Extending Storability of Persimmon Fruit cv. Karaj by Postharvest Application of Salicylic Acid

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ABSTRACT

The main postharvest problems of persimmon in Iran are severe softening and disease incidence on the fruits during storage. Therefore, delay in softening and/or control of diseases result in the storage life extension of persimmon fruit. The strategy of induced disease resistance in plants by biotic and abiotic treatments is an attractive method for controlling diseases. Salicylic acid (SA) is a well known natural inducer of disease resistance in plants. In this study persimmon fruits cv. Karaj were treated at harvest with SA at 0 (as control), 1 and 2 mM and the quality parameters of the fruit were measured during 3 months of storage at monthly intervals. The most noticeable effect of postharvest SA application on stored persimmon fruit was the reduction of disease incidence at 2 mM concentration, while 1 mM SA failed to control diseases. Results showed that SA did not affect TSS, titratable acidity, soluble tannin content, and fruit firmness. Also, SA could not suppress ethylene production compared to the control. SA treatment at 2 mM concentration reduced postharvest disease incidence of persimmon fruit by inducible defense mechanism, being suitable for increasing postharvest life of the fruit.

Keyword: Disease incidence, Ethylene, Fruit softening, Induced resistance.

INTRODUCTION

Persimmon (*Diospyros kaki* Thunb.) is an important late season fruit crop in Iran. The harvest time of persimmon in Iran is mainly between October and November, competing with more traditional horticultural crops such as apple, mandarin, and pomegranate at this time. Unfortunately, most astringent type persimmon fruit producers do not use the suitable technology to preserve the quality of the fruit and, consequently, sell their produce at lower prices. Therefore, proper postharvest handling of astringent type persimmon is important in many developing countries. The main problems in postharvest handling of persimmon in Iran are severe softening and disease incidence on the fruits (observed by authors). Delay in softening and or control of diseases result in the extension of storage life of persimmon fruit.

Spores of fungi landing on the persimmon fruit surface can penetrate through small cracks around and beneath the calyx formed as a result of fruit growth during the final period of development. The infection remains quiescent, but natural disease resistance of fruit declines gradually during fruit ripening, allowing the development of quiescent infections (Prusky *et al*., 1981; Terry and Joyce, 2004). Nowadays, the control of postharvest diseases by chemical fungicides is restricted because of the harmful effects on human health and the environment. Hence, developing safe and effective disease management strategies are highly needed (Mandal *et al*., 2009). Control of postharvest diseases by treatment with natural compounds that induce resistance to disease is an attractive

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and safe strategy, and SA is one of the well known and effective natural inducers of plant resistance to different diseases (Terry and Joyce, 2004; Yao and Tian, 2005). Accumulation of endogenous salicylic acid induces a systemic acquired resistance (SAR) mechanism, which is considered one of the classical forms of induced resistance (Durrant and Dong, 2004).

Salicylic acid (a simple phenolic compound) is an endogenous hormone, having key roles in different aspects of plant growth and development such as stomatal movement, seed germination, ion absorption, and responses to environmental stresses. It is a molecule involved in some signal transduction systems, which induce biosynthesis of defense compounds such as polyphenols, alkaloids or pathogenesis-related (PR) proteins (Raskin, 1992; Yao and Tian, 2005). Exogenously applied SA, moves inside the plant systemically and induces expression of defense genes which result in resistance to different kinds of pathogens (Oostendorp *et al*., 2001; Yao and Tian, 2005). Babalar *et al.* (2007) indicated that the treatment of 'Selva' strawberry with 1 and 2 mM SA at vegetative stage followed by postharvest stage application was the most effective strategy for decreasing ethylene production and control of postharvest diseases. Positive SA effect have also been reported for control of; *Fusarium oxysporum* in tomato (Mandal *et al*., 2009), anthracnose disease caused by *Colletotrichum gloeosporioides* in mango (Zainuri *et al*., 2001), *Penicillium expansum* in sweet cherry fruit (Xu and Tian, 2008) and gray mold decay in peach (Zhang *et al*., 2008). Xu and Tian (2008) indicated that SA treatment stimulated the activities of antioxidant enzymes (catalase, glutathione peroxidase, chitinase and ß-1, 3-glucanase) in sweet cherry fruit and they concluded that activation of antioxidant defense plays the main role in resistance against *P. expansum*.

