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Preharvest calcium applications improve postharvest quality of papaya fruits (*Carica papaya* L. cv. Eksotika II)

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ABSTRACT

This research was conducted to evaluate the effects of calcium chloride ($CaCl_2$) and calcium nitrate $Ca(NO_3)_2$ on nutrient concentrations and postharvest quality of papaya fruits. In the first experiment, plant stem height increased significantly after $Ca(NO_3)_2$ application compared to $CaCl_2$. The calcium content in the peel and pulp for both sources [$CaCl_2$ and $Ca(NO_3)_2$] significantly rose with increasing calcium concentrations, but there was a significantly higher content of calcium in fruit peel and pulp in the $CaCl_2$ treatment. Magnesium and potassium in fruits decreased with increasing calcium concentrations. A reduction in anthracnose lesion diameter in the infected fruit with increasing calcium was observed in both $CaCl_2$ and $Ca(NO_3)_2$ treatments. Ethylene production in fruits decreased with increasing calcium concentrations. In the second experiment, only $CaCl_2$ was used as the calcium source, and results showed that the calcium content in fruit peel and pulp significantly increased at higher $CaCl_2$ levels, whereas ethylene production, anthracnose lesion diameter, and magnesium content decreased compared to control.

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KEYWORDS
Anthracnose; calcium chloride; calcium nitrate; papaya; postharvest quality

Introduction

Plant nutritional status is an important parameter for determining quality at harvest and postharvest life of many fruits and vegetables. Deficiencies, excesses, or imbalances of nutrients result in disorders that can decrease the storage life of fruits and vegetables (Kader, 2002). Calcium (Ca) is particularly essential to plants as its deficiency reduces the growth of meristem and deforms the youngest leaves. Insufficient Ca levels lead to deterioration of cell walls, with the consequent death of plant cells and tissues (Mengel and Kirkby, 2001). Calcium is also used to reduce many postharvest disorders in fruits, such as bitter pit in apple, cork spot in pear, and blossom end rot in tomato (Kader, 2002). It has been estimated that only limited amounts of the Ca absorbed by roots (around 10%) are transported to fruit tissues (Wojcik, 2004). Generally, fruits with low Ca content are poor in quality and less suitable for storage (Agusti et al., 2004). In some species such as peach, stem elongation and length increase after foliar Ca spray (Alcaraz-Lopez et al., 2004).

An optimal equilibrium between Ca, magnesium (Mg), and potassium (K) is of key importance to plant nutritional status, as Mg^{2+} and K^{+} are known to compete with Ca^{2+} for binding sites at the plasma membrane (Mills and Benton-Jones, 1996; Yermiyahu et al., 1994). Therefore, high levels of K and Mg could potentially replace Ca at the plasma membrane surface, which could lead to increases in membrane permeability and fruit tissue susceptibility to Ca deficiency disorders (De Freitas and

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Mitcham, 2012; Yermiyahu et al., 1994). There are many commercial foliar Ca fertilizers in the European Union market (Wojcik, 2004). However, the efficiencies of preharvest sprays of Ca materials in improving the status of this nutrient in fruits seldom are higher compared to sprays of calcium chloride ($CaCl_2$) or calcium nitrate ($Ca(NO_3)_2$) (Wojcik and Borowik, 2013). Foliar spray of $CaCl_2$ has a significant effect on the postharvest quality of apples (Siddiqui and Bangerth, 1995). Also, $Ca(NO_3)_2$ strongly improves the postharvest quality of many fruits (Wojcik and Borowik, 2013).

Papaya (*Carica papaya* L., family Caricaceae) is a large perennial plant with a rapid growth pattern (Paull and Duarte, 2011). It is an important fruit in Malaysia, ranking third in exportation after durian and banana (Ali et al., 2010). In particular, "Eksotika II" is a high-yielding, good-quality papaya F_1 hybrid, released by Malaysian Agricultural Research and Development Institute (MARDI). Although the cultivar has gained popularity in the domestic and export markets, the harvested fruit is susceptible to anthracnose caused by *Colletotrichum gloeosporioides* Penz., one of the most devastating infections occurring during papaya postharvest storage (Madani et al., 2013; Shukor and Shokri, 1997). The most common intervention measures against the disease include the use of hot water and fungicides. Unfortunately, hot water can damage fruits, and the pathogen can progressively become resistant to some of the fungicides currently in use (Ali et al., 2010). Considering the increased interest in non-fungicidal management approaches, researchers are looking for new methods to maintain disease-free fruits in the postharvest milieu (Ali et al., 2010).

