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Effect of pre- and postharvest salicylic acid treatment on ethylene production, fungal decay and overall quality of Selva strawberry fruit

Mesbah Babalar [a], Mohamadreza Asghari [b,*], Alireza Talaei [a], Asghar Khosroshahi [c]

[a] Department of Horticulture, Faculty of Agriculture, University of Tehran, Karaj, Iran
[b] Department of Horticulture, Faculty of Agriculture, University of Uromie, Uromie, Iran
[c] Department of Food Science, Faculty of Agriculture, University of Uromie, Uromie, Iran

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Abstract

The effect of salicylic acid (SA) treatment at different concentrations and growth stages of strawberry (*Fragaria ananassa* cv. Selva) fruit on postharvest ethylene production, fungal decay and overall quality index was studied. SA at all concentrations effectively reduced fruit ethylene production and fungal decay and retained overall quality. Treatment of plants at vegetative stage and fruit development stage followed by postharvest treatment of fruits with 1 and 2 mmol L^{-1} was the most effective strategy, whilst with decrease in treatment time the effects of treatment decreased. Single stage treatment strategy of fruits with 2 mmol L^{-1} SA at postharvest stage was most effective. Postharvest treatment with 4 mmol L^{-1} SA slightly damaged the fruits and was less effective than 2 mmol L^{-1} in retaining fruit quality.

Keywords: Postharvest; Strawberry; Salicylic acid; ACC; Ethylene; Fungal decay; Marketability; Overall quality

1. Introduction

Strawberry is an important fruit produced in commercial scale in Iran. Because of high perishability and short postharvest life, the use of chemicals to retain the fruit quality during storage and transport is restricted and it is necessary to find alternate compounds for use in postharvest technology. Salicylic acid (SA), a simple ubiquitous plant phenolic, has been reported to regulate a number of processes in plants including heat production, disease resistance, seed germination, sex polarization and ethylene production (Raskin, 1992a; Zhang, Chen, Zhang, & Ferguson, 2003). It has been demonstrated that both SA and its derivative acetylsalicylic acid (ASA) inhibit ethylene production in several plants (Leslie & Romani, 1988; Romani, Hess, & Leslie, 1989; Zhang et al., 2003).

There is evidence for a positive correlation between free radical activity and ethylene production in apple fruit tissue (Kacperska & Kubacka-Zebalska, 1989). It has been shown that SA inhibits lipoxygenase (LOX) activity in kiwifruit disks and leads to a reduction in ethylene production and free radical activity (Xu, Chen, Li, & Zhang, 2000).

Botrytis fruit rot, also known as gray mold, caused by *Botrytis cinerea*, is the most serious disease of strawberry and is widespread in the environment. It can infect strawberry flowers and cause flowers to rot and also may become dormant and remain on fruit. Dormant infections resume activity on the berries later in the season anytime before

Abbreviations: SA – salicylic acid; F – fruit development stage; P – postharvest stage; V – vegetative stage; FP – fruit development stage plus postharvest stage; VF – vegetative stage plus fruit development stage; VP – vegetative stage plus fruit development stage; VFP – vegetative stage plus fruit development stage plus postharvest stage; ACC – 1-aminocyclopropane-1-carboxylic acid; NL – Nanoliter (ppb); LOX – lipoxygenase.

* Corresponding author. Tel.: +98 914 482 1054; fax: +98 481 224 1150.
E-mail address: mhamadreza@yahoo.com (M. Asghari).

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or after harvest when sugars increase and conditions become favourable to disease development (Goetza et al., 1999; Zavala, Wang, Wang, & Aguilar, 2004). To reduce postharvest decay of fruits it is necessary to improve plant defense mechanism and to eliminate or decrease fungi contamination during plant vegetative growth, flowering and fruit development stages.

The effect of SA on plant resistance to diseases has been discussed by several researchers and it is known that SA is the signal molecule in plant systemic acquired resistance (SAR) induction (Raskin, 1992b). In addition, SA exerts antifungal effects on some plants and harvested fruits (Amborabe, Lessard, Chollet, & Roblin, 2002; Cai & Zheng, 1999; Goetza et al., 1999; Huang, Deverall, Tang, Wang, & Wu, 2000). Lu and Chen (2005) have demonstrated the inhibitory action of SA on *Botrytis* rot in lily leaves. Foliar application of Asilbenzolar-S-methyl (a synthetic analogue of SA) has led to protection of postharvest Rock melons and Hami melons from diseases (Huang et al., 2000).