SA can control ethylene production and ethylene action in harvested strawberry and other fruit (Babalar *et al*., 2007; Raskin, 1992). By suppression of ethylene production and action, and therefore by delaying fruit ripening

and softening, SA can extend the shelf life of the harvested fruit by delay in development of disease incidence (Terry and Joyce, 2004). Zhang *et al.* (2003) indicated that higher SA level in the kiwifruit caused a decrease in ethylene production via suppressed ACC synthase and ACC oxidase activities and hence retarded the climacteric rise in ethylene production. If SA can reduce ethylene production, delaying fruit softening and controlling postharvest diseases, it may increase storage life of Iranian cultivars of persimmon fruit, for which no information has been reported on the possible effect of SA application during storage. Therefore, the objective of the present work was to investigate the effect of SA application on the persimmon fruit cv. Karaj after harvest.

MATERIALS AND METHODS

Fruit Samples

Persimmon fruits (cv. Karaj) were harvested at firm and orange color stage from an orchard near Karaj city and were immediately transported to postharvest laboratory of the Department of Horticultural Science, University of Tehran. Later, fruits without wounds or rots were sorted for uniform size and maturity.

Treatments

The fruits were divided into three groups (60 fruits in each group) and were treated by dipping in the solution of 0 (as control), 1 and 2 mM SA for 10 min at 25℃. Three replicates were used for each treatment. Treated fruits were then air-dried for about 30 min and stored at 1°C and 85% RH for 3 months. Following 0, 1, 2 and 3 months of cold storage, 15 random fruits of each treatment (as 3 replications each of 5 fruit) were transported to ambient condition and maintained at 25°C for 2 days (simulating as shelf life). Fruit decay, browning index, physical and chemical

properties were determined at the end of shelf life by using 5 fruits per replicate.

Fruit Assessment

Persimmon fruit samples were weighed before and after storage to calculate weight loss (%) during storage by using the following formula (1):
Skin color of each fruit as parameters of L*, a*, b* were determined objectively with a Minolta chromameter (model CR-400, Japan) and the results were expressed as skin *Colour index:*

Colour index= (1000a*)/ (L*b*)   (2)

according to Salvador *et al.* (2007). Fruit firmness (N) was determined using an Effegi, FT 327 penetrometer equipped with an 8 mm tip at 3 equatorial points. Fruit samples were pooled and juiced to determine soluble solids content (SSC) and titratable acidity (TA). SSC percent was determined by a hand refractometer (RF40) and TA was measured by titrating fruit juice with 0.1 N NaOH up to pH 8.2; using 5 ml of juice diluted to 50 ml with distilled H_2O. The results were expressed as gram of malic acid per 100 gr fresh weight. The soluble tannin content was determined by Folin-Denis method (Tiara, 1996). The rate of whole fruit ethylene production was measured by enclosing two fruits in 2 L airtight containers for 1 hour at 20°C, withdrawing 1 ml of the headspace gas, and injecting into a gas chromatograph (Shimadzu, 14A, Japan), fitted with a flame ionization detector and an activated alumina column.

The severity of fruit skin browning was assessed visually on the surface of persimmon fruits. The browning severity was divided into 5 classes; 0 as no browning, 1 as slight browning (less than 25% of fruit surface was brown), 2 as mild browning (more than 25%

and less than 50% of fruit surface was brown), 3 as severe browning (more than 50% and less than 75% of fruit surface was brown) and 4 as very severe browning (more than 75% of fruit surface was brown). From these, the browning index was expressed according to Wang *et al.* (2006) as:

The percentage of fruit decay incidence was calculated as follows formula (3&4):

Statistical Analysis

All data were analyzed by one-way analysis of variance (ANOVA). Means separation were performed by the *Fishers* protected least significant difference (*LSD*) test. Differences at *P= 0.05* were considered as significant.

RESULTS AND DISCUSSION

Ethylene production of the fruits in all treatments increased to the maximum value in the first month after storage and then dropped gradually over time in storage (Figure 1). Initial increase in ethylene production and the following decrease reflects the typical climacteric behavior of persimmon fruit (Nakano *et al.*, 2001; Salvador *et al.*, 2007;). There was no significant difference in ethylene production between SA treatments and the control during storage life. Color index of fruit skin increased gradually during storage with no significant difference among treatments (Figure 2). Skin colour change from yellow orange to red orange during storage indicated that the 'Karaj' persimmon ripened during storage (Cia, 2006; Salvador *et al.*, 2007).