Studies on preharvest Ca application in papaya are few. Ramakrishna et al. (2001) applied $CaCl_2$ and $Ca(NO_3)_2$ to papaya fruits, observing that both the compounds sprayed in the preharvest phase reduced physiological loss in weight, enhanced firmness, and increased titratable acidity. Qiu et al. (1995) found no effect of $CaCl_2$ sprays on Ca concentrations in mesocarp tissues of papaya. Madani et al. (2013) indicated that increased Ca concentration (180 mg L^{-1}) applied to the leaves caused higher leaf Ca concentration, with concomitant decreases in Mg and K levels and increases in stem height and diameter. However, this study was carried out in greenhouse on plants during the vegetative phase, and fruit quality parameters were not evaluated. Therefore, the aim of this research was to evaluate the effects of $CaCl_2$ and $Ca(NO_3)_2$ on plant growth, nutrient, and postharvest parameters of papaya fruits. Disease severity of Ca-treated papaya fruits infected with *C. gloeosporioides* was evaluated in order to assess the eventual use of $CaCl_2$ and $Ca(NO_3)_2$ as fungicidal/fungistatic agents.

Materials and methods

First experiment

Forty-two papaya plants (*C. papaya* L., cv. Eksotika II) from a 4-month-old orchard at Agro-Tech Unit, University Agriculture Park (TPU), Universiti Putra Malaysia, Serdang, Selangor, Malaysia (3° 00′ 21.34″″ N; 101° 42′ 15.06″ E; 37 m elevation) were selected in 2012. Trees were approximately 1.5 m tall and spaced 3 × 3 m with two plants in each 3 × 3 m plot. Fertilization (nitrogen (N):phosphorus (P):K:Mg 12:12:17:2) was carried out uniformly each month around the canopy periphery, according to the Malaysia Ministry of Agriculture recommendations (Basir, 2005). Irrigation was carried out by overhead sprinklers at approximately 4-day intervals. Weeds were controlled by mowing as needed. Different concentrations of Ca (0, 34, 67, 100 mM) in the form of $CaCl_2$ and $Ca(NO_3)_2$ (approximately 1.5 L per tree) were sprayed 21 days after flower anthesis onto the fruits and leaves until runoff using a 16-L knapsack sprayer (Syarikat Jun Chong Sdn Bhd, Johor, Malaysia; solid cone nozzle, nozzle cap, 0.70–0.85 L min^{-1}), and Tween 20 (0.03% v/v) was used as a surfactant. Spray applications began in February 2012 and were repeated every 2 weeks for 6 times, with the final spray applied 4 days before fruit harvesting (April 18, 2012). For each Ca treatment, 12 trees were used.

Stem height was measured from the base of plants to the apical part of plants. Stem diameter of the plants was measured as 10, 80, and 160 cm from the base of plants, and the average of the three readings was calculated.

Uniformly sized (500–550 g) and shaped fruits were harvested at fruit ripening index 2 (green with a trace of yellow), washed with water, and allowed to air dry before analysis. For each treatment, lot of

24 fruits were harvested. From each lot, six samples of the peel and pulp were taken from the middle part of the fruits using a metal blade and dried at 70°C in an air-circulating oven. Then, 5 mL of 98% sulfuric acid (H_2SO_4) was added to the ground material, followed by 2 mL of 50% hydrogen peroxide (H_2O_2). Samples were put into a flask for digestion at 285°C for 40 min. After digestion, samples were left to cool, and then the solution was made up to a volume of 100 mL with deionized water. Samples were analyzed using an atomic absorption spectrometer (Model 3110; PerkinElmer Inc., Waltham, MA, USA) to determine Ca concentration in the peel and pulp, and K and Mg concentrations in the peel. Nitrogen concentrations in the peel were determined using an autoanalyzer (Model 403; Perkin Elmer Inc.). Nutrient content was expressed as mg g^{-1} dry weight (DW).