SA, a natural compound, has a high potential in suppressing ethylene production and fungal decay in harvested fruits. The present study was conducted to determine if SA was able to decrease the ethylene production and fungal decay and increase the postharvest life of the strawberry as a nonclimactric and sensitive fruit. Unlike most of previous reports that examined the effects of postharvest SA treatment on fruit attributes, we tried to investigate its pre- and postharvest application effects to determine if it is able to trigger plant resistance against pathogens and decrease ethylene production and if successive treatment at different plant growth, fruit development and postharvest stages exerts any additional effect.

2. Materials and methods

2.1. Plant material

Three month old strawberry (*Fragaria ananassa* cv. Selva) seedlings, propagated due cutting runners from mother plants, were planted in 25 cm plastic pots filled with sterilized pit mass (1/3) and perlite (2/3) and hydroponically grown in a greenhouse (under high light and 16–18 °C night and 25–28 °C day temperature conditions).

2.2. Salicylic acid treatment

Different SA concentrations (1, 2 and 4 mmol L^{-1}) were prepared by dissolving powdered SA in hot water and then applied on plants and fruits in different combinations of SA concentration and time of application. Plants were divided into 22 groups; each group consisted of 21 plants and received SA treatments at different plant and fruit development stages as described in Table 1.

SA was applied by both spraying 110 mL of the solution on plants and fruits and directly adding to growth medium. Harvested fruits were treated by dipping them in SA solutions. Fruits were harvested at commercial ripeness (>75% of surface showed red colour) and sorted to obtain fruit of uniform size and colour. Twenty fruits of each treatment group were packaged in a 500 mL plastic jars and the open end was sealed. Three packages of each treatment group were prepared and all were stored in the dark under controlled temperature at 2 °C (±0.5 °C). After 14 days the packages were removed from cold storage and kept for 24 h at 20 °C and then subjected to quality evaluation.

Table 1
Treatment groups (22 groups each with 21 plants) with different concentrations of SA and at different stages of growth or development stages

Time of treatment[a]	Salicylic acid concentration (mmol L^{-1})			
	1	2	4	0
V	V1	V2	V4	C
F	F1	F2	F4	C
p	P1	P2	P4	C
VF	VF1	VF2	VF4	C
VP	VP1	VP2	VP4	C
PF	FP1	FP2	FP4	C
VFP	VFP1	VFP2	VFP4	C
Not treated	–	–	–	Control[b]

[a] V, vegetative growth stage; F, fruit development stage; P, postharvest stage; FP, fruit development stage plus postharvest stage; VF, vegetative growth stage plus fruit development stage; VP, vegetative growth stage plus fruit development stage; and VFP, vegetative growth stage plus fruit development stage plus postharvest stage.

[b] The control plants received no treatment during the vegetative growth and fruit development stages and the harvested fruits were dipped only in pure water.

2.3. Determination of ethylene released from fruit

Fruit ethylene production was determined following the method of Srivastava and Dwivedi (2000) with some modifications. Fruits (200 g) were placed in a 1 L sealed jar for 60 min and then headspace samples (1 mL) were collected by syringe and ethylene concentrations measured by flame ionization gas chromatography using a Shimadzu gas chromatograph model GC-14A (Shimadzu, Kyoto, Japan).

2.4. Fungal decay index

Fungal decay was visually inspected by 10 trained persons. According to the amount of the fungal mold on fruit surface scales from 1 to 5 were given to the each treatment group where; 1 = normal (no decay on fruit surface), 2 = trace (up to 5% of fruit surface were decayed), 3 = slight (5–20% of fruit surface were decayed), 4 = moderate (20–50% of fruit surface were decayed), and 5 = severe (>50% of fruit surface were decayed). Results were expressed as fungal decay index.

2.5. Overall quality

Overall quality (percentage of fruit surface area decayed, shrunken and adversely affected) was evaluated by 10 trained panelists using a 1–5 scale, where 1 = unacceptable

(>50% surface affected), 2 = bad (20–50% surface affected), 3 = acceptable (5–20% surface affected), 4 = good (up to 5% surface affected), and 5 = excellent (no decay, shrinkage or any other adverse effects on fruit surface were seen). Results were expressed as an overall quality index.