[(weight of fruits before storage – weight of fruits after storage) / weight of fruits before storage] × 100 (1)

Browning Index= ∑ [(Browning level)×(Number of fruits at each Browning level) / (5×Total number of fruits in the treatment).   (3)

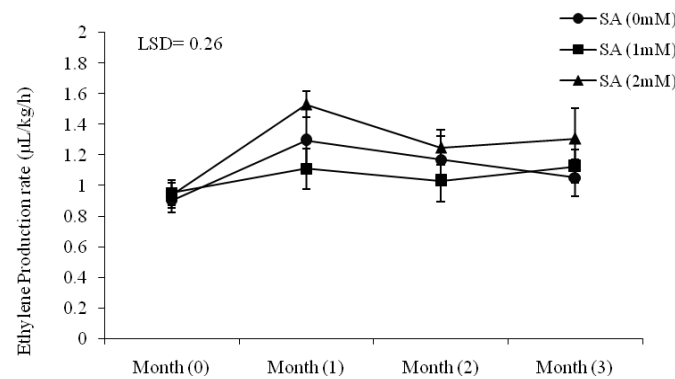The percentage of decay incidence= (Number of fruits with incidence/Total number of fruits in the treatment)×100.   (4)

Figure 1. Effect of SA treatments after harvest on ethylene production rate ($\mu l.kg^{-1}.h^{-1}$) of persimmon fruit, cv. Karaj during cold storage (+1°C) periods plus 2 days shelf-life at 25°C.

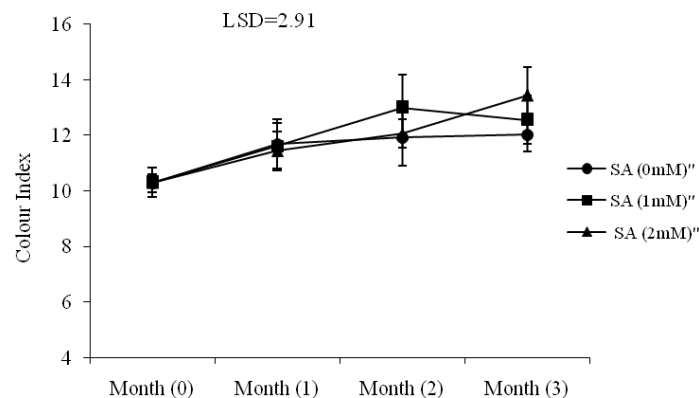**Figure 2.** Effect of SA treatments after harvest on colour index of persimmon fruit, cv. Karaj during cold storage (+1°C) periods plus 2 days shelf-life at 25°C.

The fruit firmness decreased markedly during storage, but no significant difference was observed between SA treatments and control (Table 1). Ripe persimmon fruit typically has low firmness; the degree of firmness is dependent on the cultivar. No minimum recommended degree of firmness is available for this fruit, but from a marketing and commercial point of view, firmness of less than 10 N is considered as unsuitable (Arnal and Del Rio, 2004; Salvador *et al.*, 2007). According to Table 1, fruit firmness at the end of three months storage was lower than 10 N. This is while Iranian consumers prefer persimmon fruit with soft texture (Mostofi *et al.*, 2008). Softening of persimmon as a climacteric fruit depends on the internal ethylene production. Persimmon is very sensitive to ethylene action and low concentrations of internal ethylene can accelerate fruit ripening and sharply softening the fruit (Nakano *et al.*, 2001; Salvador *et al.*, 2004). Increase in ethylene production in the first month after storage resulted in drastic softening of fruit at the same time.

The most noticeable effect of postharvest application of SA on stored persimmon fruit in this study was reduction of disease incidence at 2 mM concentration, while disease control failed in 1 mM SA treatment (Figure 3). Both treated and untreated fruit suffered from disease incidence during storage over 3 months at 1°C, but severity of disease incidence in the 2 mM SA treatment was significantly lower than that of the control and 1 mM SA treatment. Also, by using SA treatment, fungal decay was reduced or eliminated in strawberry (Babalar *et al.*, 2007), tomato (Mandal *et al.*, 2009),

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Table 1. Effect of SA treatments after harvest on fruit firmness, soluble tannin content, soluble solids contents (SSC), titratable acidity (TA) and weight loss of persimmon fruit, cv. Karaj during cold storage periods plus subsequent 2 days shelf life at 25°C. Means for each trait with the same letters are not significantly different according to *LSD* (*P*<0.05).