After fruit harvesting, papaya fruits were inoculated with a conidial suspension of *C. gloeosporioides*. Fruits were wounded on three sides to a depth of 3 mm, immersed for 30 s in a conidial suspension of 10^5 spore mm^{-1}, and drained and placed at 25 ± 2°C. Anthracnose lesion diameter of fruits was measured after 8 days. The average of three values was used for expressing the lesion diameter. Lesion diameter was measured on 12 fruits per treatment.

Ethylene production was measured on six fruits per treatment following the method of Saltveit (1982). Individual fruit was placed in a 1.9-L airtight plastic jar sealed and incubated for 2 h at room temperature (25 ± 2°C). After incubation, 1 mL of headspace gas was withdrawn with gas-tight hypodermic syringe and analyzed using gas chromatograph (GC) (model Clarus 500; Perkin Elmer Inc.). The GC was equipped with a combination of a thermal conductivity detector (TCD) and a flame ionization detector (FID) with a stainless steel column (Porapak Q, 50/80, Sigma Aldrich, Germany) to detect ethylene. The signals of TCD and FID were adjusted to >0.3 and 0.5 mV singly after ignition, respectively; H_2 and N_2 were used as the carrier gases with a flow rate of 30 mL min^{-1}. Standard ethylene (Air Products Pte. Ltd., Senoko, Singapore) was used for GC calibration. The amounts of ethylene were expressed as μL kg^{-1} h^{-1}.

Ascorbic acid measurements were carried out in six fruits per treatment using the dye method described by Ranggana (1986). Pulp tissues, 10 g from the mid-region of fruits, were homogenized with 40 mL of 3.0% (v/v) metaphosphoric acid using a kitchen blender (MX-799S; Panasonic Inc., Shah Alam, Selangor, Malaysia), and the mixture was filtered through a standard dye solution of 2,6-dichlorophenol-indophenol to pink color. The ascorbic acid content was expressed as mg 100 g^{-1} fresh weight (FW).

Peel color of fruits was measured on six fruits per treatment by a colorimeter (CR-300, Minolta Corp., Osaka, Japan) and expressed as lightness (L^*), chroma (C^*), and hue (h°). Color measurements were performed at the stem end, mid-region, and floral end of the fruit, and the average of the three readings was calculated. The L^* indicates the lightness of color with ranging values from 0 = black to 100 = white; C^* represents the hypotenuse of a right triangle with ranging values from 0 = least intense to 60 = most intense; (h°) is the angle of the tangent, where 0 = red purple, 90 = yellow, 180 = blue-green, and 270 = blue.

Second experiment

According to the performance of the first experiment, the second experiment was focused on $CaCl_2$ at higher Ca concentrations. Twenty-four papaya plants (cv. Eksotika II) from an 8-month-old orchard at Agro-Tech Unit, University Agriculture Park (TPU), Universiti Putra Malaysia, were selected in 2013. Different concentrations of Ca (0, 100, and 135 mM) in the form of $CaCl_2$ were sprayed 21 days after flower anthesis to the fruits, and Tween 20 (0.03% v/v) was used as a surfactant. Spray applications began in January 2013 and were repeated every 2 weeks for 6 times, with the final spray applied 2 days before fruit harvesting (March 28, 2013). For each Ca treatment, eight trees were used, whereas eight trees were not treated with Ca and maintained as controls.

Uniform sized (500–550 g) and shaped fruits were harvested at fruit ripening index 2 (green with a trace of yellow), washed with water, and allowed to air dry before being randomly divided into three different treatments. For each treatment, 32 fruits were harvested. Fruit samples for each treatment were taken at harvest. Ethylene production, color measurement, ascorbic acid content,

lesion diameter, and mineral content were measured in eight fruits per treatment according to the procedures described in the first experiment. The pH was measured in eight fruits per treatment by homogenizing 10 g of pulp from the mid-region of fruits in 40 mL of distilled water using a blender (Panasonic Inc.). The mixture was filtered with a cotton wool. Then, 5 mL of filtrate was used for pH measurement using a pH meter (model Micro pH 2000; Crison Instruments, Barcelona, Spain).

