

Fruit Growth and Sensory Evaluation of 'Hayward' Kiwifruit in Response to Preharvest Calcium Chloride Application and Orchard Location

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Summary

In order to receive reliable results in the effect of preharvest calcium chloride (CaCl₂) application on fruit growth and sensory characteristics of kiwifruit (*Actinidia deliciosa* cultivar 'Hayward') at the harvest time, a field experiment was carried out in two commercial orchards at different locations. The vines were sprayed with CaCl₂ (1.5%), one, two, or three times in 35, 85 and 125 days after full bloom. The results showed that CaCl₂ treatment significantly reduced fruit size, fresh weight and total dry matter content. Moreover, fruit growth relative attributes such as relative growth index, daily relative growth rate, daily transpiration rate, total carbon received by fruit and yield threshold pressure significantly decreased by thrice application. After thrice application of CaCl₂, fruits showed better sensory quality. Overall, one time preharvest CaCl₂ application had no-significant effect on the most fruits characteristics, while thrice application of CaCl₂ could delay fruit ripening process.

Key words

Actinidia deliciosa, daily transpiration, fruit size, growth index, ripening

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Introduction

The appearance and quality of fresh fruits is a primary criterion in making purchasing decisions. Consumer's acceptance for ripe kiwifruits (*Actinidia deliciosa* cultivar 'Hayward') is fruit size and shape, sugar concentration, sugar-acid ratio, green pulp colour, and the sensory properties such as volatile content, texture, sweet-nectar odour, sweet pulp taste and appearance (Jaeger et al., 2003; Ghasemnezhad et al., 2013).

Previous studies showed that, there are three main stages in terms of the dominating metabolism in kiwifruit: (1) cell division [from 0 to 45 day after full bloom (DAFB)], (2) starch accumulation (from about 45 to about 120 DAFB) and (3) fruit maturation (from about 120 DAFB to harvest) (Richardson et al., 2004; Moscatello et al., 2011). During kiwifruit growth and development, numerous biochemicals, physiological and structural modifications happen and these changes determine the final fruit quality (Tavarini et al., 2009). Since the quality characteristics of fruits are strongly influenced by different factors, foliar application of macro and micro-nutrients during fruit development have very important role in improving quality of fruits (Shukla et al., 2011; Singh et al., 2007). Calcium is an essential element as well as a crucial regulator of growth and development in plants (Hepler, 2005), and participates in cross-linking negative charges, especially on the carboxylic residues of pectin, imparting significant structural rigidity to the cell wall (Hepler and Winship, 2010; Li et al., 2012).

Since, during fruit growth and development, small amount of calcium is transferred from leaves to fruits, and on the other hand, lenticels, cracks and surface discontinuities seem to have a significant positive effect on calcium penetration, direct calcium applications on the fruit surface is recommended and calcium sprays have been reported to be particularly effective in increasing calcium levels in many fleshy fruits (Manganaris et al., 2005; Manganaris et al., 2006). Preharvest treatment with calcium changes intracellular and extracellular processes such as fruit respiration rates and ethylene production, thereby causes retarding fruit ripening, softening and finally delays senescence (Tsantili et al., 2007).

Considerable attention has been given to calcium application to kiwifruit, since it was found that calcium application is effective way to maintain fruit quality, extend storability and potentially is related with the appearance of pitting incidence (Basiouny and Basiouny, 2000; Xie et al., 2003; Gerasopoulos and Drogoudi, 2005). Moreover, calcium makes fruit more acceptable by reducing colour change rate, maintain membrane permeability and slow ripening processes (Gerasopoulos and Drogoudi, 2005). It should also be noted that the beneficial effects of preharvest calcium sprays depend on many factors, including calcium source, frequency, method and timing of treatment, as well as orchard location, growth and environmental conditions and cultivar (Serrano et al., 2004; Manganaris et al., 2006).

