenced by ripening. As non-climacteric fruit, strawberries must be harvested at full ripening stage since they do not develop quality attributes suitable for fresh consumption following detachment (Nunes *et al.,* 2006). However, ripening dynamics depends on cultivar type and cultivation mode, among other factors (Voca *et al.,* 2009).

Attempts have been made to improve its quality by adopting cultural practices such as foliar application of natural compounds. Salicylic acid (SA), which belongs to a group of phenolic compounds, is widely distributed in plants (Ghasemzadeh *et al.,* 2012). SA acts as a potential non-enzymatic antioxidant, as well as a plant growth regulator, and plays an important role in regulating a number of plant physiological processes, including heat production or thermogenesis, ion uptake and transport, disease resistance, seed germination, sex polarization, crop yield and glycolysis (Klessig and Malamy, 1994; Zhang *et al.,* 2003).

The known effects of SA on stomatal function, chlorophyll content, transpiration rate and respiratory pathways indicate that SA and related phenolic compounds may be involved in regulation of some photosynthetic reactions (Ghasemzadeh and Jaafar, 2013). Moreover, SA has been shown to interfere with the biosynthesis and action of ethylene, abscisic acid and cytokinins in plants (Manoj *et al.,* 2000).

As evidenced by recent research reports, SA can enhance physical properties of fruits such as size (Marzouk and Kassem, 2011), weight (Elwan and El-Hamahmy, 2009) and firmness (Shafiee *et al.,* 2010). In addition, SA was found to affect maturity of both climacteric and non-climacteric fruits like strawberry (Karlidag *et al.,* 2009) and tomato (Mady, 2009). Additionally, SA positively effect on reducing fruit respiration, ethylene biosynthesis (Srivastava and Dwivedi, 2000) weight loss, decay and softening rate (Babalar *et al.,* 2007; Shafiee *et al.,* 2010) and reduce lipid peroxidation of navel orange (Huang *et al.,* 2008).

Many studies reported the possible ameliorative effects of exogenously applied of SA on plant growth and development processes. Furthermore, there is little or no reliable information on the physiological and morphological effects of SA in strawberry plant at the harvest time. Therefore, the objective of this study was to evaluate the effects of SA on the changes of chlorophylls composition and some morphological attributes of strawberry (*Fragaria × ananassa* Duch cv. Camarosa) plant.

Materials and methods

Plant material and treatments

A field experiment was conducted during 2011 and 2012 on strawberry (*Fragaria × ananassa* Duch. cv. Camarosa) plants in Behshahr, Mazandaran province, Iran. Seedlings were planted in growing media containing peat and perlite (50:50 v/v). Strawberry plants were grown under natural light conditions and were managed according to standard cultural practices. The plants were regularly irrigated during the season, water was supplied based on field capacity (FC).

Different SA concentrations (0, 0.5 and 1.0 mmol L^{-1}) were prepared by dissolving powdered SA in water and then spray on plants in different combinations of SA concentration and number of application (one, two and three times).

Fresh and dry weights of leaf and root

Plants were harvested at commercial ripeness (>75% of fruit surface showed red colour) and transferred to laboratory. Immediately after harvest, leaves and roots were separated and their fresh weights (FWs) were measured by digital scale. The shoots and roots were put in the oven at 70 °C. After 48 h their dry weights (DWs) were measured.

Chlorophylls content

The amount of chlorophyll a, chlorophyll b and total chlorophyll were determined by UV-visible spectrophotometry as described by Zhang (1990). Briefly, 10 ml of acetone 80% was added to 0.02 g of homogenized freeze-dried herbage samples. The supernatant was separated and the absorbances were read at 470.0 nm, 646.8 nm and 663.2 nm using UV-visible spectrophotometer. The amount of chlorophylls was calculated according to the following formulas:

Chlorophyll a	$12.25\ A_{663} - 2.79\ A_{647}$
Chlorophyll b	$21.50\ A_{646.8} - 5.10\ A_{663.2}$
Total chlorophyll	Chlorophyll a + Chlorophyll b

Where: A663 = Absorbance at 663.2 nm

A646.8 = Absorbance at 647 nm.

Statistical analysis

Two-factorial (SA concentrations and number of application) field experiment established according to completely randomized design with three replications. Data were analyzed as a combined experiment model by SAS software (Ver. 9.1 2002–2003, SAS Institute, Cary, NC, USA). Before analysis of variance, data were tested for normality and homoscedasticity using the Kolmogorov–Smirnov and Cochran tests, respectively. least significant difference (LSD) test was calculated to compare differences between means when F values were significant.