Statistical analysis

For the first experiment, three replications arranged in a randomized complete block design with two factors (Ca sources and concentrations) were used. For the second experiment, four replications arranged in a randomized complete block design were used. Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System version 8.2 (SAS Institute Inc., Cary, NC, USA). The means were compared using Duncan's multiple range test (DMRT) at a significant level of $p \leq 0.05$.

Results

First experiment

The application of $Ca(NO_3)_2$ better increased stem height compared to $CaCl_2$ (Table 1). However, no differences were found between $CaCl_2$ and $Ca(NO_3)_2$ for stem diameter (Table 1). Although stem height significantly increased with increasing Ca (17% higher at 100 mM Ca compared to control), stem diameter was not affected by Ca concentrations (Table 1). The use of $CaCl_2$ significantly raised the Ca levels in the peel and pulp compared to $Ca(NO_3)_2$. At 100 mM Ca concentration, Ca levels in the peel and pulp were 43% and 32% higher than those in controls (Table 1). The Mg and K peel levels were not significantly affected by Ca source (Table 1). However, with increasing Ca concentrations, the levels of both elements decreased significantly (Table 1). Moreover, there was a significant interaction at $p \leq 0.05$ between Ca sources and concentrations for peel N content (Table 1). Peel N content increased with increasing Ca concentration when $Ca(NO_3)_2$ was used as Ca source, whereas no changes were observed with increasing of $CaCl_2$ (Figure 1). However, the highest N was recorded in $Ca(NO_3)_2$ at 100 mM, whereas $CaCl_2$ decreased it at this rate of Ca (Figure 1).

Table 1. Effects of different Ca sources and concentrations on plant growth parameters ($n = 12$) and fruit mineral content of papaya ($n = 6$).

	Growth parameters		Mineral content (mg g^{-1} DW)				
	Stem		Ca		N	Mg	K
Factor	Height (cm)	Diameter (cm)	Peel	Pulp	Peel		
Ca source (S)							
$CaCl_2$	202.5 ± 4.8^{b}	9.2 ± 1.1^{a}	22.5 ± 1.0^{a}	11.1 ± 0.4^{a}	12.9 ± 0.3^{b}	6.6 ± 1.00^{a}	6.4 ± 0.6^{a}
$Ca(NO3)_2$	213.8 ± 3.1^{a}	9.4 ± 0.8^{a}	19.8 ± 2.5^{b}	10.5 ± 0.4^{b}	14.4 ± 1.3^{a}	6.5 ± 1.13^{a}	6.4 ± 1.2^{a}
Ca concentration (C) (mM)							
0	190.0 ± 2.0^{d}	9.3 ± 0.5^{a}	18.1 ± 3.8^{b}	9.7 ± 0.0^{c}	13.8 ± 1.7^{ab}	7.7 ± 1.4^{a}	6.7 ± 1.1^{a}
34	206.2 ± 9.2^{c}	9.1 ± 1.1^{a}	20.3 ± 2.3^{b}	9.9 ± 0.2^{c}	12.4 ± 0.5^{b}	7.1 ± 0.5^{ab}	6.3 ± 2.1^{b}
67	214.5 ± 14.1^{b}	9.4 ± 1.2^{a}	20.7 ± 2.3^{b}	10.9 ± 0.2^{b}	13.6 ± 0.9^{ab}	6.2 ± 0.1^{bc}	6.3 ± 0.5^{b}
100	222.6 ± 8.5^{a}	9.4 ± 1.2^{a}	25.5 ± 3.0^{a}	12.8 ± 1.3^{a}	15.0 ± 3.7^{a}	5.3 ± 0.2^{c}	6.4 ± 0.8^{b}
Interaction (S×C)	ns	Ns	ns	ns	*	ns	ns

Different letters within a column indicate significant differences between Ca sources and concentrations, according to DMRT at a significant level of $p \leq 0.05$. Values represents means ± standard deviation.
ns: non-significant at $p \leq 0.05$.
*Significant at $p \leq 0.05$.