2.6. Statistical analysis

The experiment was designed as a completely randomized design with 3 (SA concentration) × 7(time of SA application) = 21 factors with 3 replicates (each replication included of 7 plants). ANOVA was performed for the experiment using MSTATC. Differences among means of data were compared by Duncan's Multiple Range Test. Differences at $p \leqslant 0.05$ were considered significant.

3. Results

3.1. Ethylene production

As shown in Table 1, SA significantly affected fruit postharvest ethylene production. The most effective SA concentration was 2 mmol L^{-1}, which led to more than 30% reduction in fruit ethylene production in comparison to the control fruit, while 4 mmol L^{-1} was less effective than 1 and 2 mmol L^{-1}.

SA was most effective on fruit ethylene production decrease when applied consequently at 3 stages of vegetative growth (V), fruit development (F) and postharvest (P) (VFP). In the single application method postharvest treatment of fruit was more effective than application at V and F stages alone (Tables 1 and 2).

3.2. Fungal decay

Fungal decay decreased rapidly in berries treated with SA in a concentration dependent manner from 1 to 2 mmol and did not differ from 2 to 4 mmol (Table 1). When applied at 3 stages of V, F and P in all concentrations SA completely controlled fruit decay and the effect became lower with a decrease in the treatment time (Table 2). Treatment at vegetative stage was less effective than at other two stages alone. SA application at postharvest stage was as effective as application at both stages of vegetative growth and postharvest and was more effective than treatment at both stages of vegetative growth and fruit developmental stages. Application of SA at vegetative stage was effective only when plants were treated with 4 mmol L^{-1} while differences between different SA concentrations applied at fruit developmental stage were not significant (Table 2). SA applied at 3 stages (VFP) at all concentrations completely suppressed fruit fungal decay (Table 3).

3.3. Overall quality

Table 1 shows the effects of different SA concentrations on fruit overall quality index. SA significantly affected fruit overall quality index. SA treatment at all three concentrations resulted in high overall quality and the 2 mmol L^{-1} SA was the most effective without any adverse effects while SA at 4 mmol L^{-1} slightly caused damage to fruit. As evident from Tables 1 and 2, SA applied at 3 stages of

Table 2
Effects of salicylic acid concentration and time of SA treatment on Selva strawberry fruit fungal decay, overall quality index and ethylene production

Treatment		Fungal decay index*	Overall quality index	Ethylene (NL $kg\ h^{-1}$)
SA concentration (mmol L^{-1})	0	5^{a}	1^{d}	32.07^{a}
	1	2.9^{b}	2.8^{c}	15.69^{c}
	2	2.57^{c}	3.23^{a}	10.42^{d}
	4	2.6^{c}	3^{b}	17.13^{b}
Significance		***	***	***
Time of SA application	V	4.6^{a}	1.2^{e}	23.73^{a}
	F	4.1^{b}	1.3^{e}	20.68^{b}
	P	2.93^{c}	3^{c}	18.97^{c}
	VF	3.73^{d}	1.7^{d}	19^{c}
	VP	2.85^{c}	3.22^{c}	16.75^{d}
	FP	2.55^{e}	3.48^{b}	16.67^{d}
	VFP	2.1^{e}	3.7^{a}	16.02^{d}
Significance		***	***	***

*Means followed by different letters within a group are significantly different at the 5% level.
***Indicates significance at $P > 0.001$.

Table 3
Effects of different combinations of SA concentration and time of application on Selva strawberry fruit fungal decay, overall quality index and ethylene production

SA concentration (mmol L^{-1})	Time of SA application	Fungal decay index	Overall quality index	Ethylene (NL $kg\ h^{-1}$)
1	V	4.7^{ab}	2.73^{gh}	25^{c}
	F	4^{cde}	2.8^{gh}	18.29^{de}
	P	2.5^{hi}	2.97^{e}	16.25^{ef}
	VF	3.7^{def}	3.1^{g}	16.65^{ef}
	VP	2.2^{ij}	3.16^{e}	15.81^{f}
	FP	2^{ijk}	3.61^{cd}	10.41^{h}
	VFP	1.2^{lm}	4.31^{a}	7.4^{i}
2	V	4.5^{ab}	3^{gh}	10.77^{gh}
	F	3.6^{ef}	3.16^{g}	16.65^{ef}
	P	2^{ijk}	3.26^{cd}	12.71^{g}
	VF	3^{gh}	3.37^{f}	315^{f}
	VP	2.3^{i}	3.58^{bc}	7.5^{i}
	FP	1.5^{klm}	4.2^{ab}	5.17^{j}
	VFP	1.06^{m}	4.8^{a}	5.17^{j}
4	V	4.2^{bcd}	2.94^{gh}	27.07^{b}
	F	3.8^{def}	2.82^{g}	15.7^{f}
	P	2.2^{ij}	2.17^{de}	14.85^{f}
	VF	3.23^{fg}	3.75^{f}	12.27^{gh}
	VP	2^{ijk}	2.5^{cd}	11.6^{gh}
	FP	1.7^{jkl}	2.42^{bc}	19.01^{d}
	VFP	1.1^{m}	2.54^{cd}	19.43^{d}
0 (control)	–	5^{a}	2.6^{h}	32.07^{a}
Significance		***	***	***