Results

Chlorophylls content

The results showed that SA concentration and the number of SA application had significantly effect on the chlorophyll a content but their interaction had no significantly effect (Table 1). Chlorophyll a content significantly increased with increasing the SA concentration and the number of application as the highest amount was found in 1% SA (7.59 mg g^{-1} FW) and thrice sprays (7.90 mg g^{-1} FW).

Table 1. Influence of salicylic acid treatment and number of application on chlorophyll a and b concentration of strawberry (*Fragaria* × *ananassa* Duch) cv. Camarosa.

		Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)
Salicylic acid (SA)		**†	**
Number of treatment (NT)		**	**
SA × NT		ns	ns
Salicylic acid (mM)	0.0	6.41 ± 0.33 b‡	3.15 ± 0.20 b
	0.5	7.27 ± 0.28 a	3.61 ± 0.15 a
	1.0	7.59 ± 0.25 a	3.77 ± 0.13 a
Number of treatment	1	6.41 ± 0.29 b	3.12 ± 0.18 b
	2	6.96 ± 0.24 b	3.45 ± 0.11 b
	3	7.90 ± 0.25 a	3.96 ± 0.12 a

† ns and ** indicates non-significant and significant at $P \leq 0.01$, respectively.

‡ For each column and each factor means within each column followed by the same letter are not different at $P \leq 0.01$ based on LSD test.

As the Table 1 illustrates, simple effects of SA concentration and the number of SA application significantly affect chlorophyll b content but their interaction had no significantly effect. Along with increasing the SA concentration and the number of application, chlorophyll b content significantly increased (Table 1).

SA sprays and the number of SA application significantly enhanced total chlorophyll content of strawberry (*Fragaria* × *ananassa* Duch. cv. Camarosa) plants (Figure 1). The highest total chlorophyll content was obtained in 1% SA and thrice sprays (11.36 and 11.86 mg g^{-1} FW respectively).

Table 2. Change of leaf and root fresh and dry weights of strawberry (*Fragaria* × *ananassa* Duch) cv. Camarosa in response to salicylic acid treatment and number of application.

		Leaf fresh weight (g)	Leafdry weight (g)	Root fresh weight (g)	Root dry weight (g)
Salicylic acid (SA)		ns[†]	ns	ns	ns
Number of treatment (NT)		*	*	ns	ns
SA × NT		ns	ns	ns	ns
Salicylic acid (mM)	0.0	14.3 ± 0.5 a[‡]	0.143 ± 0.005 a	9.48 ± 0.423 a	0.953 ± 0.044 a
	0.5	14.5 ± 0.4 a	0.145 ± 0.004 a	9.69 ± 0.440 a	0.971 ± 0.045 a
	1.0	15.3 ± 0.5 a	0.154 ± 0.005 a	10.22 ± 0.430 a	1.030 ± 0.041 a
Number of treatment	1	14.1 ± 0.4 b	0.141 ± 0.004 b	9.48 ± 0.432 a	0.948 ± 0.042 a
	2	14.2 ± 0.6 b	0.142 ± 0.006 b	9.96 ± 0.451 a	1.002 ± 0.044 a
	3	15.9 ± 0.3 a	0.159 ± 0.004 a	9.94 ± 0.421 a	1.004 ± 0.045 a

[†] ns and * indicates non-significant and significant at $P \leq 0.05$, respectively.

[‡] For each column and each factor means within each column followed by the same letter are not different at $P \leq 0.05$ based on LSD test.

Fresh and dry weights of leaf and root

Both leaf FW and DW of strawberry plants showed similar results by SA sprays (Table 2). The numbers of SA application significantly enhanced leaf FW and DW, whereas different SA concentration had no significantly effect. The highest leaf FW and DW was observed in thrice sprays (15.9 and 0.159 g respectively).

No significant differences were found between different SA concentration and numbers of SA application on the root FW and DW (Table 2). With a few exceptions, root FW and DW slightly increased with increasing the SA concentration and the number of application but this increase was not significant as compared with control.

Discussion

Chlorophylls content

Our results showed that chlorophylls content significantly increased by increasing the SA sprays as compared with control. The increase in chlorophyll content with SA confirmed the reports of El-Tayeb (2005) for barley, Gunes *et al.* (2007) for maize and Yildirim *et al.* (2008) for cucumber.