Months / SA treatments	Fruit firmness (N)				Soluble tannin (ppm)				SSC (%)				TA (%)				Weight loss (%)			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
0 mM	63.6 a	18.16 bc	12.46 cde	2.86 e	6883 a	4638 b	4395 b	1959 c	20.53 a	17.07 bcd	18.00 bc	18.5 abc	0.235 a	0.15 b	0.14 b	0.15 b	0 f	2.75 cde	4.40 bc	8.17 a
1 mM	62.6 a	21.33 bc	17 bcd	4.7 de	6893 a	4920 b	4503 b	2492 c	21.03 a	16.13 d	16.67 cd	17.47 bcd	0.223 a	0.14 b	0.15 b	0.14 b	0 f	2.66 de	4.36 bcd	8.76 a
2 mM	60.6 a	26 b	15.33 bcde	4.13 de	6873 a	4590 b	4515 b	2547 c	20.83 a	16.33 cd	16.5 cd	17.33 bcd	0.233 a	0.15 b	0.14 b	0.16 b	0 f	1.58 ef	4.74 b	8.53 a

sweet cherry (Yao and Tian, 2005) and peach (Zhang *et al.*, 2008). Resistance to pathogens is based on both constitutive and inducible defense mechanisms that are induced by biotic or abiotic agents such as SA (Yao and Tian, 2005). Considering the lack of SA treatments effect on maintaining fruit firmness in this study, SA decreased fugal decay by inducible resistance. A study by Chan *et al.* (2007) on the peach fruit showed that the SA treatment at concentration of 0.5 mM could significantly enhance resistance against infection by *Pencillium expansum*. By proteomics analysis, they confirmed that, in SA-treated fruit, 18 proteins were up-regulated in comparison to the control. Some of these proteins were antioxidant enzymes, being considered as the main enzymatic systems for protecting cell against oxidative damage and responsible for the disease resistance. The other up-regulated proteins were pathogen-related proteins and SAR was associated with their production. Also, in another research by Chan *et al.* (2008) on the sweet cherry fruit, results indicated that SA treatment increased the level of five heat shock proteins (HSP), in addition to antioxidant and pathogen related proteins. HSP are of the major classes of chaperone molecules and play many roles inside the cells. These proteins may act as a primary defense mechanism during oxidative stresses caused by pathogens, thus preventing damage of ROS to cellular membrane. In both research, some enzymes involved in metabolism and energy pathway were up-regulated by SA; but, the roles of these enzymes in resistance against disease infection are unclear (Chan et al., 2007 and 2008).

No noticeable browning symptoms were observed after 2 months storage at 1°C, but browning symptoms subsequently developed on all treatments at 3 months (Figure 4). The 1 mM SA treatment compared with the control and 2 mM SA had significantly higher browning index. Internal and external browning has been reported as chilling injury symptoms in persimmon (Collins and
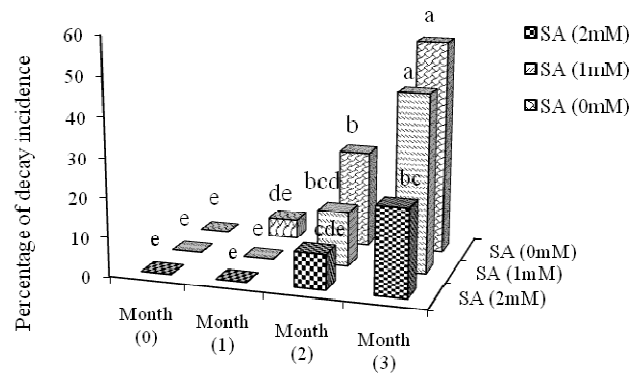
Figure 3. Effect of SA treatments after harvest on fungal decay incidence (%) of persimmon fruit, cv. Karaj during cold storage (+1°C) periods plus 2 days shelf-life at 25°C. Columns with different letters are significantly different according to LSD (P< 0.05).