Means followed by different letters within a group are significantly different at the 5% level.
***Indicates significance at $P > 0.001$.

V + F + P was most effective in overall quality retention and with decrease in treatment times, the positive effects of SA decreased. In single application strategy postharvest treatment of fruits was more effective than treatment at fruit development stage and it was more effective than treatment at vegetative stage. Application of SA at concentrations of 1 and 2 mmol L^{-1} at 3 stages (VFP) resulted in fruits with excellent overall quality index at the end of the storage.

4. Discussion

SA prevents 1-aminocyclopropane-1-carboxylic acid (ACC) conversion to ethylene by decreasing ACC oxidase production and activity (Leslie & Romani, 1988). Zhang et al. (2003) reported that postharvest treatment of kiwifruit with ASA (a synthetic analogue of SA) results in lower ACC oxidase and ACC synthase activity and decreases ethylene production during the early stages of fruit ripening. They also pointed out that factors leading to decrease in fruit softening also lead to high internal free SA content in fruits. They postulated that ASA applied to fruit immediately converts to SA. Our results on whole plant and fruit confirm the published data. Decrease in ethylene production in fruits harvested from plants treated with SA at stages of vegetative growth and fruit development is due to decreased ACC synthase production rate and activity which leads to a decrease in ACC and ethylene accumulation in fruit tissue. With increase in treatment times, these effects become more noticeable. Since the inhibitory effect of SA on ethylene production was more prevalent upon application at all 3 stages, the effect of SA on ethylene production seems to be reversible.

In comparison to fruits treated with 2 mmol L^{-1} SA, fruit treated with 4 mmol L^{-1} SA showed high ethylene production because 4 mmol L^{-1} SA slightly damaged and any damage to fruit leads to increase in ACC synthase activity and ethylene production.

Botrytis fruit rot, also known as gray mold, caused by *Botrytis cinerea*, is the most serious disease of strawberry (Zavala et al., 2004) and is widespread in the environment. It can infect strawberry flowers and cause flowers to rot and also may become dormant and remains on fruits. Dormant infections resume activity on the berries later in the season anytime before or after harvest when sugars increase and conditions become favourable to disease development. It is well known that SA and its derivatives trigger plant resistance system against diseases (Raskin, 1992a, 1992b). In this study plant treatment at both vegetative and fruit development stages resulted in a significant decrease in fungal decay confirming the fact that SA leads to plant defense system activation against pathogens. SA causes a rapid increase in H_2O_2 production in plants and H_2O_2, as a signal molecule, activates the plant's systemic resistance against pathogens (Cai & Zheng, 1999). The inhibitory effects of fruit postharvest treatment with SA on fungal decay confirm the previous reports about its antifungal effects (Amborabe et al., 2002; Goetza et al., 1999; Huang et al., 2000; Lu & Chen, 2005) showing that SA directly prevents decay fungi growth and extension in strawberry fruits. Our findings showed that the best strategy to prevent postharvest decay of strawberry fruit is pretreatment of plants at both vegetative and fruit development stages followed by postharvest treatment of fruit before storage.

Overall quality is the most important factor in fruit marketability assessment. Fruits lacking any kinds of decay and shrivels with high red colour are considered as marketable. SA treatment effectively controlled fruit fungal decay and decreased ethylene production leading to absence of any decay on fruit and noticeable decrease in metabolic activity almost including respiration. It is well known that any factor increasing ethylene production or activity leads to increase in respiration rate and any factor increasing respiration rate leads to increase in ethylene production and activity (Wills, McGlasson, Graham, & Joyce, 1998). It has been demonstrated that SA in a concentration dependent manner effectively reduces respiration in plants and harvested fruits (Han, Wang, Li, & Ge, 2003; Srivastava & Dwivedi, 2000; Wolucka, Goossens, & Inze, 2005). Decrease in fruit metabolic activities results in a decrease in fruit water loss and carbohydrate depletion rate and consequently, effectively delays fruit senescence process (Wills et al., 1998). The effect of SA on fruit overall quality index retention, when applied at growth stages, is mostly due its adverse effect on ethylene production and fungal decay extension. Since it was more effective when successively applied at vegetative + fruit development + postharvest stages, like its effects on other fruit attributes, the effect of SA on overall quality is reversible and also cumulative.