Despite the importance of calcium content and the effects of calcium applications in many fruit species, there is little or no reliable information on the effect of CaCl_2 treatment on fruit relative growth characteristics and sensory quality of kiwifruit. Discussions in the papers outlined previous conclusion that application of CaCl_2 could change and/or enhance the postharvest

quality of kiwifruit by changing fruits physiological and biochemical characteristics, with no detrimental effect on consumer's acceptance. Therefore, the main objective of this study was to define the influence of preharvest CaCl_2 applications on kiwifruit quality, with an emphasis on the growth relative and sensory attributes at the harvest time.

Materials and methods

Plant material and handling

The experiment was conducted during the 2013 growing season in two commercial full yieldness 'Hayward' kiwifruit orchards. The orchards located in Rasht (latitude of $37^{\circ}21'$ N, longitude of $49^{\circ}57'$ E and 5 m altitude) and Ramsar at the Iran Citrus Research Institute (latitude of $36^{\circ}90'$ N, longitude of $50^{\circ}65'$ E and 21 m altitude) in Iran. The both orchards are located in subtropical regions, where the mean annual temperature is about 15.9 and 21°C , rainfall 1359 and 1200 mm per year in Rasht and Ramsar respectively.

Two orchards were selected assuming the climate, vines age, training and management system, vegetative and reproductive characteristics to be similar, although there are some differences because of their geographical separation. The 10-years old vines were spaced 6×4 m (417 vines ha^{-1}) and trained on a T-bare system in a medium-textured soil with 'Tomori' as pollinizer (8:1). The vines and the soil were managed according to standard cultural practices. The vines were regularly drip-irrigated during the season, water was supplied based on evaporative demand; meteorological data were recorded throughout the study.

In each orchard 54 vines were selected for preharvest CaCl_2 treatment. According to three main stages of the dominating metabolism in kiwifruit (Richardson et al., 2004; Moscatello et al., 2011), CaCl_2 (1.5%) application was performed at three times (35, 80 and 125 DAFB) during the growing season. A surfactant (0.01% Tween 20) was added during sprays for maximum calcium absorption. Treatments were arranged in a completely randomized block design with three replicates. Each replication consisted of three vines. Treatments were identified as follows: T0 (control, non-treated), T1: (CaCl_2 sprayed at 35 DAFB), T2: (CaCl_2 sprayed at 80 DAFB), T3: (CaCl_2 sprayed at 125 DAFB), T4: (CaCl_2 sprayed at 35 + 80 DAFB), T5: (CaCl_2 sprayed at 35 + 80 + 125 DAFB).

Thirty uniform and defect-free fruits from each treated and untreated vines were harvested when total soluble solids (TSS) content reached an average of 6.2-6.5% (Gerasopoulos and Drogoudi, 2005). Immediately, fruits were transferred to the laboratory and some quantitative and qualitative parameters were determined.

Quality parameters related to fruit physical dimensions and fresh weight

In each treatment, ninety fruits were selected to determine fruit quality. Physical dimensions of length, diameter and volume of all fruits were measured. Fruits were weighed (fresh weight), percentage of fruit dry matter (%DM) (skin plus flesh) was determined after 48 h drying (60°C) using a ventilated oven. Water content was calculated as the difference between fresh weight (FW) and dry weight (Montanaro et al., 2006).

Physical dimensions, fresh and dry weights were measured at two stages, 10 days after fruit set (DAFS) and immediately after harvest. Using these data, %DM, fruit DM content (FDMC) (g per fruit), percentage of excess DM per fruit (%EDMF), daily dry matter accumulation (DDMA) (mg DM per fruit⁻¹ d⁻¹) and the specific rates of fruit DM accumulation (SRFDMA) (g DM gFW⁻¹ d⁻¹) were calculated (Montanaro et al., 2010). Fruit density was calculated by water displacement method (Jan et al., 2013):

$$\text{Fruit density (g cm}^{-3}\text{)} = M/V$$

where M is the mass of fruit and V is the volume of fruit.

Fruit surface area (FSA) (cm² fruit⁻¹) was calculated as L×W×3.14; where L is the fruit length and W is the maximum fruit diameter (Montanaro et al., 2012).