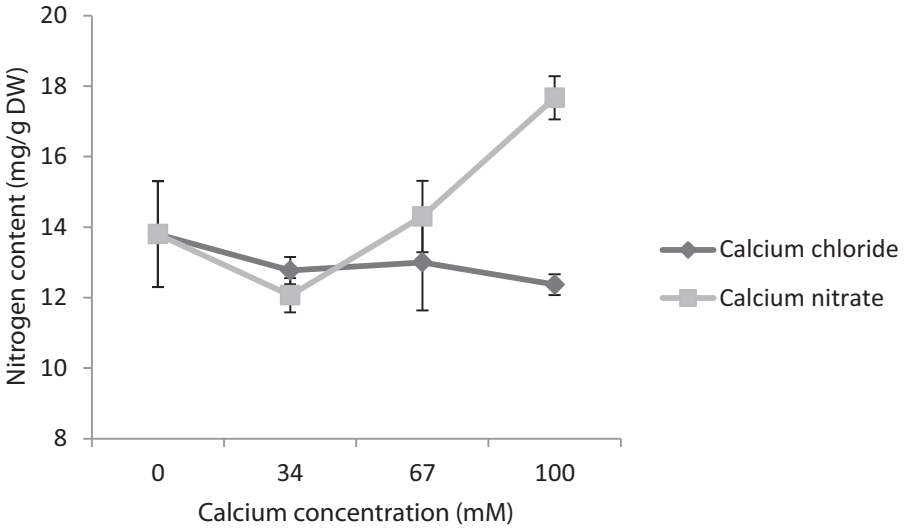

Figure 1. Interaction effects of Ca sources and concentrations on N content in the peel of papaya fruits. Vertical bars indicate the standard error of the means. Each mean value represents six fruits per treatment.

Ethylene and ascorbic acid levels were little affected by Ca source (Table 2). However, increasing Ca concentrations caused a significant drop in ethylene production and an increase in ascorbic acid content (Table 2). No statistical differences were observed between the two Ca sources for color parameters (L^*, C^*, h°). However, with increasing Ca concentrations, L^* and C^* decreased, whereas h° did not statistically change (Table 2).

There was a significant interaction at $p \leq 0.05$ between Ca concentrations and Ca sources for anthracnose lesion diameter (Table 2). Results of interaction showed that anthracnose lesion diameter decreased with increasing Ca concentration for both Ca sources (Figure 2). Also, data on the interaction effects for lesion diameter indicate that at all Ca concentrations, anthracnose lesion diameter was higher in the $Ca(NO_3)_2$ treatment than in $CaCl_2$ treatment (Figure 2).

Table 2. Effects of different Ca sources and concentrations on ethylene and ascorbic acid levels ($n = 6$), antrachnose damage ($n = 12$), and colometric parameters ($n = 6$) of papaya fruits.

				Color		
Factor	Ethylene (μL kg^{-1} h^{-1})	Ascorbic acid (mg 100 g^{-1} FW)	Anthracnose lesion diameter (mm)	Lightness (L^*)	Chroma (C^*)	Hue (h°)
Ca source (S)						
$CaCl_2$	4.9 ± 0.4^a	40.0 ± 5.4^a	16.1 ± 1.4^b	55.3 ± 3.7^a	45.0 ± 3.8^a	158.4 ± 11.5^a
$Ca(NO3)_2$	5.1 ± 0.8^a	39.9 ± 5.3^a	19.3 ± 2.6^a	54.8 ± 3.7^a	44.6 ± 7.3^a	157.5 ± 20.2^a
Ca concentration (C) (mM)						
0	6.1 ± 0.9^a	36.3 ± 1.7^c	22.0 ± 3.5^a	58.1 ± 5.1^a	47.1 ± 5.0^a	155.4 ± 10.4^a
34	5.5 ± 1.2^a	36.0 ± 0.5^c	18.7 ± 2.9^b	57.6 ± 4.7^a	45.9 ± 4.8^a	159.2 ± 11.6^a
67	5.1 ± 0.3^a	39.8 ± 1.0^b	16.1 ± 2.8^c	52.9 ± 6.3^b	43.1 ± 6.9^b	157.6 ± 9.7^a
100	3.4 ± 0.3^b	47.5 ± 3.2^a	14.4 ± 3.3^d	51.0 ± 7.2^b	43.1 ± 4.6^b	159.3 ± 11.0^a
Interaction (S × C)	ns	ns	*	ns	ns	ns