The responses of growth are most important phenomenons of plant physiology. In growth, which is a result of accelerated anabolic reactions in the cells and consequent lipid, protein, chlorophyll, DNA and RNA synthesis were studied in detail (Cag *et al.,* 2009). Altman (1982) showed that ethylene accelerated chlorophyll loss. Li *et al.*, (1992) established that SA inhibited the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase enzyme, preventing the formation of ethylene and chlorophyll loss.

According to our results accumulations of chlorophylls were found highest in 1 mM of SA spray (Table 1 and figure 1). These results are in agreement with those obtained by Khandaker *et al.* (2011), who found that in *Amaranthus tricolor* SA at the highest concentration stimulated total chlorophyll synthesis.

Gharib (2006) also obtained similar results, who found high total chlorophyll synthesis in sweet basil and marjoram plants increased by the highest concentration of SA treatment.

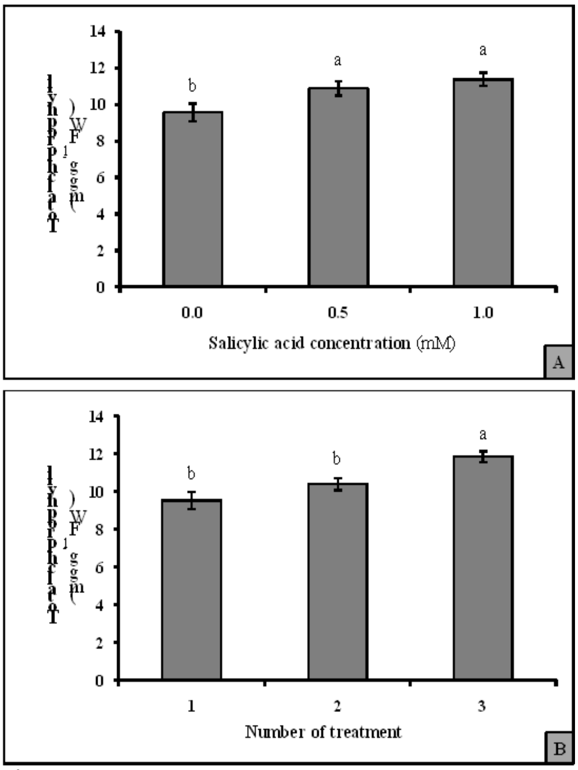

Fig. 1. Effect of salicylic acid treatment (A) and number of application (B) on total chlorophyll content of strawberry (*Fragaria* × *ananassa* Duch) cv. Camarosa.

Moreover, it was reported that exogenously applied SA significantly enhanced net photosynthetic rate which could be due to improving the functional state of the photosynthetic machinery in plants either by the mobilization of internal tissue nitrate or by chlorophyll biosynthesis (Shi *et al.,* 2006). Furthermore, SA has been reported to have stimulatory effects on photosynthetic capacity in maize plants through the induction of rubisco activity (Khodary, 2004).

Fresh and dry weights

In our study, SA sprays enhanced vegetative growth by increasing fresh and dry biomass. These results are correlate with those of El-Tayeb (2005) and Gautam and Singh (2009) who documented that foliar applied SA enhanced biomass production in barley and wheat. Improved plants fresh and dry weights might be due to increased cell division. Salicylic acid treatments maintain the IAA and cytokinin levels in the plant tissues, which enhanced the cell division (Shakirova *et al.,* 2003).

Likewise, the effect of improvement by SA on growth of strawberry plants is considered to be related to hydrophilicity (Barkosky and Einhelling, 1993), regulation of stomata (Arfan *et al.,* 2007), nutrient absorption (Glass, 1974), and photosynthesis (Khan *et al.,* 2003). Moreover, Singh and Usha (2003) revealed that plants treated with SA generally exhibited higher moisture content, dry mass, carboxylase activity of rubisco, superoxide dismutase (SOD) activity and total chlorophyll, compared to untreated seedlings.

As conclusion, strawberry (*Fragaria* × *ananassa* Duch) plants cv. Camarosa sprayed three times with 1 mM concentration of SA had the highest chlorophylls content and better morphological attributes. Therefore, thrice sprays of with 1 mM of SA was found more effective to improve growth indices of strawberry plants.

Acknowledgment

The support of this work by the Islamic Azad University, Science and Research Branch, Tehran, Iran is gratefully acknowledged.

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