Fruit growth relative attributes

Relative growth index (RGI) was calculated as:

$$\text{RGI} = (\text{FW}_{t_1} - \text{FW}_{t_0}) / [(\text{FW}_{t_1} + \text{FW}_{t_0}) / 2]$$

where FW_{t₁} and FW_{t₀} are stands for fresh weights (g) measured at harvest (t₁) and 10 DAFS (t₀), respectively (Gallego et al., 1997).

Daily relative growth rate (DRGR, g g⁻¹ day⁻¹) was calculated using the following equation (Morandi et al., 2010):

$$\text{DRGR}_{t_1} = (\text{FW}_{t_1} - \text{FW}_{t_0}) / [(t_1 - t_0) \text{FW}_{t_0}]$$

where FW_{t₁} and FW_{t₀} are stands for fruit fresh weights (g) measured at harvest (t₁) and 10 DAFS (t₀), respectively.

The total carbon received (TCR) by fruit during the growing season (C_t) was estimated by assuming that the daily respiration rate (C_r) for a kiwifruit berry decreased from 1.4 to 0.1 mM CO₂ g⁻¹ dry matter day⁻¹ during the first 150 days after fruit set, following an exponential decay pattern and by assuming that the carbon remaining in fruit DM was approximately 48% (Montanaro et al., 2006).

$$C_t = \text{DM} (0.48 \times C_r)$$

$$C_r = (0.10635 + 1.31078 e^{-(x/29.13114)})$$

where, DM is dry matter and x is day after fruit set.

The threshold pressure (Y) reflects properties of the cell wall and may vary during fruit growth and it was calculated according to Green et al. (1971) and Lechaudel et al. (2007).

Fruit sensory quality analysis

Ten panelists were trained for kiwifruit sensory evaluation based on the following criteria: willingness to consume kiwi, knowledge of sensory tests, availability and no history of negative allergic reactions. The sensory panel underwent 8 h of training, during which they developed and defined a descriptive vocabulary of 10 attributes to establish differences in the sensory properties, color, texture, flavor and taste (Table 1), for comparing the treatments (Fernández-Sestelo et al., 2013; Shiri et al., 2013a; Shiri et al., 2013b).

Statistical analysis

A randomized complete block experimental design with three replications was used. Data were analyzed as a combined experiment model by PROC ANOVA procedure by SAS software (Ver. 9.1 2002–2003, SAS Institute, Cary, NC, USA). Before analysis of variance, data were tested for normality and homoscedasticity

Table 1. Fruit sensory attributes used in the presented quantitative descriptive analysis

Attributes	Descriptors	Grouped and associate descriptors	Anchoring points(left-right)
Skin color	Brown		Dark brown – pale brown
	Green		Verdant – greeny
Pulp texture	Pulp consistency	Degree of firmness of fruit flash	Soft, firm – hard
	Juicy		Watery – semi dry
Taste	Sweet	Sugar, honey	None – very intensive
	Acid	Sour	None – very intensive
	Bitter	Metallic	None – very intensive
Flavor	Fruity, natural	Flavor characteristic for fully ripe kiwifruit or gooseberry fruits	None – very intensive
Skin	Skin resistance	Resistance of fruit skin when chewed	Soft, firm – hard

The abbreviation, description and anchoring points for each attribute given

using the Kolmogorov–Smirnov and Cochran tests, respectively. Least significant difference (LSD) at $P \leq 0.01$ was calculated to compare differences between means following a significant ANOVA effect.

Results

Fruit size and growth relative attributes

The results showed that the orchards and CaCl₂ treatment significantly affected the fruit length and FSA, but their interaction has no significant effect (Table 2). Fruits harvested from Rasht orchard were longer in size and had higher FSA than those from Ramsar, additionally CaCl₂ application significantly

Table 2. Influence of CaCl₂ (1.5%) treatment and orchard location on fruit length, fruit surface area and dry matter (DM) of 'Hayward' kiwifruit