Different letters within a column indicate significant differences between Ca sources and concentrations, according to DMRT at a significant level of $p \leq 0.05$. Values represents means ± standard deviation.
ns: non-significant at $p \leq 0.05$.
*Significant at $p \leq 0.05$.

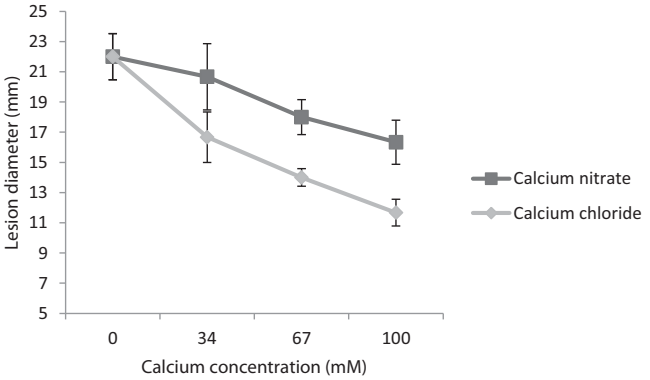

Figure 2. Interaction effects of different Ca sources and concentrations on anthracnose lesion diameter in papaya fruits after 8 days at 25 ± 2°C. Vertical bars indicate the standard error of the means. Each mean value represents 12 fruits in each treatment.

Second experiment

At the highest $CaCl_2$ concentration (135 mM), a significant rise in the fruit ascorbic acid content (+46%) and decreased ethylene production (−38.4%) were observed, compared to controls (Table 3). The pH was not significantly affected by Ca levels (Table 3). Lesion diameters noticeably decreased at 100 and 135 mM Ca compared to controls (Table 3). Regarding color parameters (L^*, C^*, h°), Ca concentrations of 100 and 135 mM provoked significant decreases in the values of L^* and C^*, whereas h° was not significantly influenced by any treatment (Table 3).

Calcium content in fruit peel and pulp and ascorbic acid levels significantly increased at higher $CaCl_2$ levels. In particular, the treatment with 135 mM Ca significantly increased Ca in fruit peel. However, significant decreases occurred at 100 and 135 mM $CaCl_2$ in Mg and K content, whereas no differences among $CaCl_2$ concentrations for peel N content were observed (Table 4).

Discussion

The primary goal of this research was to determine the effects of preharvest Ca application on plant growth, nutrient content, and postharvest quality of papaya fruits, as the effects of $CaCl_2$ and $Ca(NO_3)_2$ have not been studied in papaya trees grown in the field.

The increase in stem height observed in this study was significantly related to the concentrations of Ca applied (Table 1). Calcium is required for cell division and elongation, and it increases plant height by enhancing the mitotic activity in plant shoot meristems (Dodd et al., 2010; Mengel and Kirkby, 2001; Rab and Haq, 2012). Dordas (2009) found that $CaCl_2$ sprays are able to increase plant height in oregano. Similar results were found by Rab and Haq (2012) for tomato. Higher stem height for $Ca(NO_3)_2$ compared with $CaCl_2$ could be due to the fact that $Ca(NO_3)_2$ has the potential to encourage a more vigorous shoot growth, as observed by Elmer et al. (2007) for peach trees.

Table 3. Effect of different concentrations of $CaCl_2$ on ethylene and ascorbic acid levels ($n = 6$), pH ($n = 6$), antrachnose damage ($n = 12$), and colorimetric parameters ($n = 6$) of papaya fruits.