Figure 4. Effect of SA treatments after harvest on browning index of persimmon fruit, cv. Karaj during cold storage (+1°C) periods plus 2 days shelf-life at 25°C. Columns with different letters are significantly different according to LSD (P< 0.05).
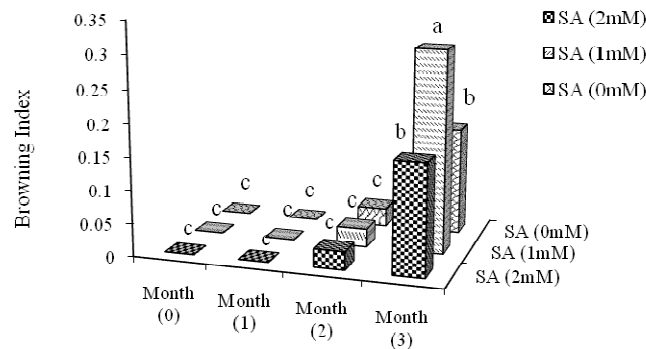
Tisdell, 1995; Salvador *et al*., 2004). In the present report on the 'Karaj' persimmon, we have demonstrated that this cultivar is insensitive to chilling injury at low temperatures (Khademi *et al*., 2010). Also, Sayyari *et al*. (2009) and Wang *et al*. (2006) showed that treatment with SA was effective in alleviating chilling injury in pomegranate and peach fruits. Wang *et al*. (2006) suggested that the effect of SA on alleviating chilling injury in peaches during cold storage may be attributed to its ability to induce antioxidant systems and production of heat shock proteins (HSP).

No significant changes were observed in SSC and titratable acidity (TA) during storage between the treatments (Table 1); similarly, SSC or TA in mango and pomegranate were not affected by SA treatment (Ding *et al*, 2007; Sayyari *et al*., 2009). Soluble tannin content drastically decreased and weight loss significantly increased during postharvest life of persimmon in this study, however, no significant difference among treatments were observed for these traits (Table 1). In conclusion, SA treatment at 2 mM concentration increased postharvest life of persimmon fruit cv. Karaj by inducing

resistance to different diseases without noticeable effect on the fruit quality.

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افزایش عمر انبارمانی میوه خرمالو رقم کرج با استفاده از مصرف پس از برداشت اسید سالیسیلک

ا. خادمی، ذ. زمانی، ی. مستوفی، س. کلانتری، و ا. احمدی

چکیده

نرم شدن سریع و آلودگی به بیماریهای متعدد در طول انبارداری مشکلات اصلی پس از برداشت میوه خرمالو در ایران می‌باشند. بنابراین تاخیر در نرم شدن و یا کنترل بیماری‌ها می‌تواند منجر به افزایش عمر پس از برداشت میوه خرمالو گردد. استراتژی القا مقاومت به بیماری در گیاهان تحت تاثیر تیمارهای زیستی و غیر زیستی یک روش بسیار مناسب در کنترل بیماری می‌باشد. اسید سالیسیلک یک ترکیب طبیعی شناخته شده است که مقاومت به بیماری را در گیاهان القا می‌کند. در این مطالعه میوه خرمالو رقم کرج با غلظت‌های اسید سالیسیلک صفر (به عنوان شاهد)، یک و دو میلی‌مولار تیمار شد و خصوصیات کیفی میوه در طول سه ماه انبارداری، به فاصله یک ماه از یکدیگر مورد بررسی قرار گرفت. مهمترین اثر کاربرد پس از برداشت تیمار اسید سالیسیلک روی میوه خرمالو در طول انبارداری کاهش شدت آلودگی بیماری در غلظت دو میلی مولار بود در حالی که غلظت یک میلی‌مولار اسید سالسیلک در کنترل بیماری ناموفق بود. نتایج نشان داد که تیمار با اسید سالیسیلک تاثیر قابل ملاحظه‌ای بر مقدار مواد جامد محلول، اسیدیته قابل تیتراسیون و مقدار تانن محلول نداشتند. همچنین، تیمار با اسید سالیسیلک در مقایسه با شاهد اثر کنترل کننده‌ای بر تولید اتیلن نداشت. بنابراین تیمار اسید سالیسیلک در غلظت دو میلی‌مولار شدت بروز بیماری‌های پس از برداشتی میوه خرمالو را از طریق القا مقاومت کاهش داده است و می‌تواند برای افزایش عمر پس از برداشت میوه خرمالو مناسب باشد.