		Fruit length (mm)	Fruit surface area (cm ² fruit ⁻¹)	DM (%)
Orchard (O)		***	***	***
Treatment (T)		***	***	NS
O × T		NS	NS	NS
Orchard	Rasht	68.4 a	117.0 a	16.6 b
	Ramsar	66.4 b	111.8 b	18.3 a
Treatment	T0	69.7 a	121.9 a	17.6 a
	T1	67.7 bc	115.3 bc	17.4 a
	T2	67.0 c	113.8 c	17.5 a
	T3	69.0 ab	117.6 b	17.7 a
	T4	66.7 c	113.8 c	17.2 a
	T5	63.7 d	103.3 d	17.3 a

NS and *** indicates non-significant and significant at $P \leq 0.001$, respectively. Means within each column followed by the same letter are not different at $P \leq 0.01$ based on LSD test.

Table 3. Influence of CaCl₂ (1.5%) treatment and orchard location on fruit diameter, length/diameter (L/D), volume, fresh weight (FW), fruit dry matter content (FDMC) and daily dry matter accumulation (DDMA) of 'Hayward' kiwifruit

		Fruit diameter (m)	L/D	Volume (cm ³)	FW (g)	FDMC (g DM fruit ⁻¹)	DDMA (mg DM fruit ⁻¹ d ⁻¹)
Orchard (O)		***	*	***	***	NS	*
Treatment (T)		***	**	***	***	***	***
O × T		*	**	***	***	**	***
Rasht	T0	560 a	1.27 a	112.2 a	118.2 a	19.8 a	123.1 a
	T1	54.1 ab	1.26 a	102.2 cd	107.1 b	17.8 b	110.6 b
	T2	54.6 ab	1.25 a	106.0 bc	108.9 b	18.1 b	112.4 b
	T3	55.5 a	1.24 b	107.2 b	110.5 b	18.6 ab	115.7 ab
	T4	54.9 a	1.24 b	106.8 b	108.4 b	17.8 b	110.5 b
	T5	52.3 b	1.25 ab	99.6 d	109.9 b	18.1 b	112.4 b
Ramsar	T0	55.4 a	1.24 b	108.4 a	108.3 a	20.0a	124.1 a
	T1	54.9 ab	1.22 b	106.2 a	105.9 ab	19.1 a	118.6 ab
	T2	53.6 bc	1.22 b	99.8 c	99.8 c	18.4 a	114.4 b
	T3	53.0 c	1.30 a	103.3 bc	102.5 bc	19.1a	118.5 ab
	T4	53.7 bc	1.22 b	106.3 ab	105.4 ab	18.9a	117.1 b
	T5	51.0 d	1.22 b	86.3 d	91.2 d	16.5 b	102.8 c

NS, *, **, or *** indicates non-significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively. Means within each column followed by the same letter are not different at $P \leq 0.01$ based on LSD test.

Table 4. Influence of CaCl₂ (1.5%) treatment and orchard location on specific rates of fruit dry matter accumulation (SRFDMA), excess dry matter per fruit (EDMF), fruit water content (FWC), fruit density (FD), relative growth index (RGI) and daily relative growth rate (DRGR) of 'Hayward' kiwifruit

		SRFDMA (mg DM gFW ⁻¹ d ⁻¹)	EDMF (%)	FWC(g)	FD (g cm ⁻³)	RGI	DRGR (g g ⁻¹ day ⁻¹)
Orchard (O)		*** †	***	***	***	***	***
Treatment (T)		***	***	***	***	***	***
O × T		***	**	***	***	***	***
Rasht	T0	1.041 a	99.490 a	98.4 a	1.054 b	1.938 a	0.417 a
	T1	1.032 b	99.433 b	89.3 b	1.049 b	1.931 b	0.378 c
	T2	1.031 b	99.442 b	90.9 b	1.028 c	1.933 b	0.384 bc
	T3	1.047 a	99.457 ab	91.8 b	1.027 c	1.934 b	0.389 b
	T4	1.025 cd	99.431 b	90.6 b	1.019 c	1.932 b	0.382 bc
	T5	1.022 d	99.441 b	91.8 b	1.107 a	1.930 b	0.386 bc
Ramsar	T0	1.145 a	99.414 a	88.4 a	0.999 b	1.927 a	0.349 a
	T1	1.126 b	99.386 a	86.2 a	0.971 c	1.924 ab	0.339 b
	T2	1.146 a	99.365 a	81.4 b	0.999 b	1.920 c	0.321 c
	T3	1.159 a	99.387 a	83.4 b	0.992 bc	1.922 bc	0.330 bc
	T4	1.118 bc	99.379 a	86.5 a	0.991 bc	1.925 ab	0.339 b
	T5	1.110 c	99.292 b	74.7 c	1.057 a	1.913 d	0.293 d