					Color		
Ca concentration (mM)	Ethylene (μL kg^{-1} h^{-1})	Ascorbic acid (mg 100 g^{-1} FW)	pH	Anthracnose lesion diameter (mm)	Lightness (L^*)	Chroma (C^*)	Hue (h°)
0	5.4 ± 0.4^a	33.1 ± 1.7^c	4.6 ± 1.5^a	27.5 ± 1.8^a	59.7 ± 2.1^a	47.2 ± 6.2^a	154.5 ± 14.1^a
100	4.3 ± 0.3^b	43.7 ± 2.2^b	3.6 ± 1.0^a	20.4 ± 2.2^b	53.7 ± 3.5^b	41.8 ± 4.2^b	160.2 ± 12.5^a
135	3.9 ± 0.6^b	48.4 ± 3.6^a	3.6 ± 1.4^a	17.2 ± 1.4^b	53.6 ± 2.4^b	41.2 ± 4.1^b	160.4 ± 13.6^a

Different letters within a column indicate significant differences among Ca concentrations, according to DMRT at $p \leq 0.05$. Values represents means ± standard deviation.

Table 4. Effect of different concentrations of $CaCl_2$ on the mineral content of papaya fruits ($n = 6$).

	Mineral content (mg g^{-1} DW)				
	Ca		N	Mg	K
Ca concentration (mM)	Peel	Pulp	Peel		
0	16.7 ± 2.1^{c}	5.1 ± 0.3^{b}	13.0 ± 1.9^{a}	7.7 ± 0.8^{a}	6.3 ± 0.6^{a}
100	24.2 ± 1.3^{b}	$6.0. \pm 0.5^{a}$	11.8 ± 1.6^{a}	5.7 ± 0.5^{b}	5.4 ± 0.5^{b}
135	29.6 ± 1.5^{a}	6.7 ± 1.1^{a}	11.2 ± 1.0^{a}	5.9 ± 0.6^{b}	5.1 ± 0.6^{b}

Different letters within a column indicate significant differences among Ca concentrations, according to DMRT at $p \leq 0.05$. Values represents means ± standard deviation.

In the first study, $CaCl_2$ appeared to be more efficient than $Ca(NO_3)_2$ in increasing Ca in the peel and pulp of fruits (Table 1). There are contradictory results in the literature about the Ca content in the fruit after different Ca treatments. Although Manganaris et al. (2005) reported that $CaCl_2$ is more effective in determining the increase of Ca in the peel and pulp of peaches than other Ca sources (e.g., Ca chelates), Wojcik and Borowik (2013) found no differences between $CaCl_2$ and $Ca(NO_3)_2$ in determining the Ca content in apples. Thus, it seems that diverse plant species react differently to different sources of Ca. Other findings showed that $CaCl_2$ foliar sprays have the effects of increasing the Ca content in the skin and pericarp of kiwifruit and in the peel of pomegranate fruit (Gerasopoulos et al, 1996; Ramezanian et al., 2009). Marked increases in the Ca content in the peel and pulp of papaya fruits (Table 1) suggest that exogenous Ca was able to cross fruit epidermis and was incorporated into the cell wall matrix of fruit cells Dodd et al. (2010) and Mengel and Kirkby (2001).

Calcium has a direct and positive effect on N assimilation and determines the increased N content by activating the enzymes responsible for N assimilation (Lopez-Lefebre et al., 2000). Higher levels of $Ca(NO_3)_2$ are able to determine higher N content in tomato fruits (Peyvast et al., 2009). Nitrogen is an important nutrient for plant growth. However, Fallahi et al. (1997) indicated that high N decreased fruit firmness and increased ethylene production and respiration rate in apple fruits. Furthermore, increasing ethylene production increased fruit decay that decreased storage life. In addition, high N triggers rapid fruit cell expansion, which resulted in further dilution of Ca in fruit (De Freitas and Mitcham, 2012). In the first experiment, the drops in Mg and K content with increasing Ca levels observed in papaya fruits (Table 1) could be relevant for understanding the antagonistic effects between Ca and the other two elements (Mills and Benton-Jones, 1996). Indeed, high K^+ and Mg^{2+} levels are able to replace the Ca^{2+} trapped in the cell wall polymers. This, in turn, increases plasma lemma permeability, produces leaky cell walls and membranes, and makes fruit tissues susceptibility to disorders and diseases (De Freitas and Mitcham, 2012).