Thus salicylic acid, a natural and safe phenolic compound, exhibits a high potential in controlling postharvest losses of Selva strawberry fruits. In this study, SA was able to prevent fruit postharvest loss for 15 days of cold storage without any additional need for use of chemicals.

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References

Amborabe, B. E., Lessard, P. F., Chollet, J. F., & Roblin, G. (2002). Antifungal effects of salicylic acid and other benzoic acid derivatives towards Eutypa lata: Structure–activity relationship. *Plant Physiology and Biochemistry, 40*, 1051–1060.

Cai, X. Z., & Zheng, Z. (1999). Induction of systemic resistance in tomato by and incompatible race of *Cladosporium fulvum* and the accumulation dynamics of salicylic acid in tomato plants. *Acta Horticulturae Sinica, 29*, 261–264.

Goetza, G., Fkyerat, A., Meatais, N., Kunz, M., Tabacchi, R., Pezet, R., et al. (1999). Resistance factors to grey mould in grape berries: Identification of some phenolics inhibitors of *Botrytis cinerea* stilbene oxidase. *Phytochemistry, 52*, 759–767.

Han, T., Wang, Y., Li, L., & Ge, X. (2003). Effect of exogenous salicylic acid on postharvest physiology of peaches. *Acta Horticulture*, 628.

Huang, Y., Deverall, B. J., Tang, W. H., Wang, W., & Wu, F. W. (2000). Foliar application of Asilbenzolar-S-methyl and protection of post-harvest rock melons and Hami melons from disease. *European Journal of Plant Pathology, 106*, 651–656.

Kacperska, A., & Kubacka-Zebalska, M. (1989). Formation of stress ethylene depends both on ACC synthesis and on the activity of free radical-generating system. *Physiologia Plantarum, 77*, 231–237.

Leslie, C. A., & Romani, R. J. (1988). Inhibition of ethylene biosynthesis by salicylic acid. *Plant Physiology, 88*, 833–837.

Lu, Y. Y., & Chen, C. Y. (2005). Molecular analysis of lily leaves in response to salicylic acid effective towards protection against *Botrytis elliptica*. *Plant Science, 169*, 1–9.

Raskin, I. (1992a). Salicylate, a new plant hormone. *Plant Physiology, 99*, 799–803.

Raskin, I. (1992b). Role of salicylic acid in plants. *Annual Review of Plant Physiology and Plant Molecular Biology, 43*, 439–463.

Romani, R. J., Hess, B. M., & Leslie, C. A. (1989). Salicylic acid inhibition of ethylene production by apple discs and other plant tissues. *Journal of Plant Growth Regulation, 8*, 63–69.

Srivastava, M. K., & Dwivedi, U. N. (2000). Ripening of banana fruit by salicylic acid. *Plant Science, 158*, 87–96.

Wills, R., McGlasson, B., Graham, D., & Joyce, D. (1998). *Postharvest, an introduction to the physiology and handling of fruit & vegetables and ornamentals* (fourth ed.). Sydney 2052, Australia: University of New South Wales Press Ltd., University of New South Wales.

Wolucka, B. A., Goossens, A., & Inze, D. (2005). Methyl jasmonate stimulates the de novo biosynthesis of vitamin C in plant cell suspensions. *Journal of Experimental Botany, 56*, 2527–2538.

Xu, W. P., Chen, K. S., Li, F., & Zhang, S. L. (2000). Regulation of lipoxygenase on jasmonic acid biosynthesis in ripening kiwifruit. *Acta Phytophysiologica Sinica, 26*, 507–514.

Zavala, J. F. A., Wang, S. Y., Wang, C. Y., & Aguilar, G. A. G. (2004). Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. *Food Science & Technology, 37*, 687–695.

Zhang, Y., Chen, K., Zhang, S., & Ferguson, I. (2003). The role of salicylic acid in postharvest ripening of kiwifruit. *Postharvest Biology and Technology, 28*, 67–74.