NS, *, **, or *** indicates non-significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively. Means within each column followed by the same letter are not different at $P \leq 0.01$ based on LSD test.

($P \leq 0.001$) reduced fruit length and FSA in both orchards. The inhibitory effects of CaCl₂ treatment increased along with increasing in application frequency.

As the Table 2 illustrates, fruits dry DM percent was higher in Ramsar orchard (18.28%) than in Rasht (16.62%), however preharvest calcium application did not have significant effect on fruit DM content.

The fruit physical dimensions (diameter, volume and length/diameter) also showed a significant difference between two orchards and CaCl₂ application. Furthermore, a significant difference was found between interaction effects of orchards and calcium treatment (Table 3). In both orchards, application of CaCl₂ significantly reduced fruit physical dimensions, whereas in Ramsar orchard, higher length/diameter was obtained in T3 (1.30) than T0 (1.24).

Percentage of fruit fresh weight was significantly ($P \leq 0.001$) affected by the orchard, calcium treatment and the interaction between orchard and calcium application. Preharvest application of calcium significantly reduced percentage of fruit FW from 118.2 to 106.9 g and from 108.3 to 91.2 g in control as compared with T5 in Rasht and Ramsar orchards, respectively (Table 3).

No significant difference was found between two orchards in fruit DM content (FDMC) (18.37 and 18.66 g DM fruit⁻¹ in Rasht and Ramsar, respectively). In contrast, CaCl₂ application significantly reduced FDMC, as the highest amount was found in control (19.82 and 19.97 g DM fruit⁻¹ in Rasht and Ramsar, respectively) (Table 3).

The results showed that along with decreasing DM content by CaCl₂ treatment (Table 2), SRFDMA (mg DM gFW⁻¹ d⁻¹), DDMA (mg DM fruit⁻¹ d⁻¹) and % EDMF also decreased by

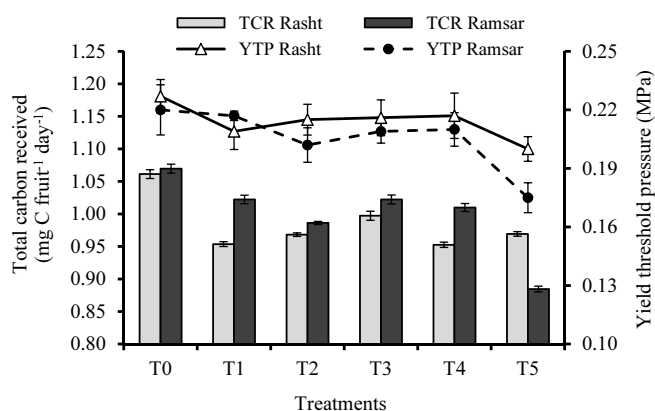


Figure 1. Effect of CaCl₂ (1.5%) application and orchard location on total carbon received (TCR) and yield threshold pressure (YTP) of 'Hayward' kiwifruit. The values are the means ± SE

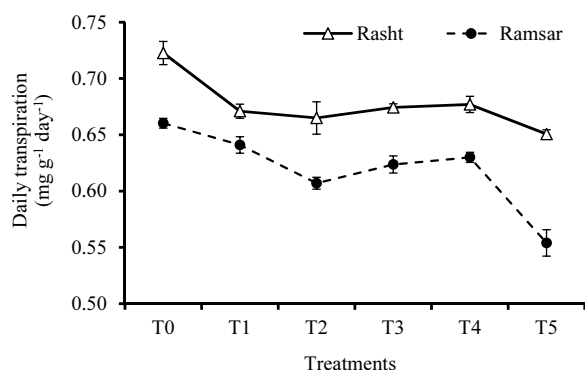


Figure 2. Effect of CaCl₂ (1.5%) application and orchard location on daily transpiration of 'Hayward' kiwifruit. The values are the means ± SE

CaCl₂ treatment (Tables 3 and 4). Moreover, it was revealed that inhibitory effect increased along with increase of CaCl₂ application times, as the thrice application had the lowest amount of above mentioned attributes.