It is known that ethylene is a ripening hormone, and its synthesis after harvest results in softening of fruits, which leads to the attack of postharvest pathogens. Decrease in ethylene production is one factor to delay the senescence of fruits (Bakshi et al., 2005). Reduction in ethylene production with increasing Ca concentration in papaya fruits (Table 2) suggests that Ca can delay fruit ripening by reducing ethylene emissions, thereby slightly retarding the climacteric rise (Hansford, 1994). Tzoutzoukou and Bouranis (1997) reported a strong decrease in ethylene production in apricots treated with $CaCl_2$. Calcium ions are often bound to calmodulin, which is one of the most common intracellular Ca receptors, and the accruing Ca–calmodulin complex modulates many physiological processes. Njoroje et al. (1998) found that calmodulin is involved in inhibition of ethylene biosynthesis, as Ca is able to inactivate some key regulator enzymes of the ethylene biosynthetic pathway via Ca–calmodulin-mediated reactions.

The delay in papaya fruits coloration after Ca application (Table 2) suggests that this element has the ability of delaying fruit ripening by inhibiting the biochemical patterns involved in this physiological process, as suggested by Haard and Chism (1996) and Bhat et al. (2012). These latter authors also reported a delay in the coloration of pears with $CaCl_2$ applications at increasing concentrations.

More than 90% of vitamin C in human regimes is delivered by fruits and vegetables (Lee and Kader, 2000). Significantly higher levels of ascorbic acid observed in papaya fruits after treatment with Ca (Table 2) could be caused by the inhibiting action of Ca on the activities of many oxidizing enzymes (e.g., ascorbic acid oxidase, peroxidase, catalase, and polyphenol oxidase) that use ascorbate as a substrate (Singh et al., 2005). Ramezanian et al. (2009) reported that preharvest $CaCl_2$ foliar sprays increased ascorbic acid concentration in pomegranate. Considering the antioxidative role of vitamin C and its beneficial effects on human health, the positive relationship between exogenous Ca application and ascorbic acid fruit content can be of particular interest for nutraceutical purposes.

The reduced fungal infection in Ca with increasing Ca concentrations might be due to the fact that the higher level of Ca in the fruit that stabilizes the cell wall structure and protects it from the pectinolytic enzymes secreted by the pathogen (Conway et al., 1994). Calcium could decrease the incidence and severity of the disease directly by inhibiting fungal spore germination and the activity of the cell wall-degrading enzymes produced by the fungus, thus preserving the host cell wall integrity (Biggs, 1999; Wisniewski et al., 1995). In addition, higher concentrations of cytosolic Ca have been shown to induce endogenous resistance mechanisms through the synthesis of phytoalexins and phenolic compounds that decrease the activity of pathogen pectolytic enzymes (Miceli et al., 1999). For example, Manganaris et al. (2005) used two different sources ($CaCl_2$ and calcium chelate) to investigate the effects of Ca on fungistatic protection in peaches, concluding that Ca salts decreased brown rot symptoms compared to non-treated fruits.

Based on the results of the first experiment, in which $CaCl_2$ increased the Ca content in fruits and decreased anthracnose disease in fruits compared to $Ca(NO_3)_2$, the second experiment was carried out only with $CaCl_2$. In this experiment, higher concentrations of $CaCl_2$ were used (up to 135 mM). The high $CaCl_2$ levels improved papaya fruit quality (in terms of increased Ca in the peel and ascorbic acid content), although no remarkable changes were found for ethylene production, anthracnose disease, peel color, and K and Mg content (Tables 3 and 4).

Conclusions

In conclusion, preharvest Ca sprays increased the Ca content and postharvest quality of papaya fruit. It seems that $CaCl_2$ could be a more suitable source of Ca for increasing the Ca content in fruit. Also, anthracnose lesion diameter was better controlled by this source than $Ca(NO_3)_2$. In contrast, $Ca(NO_3)_2$ had better effects on plant growth than $CaCl_2$. Calcium at 135 mM was better than other Ca concentrations to increase ascorbic acid and calcium content in the peel.

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