Table 3 illustrates the comparison of fruit water content (FWC) under CaCl₂ treatment. Fruits harvested from Rasht orchard had higher FWC than Ramsar (19.14 and 83.41 g, respectively). Furthermore, CaCl₂ application gradually reduced FWC, as the highest FWC was found in control (98.38 and 88.39 g in Rasht and Ramsar, respectively).

Fruit density was significantly affected by orchard location, CaCl₂ treatment and their interaction ($P \leq 0.001$). Compared with the control, T5 significantly enhanced fruit density from 1.054 to 1.107 g cm⁻³ and from 1.005 to 1.057 g cm⁻³ in Rasht and Ramsar, respectively.

Fruit growth relative attributes

Orchard, CaCl₂ treatment and their interaction ($P \leq 0.001$) had significant effect on RGI and DRGR (Table 4). CaCl₂ treatment slightly reduced fruit relative growth characteristics, as the lowest amount was found in thrice application of CaCl₂ (T5).

Changes in TCR, yield threshold pressure (YTP) and daily transpiration showed the similar patterns (Fig. 1 and 2). It was found that along with increase in application times, these characteristics significantly reduced, as the T5 had the lowest amount as compared with others.

Sensory quality parameters

Sensory quality was assessed as a consequence of CaCl₂ treatment. No significant difference was found for fruits sensory quality in CaCl₂ treated and untreated fruits with the exception in T5. Both orchards showed similar results by CaCl₂ application. As Fig. 3 A and B showed when kiwifruit vines were sprayed three times with 1.5% CaCl₂ the sensory quality properties such as fruit pulp and flesh colour, flavor and firmness were improved.

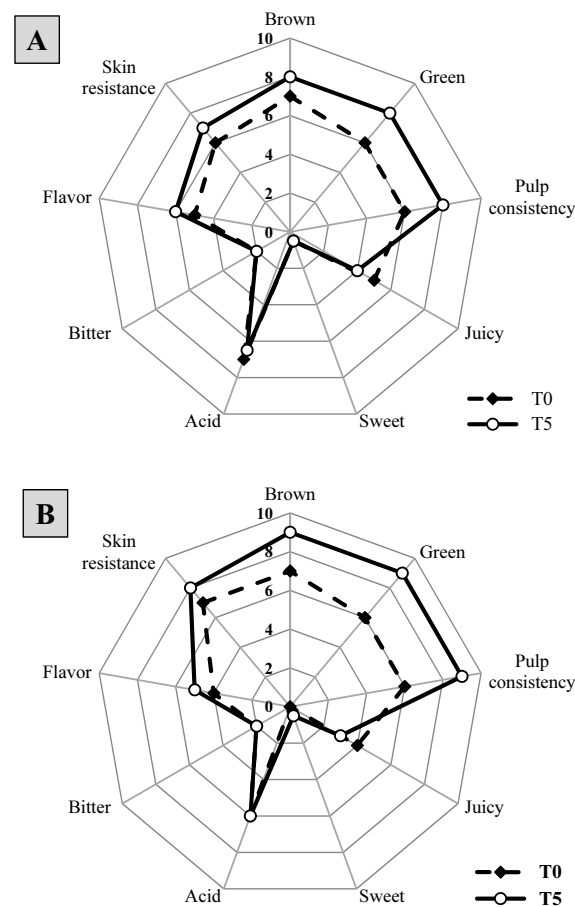


Figure 3. Fruit sensory attributes of thrice application of CaCl₂ (1.5%) treatment (T5) and control (T0) of 'Hayward' kiwifruit growing in Rasht (A) and Ramsar (B) orchards. For each variable, the possible range of values was 0-10

Discussion

The fruit size at harvest depends on a number of environmental and management factors and complex interactions between physiological processes (Basile et al., 2012). This study showed that thrice application of CaCl₂ reduced fruit size and growth related parameters. In part, it could be due to the role

of calcium in increasing mechanical strength of cell walls that can limit cell expansion. Cell elongation is influenced by external factors such as light, temperature, and plant growth regulators such as auxin and gibberellic acids (Heggie and Halliday, 2005). The ability of high concentrations of calcium ions to inhibit cell elongation has long been recognized. Furthermore, plastic extensibility was closely correlated with the growth rate of plant cells and decreasing in calcium content has the direct effects on increasing plastic extensibility and cell wall loosening (Virk and Cleland, 1988).

These results also showed that preharvest CaCl_2 application significantly reduced fruit growth relative attributes, as these inhibitory effects increased along with increasing in spraying times. The most negative effect was found when fruits were sprayed thrice with CaCl_2 during 35, 80 and 125 days after full bloom. The inhibitory effect of calcium on extensibility of the cell walls and growth would appear to be indirect. It could be a response to a calcium-mediated change in the cell wall metabolism, or it could simply be a consequence of the growth inhibition; inhibition of auxin-induced growth by respiratory inhibitors such as potassium cyanide (KCN), 2,4-dinitrophenol (DNP) and N-ethylmaleimide has been shown to result in a stiffening of the cell wall (Cleland and Rayle, 1977; Cunninghame and Hall, 1986; Jackson and Hall, 1993).

Alternatively, calcium may simply alter proteins or polysaccharides so that an H^+ -enhanced enzymatic wall-loosening reaction cannot occur (Holdaway-Clarke and Hepler, 2003; Hepler, 2005). Furthermore, recently calcium was suggested to inhibit cell elongation by destabilizing cortical microtubules via regulating a microtubule-destabilizing protein 25 (MDP25) (Li et al., 2011).

The import of sugars into the fruit is strongly related to fruit transpiration, indicating the importance of water loss through the fruit surface to fruit growth quality (Li et al., 2001). In addition, the relationships have been found between fruit calcium accumulation and fruit transpiration as affected by fruit microenvironment. It seems that decreasing in fruit transpiration by CaCl_2 application may be attributed to change in fruit cell properties (such as fruit surface area and fruit density) and decrease in fruit size. Furthermore, decrease in fruit transpiration could be caused by reduction in total carbon received by fruit and yield threshold pressure.

It was found that one and two times application of CaCl_2 had no significant effect on fruit sensory quality, while thrice application had better quality as compared with others. Sugar metabolism and carbon fluxes have important role in fruit sensory quality and their effects on quality traits may be opposite (e.g., enhancing water fluxes into fruit increases fruit size but decreases sugar concentration) (Génard and Lescourret, 2004; Lescourret and Génard, 2005). Improvement of sensory quality in thrice treated fruit by CaCl_2 (such as pulp and flesh colour, flavor and firmness) may be related to decrease in fruit size, higher sugar concentration and fruit density.

Conclusion

This study revealed that in most characteristics, there was no significant difference between one time application of CaCl_2 with untreated fruits, in contrast, thrice application of CaCl_2 had significant effect on fruit growth attributes and was more

effective in retarding fruit ripening process. Furthermore, fruit size, FW and total fruit DM content significantly decreased. The inhibitory effects of CaCl_2 application on fruit growth characteristics increased along with increasing in spraying times, while the thrice application of CaCl_2 (T5) showed the lowest values as compared with the control and other calcium treatments. Quality of a product encompasses sensory properties (appearance, texture, taste) and functional properties and defects. Thrice application of CaCl_2 produced firmer fruit and improved fruit sensory quality. In the light of these results, commercial application of thrice spraying with CaCl_2 (1.5%) can be considered for the retarding fruit ripening and maintenance of quality.

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