Improvement of Cold Tolerance in ‘Tahiti’ Lime Through Heat Treatments

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Abstract

The influence of heat treatments on the induction of cold tolerance in ‘Tahiti’ lime and their effects on ethylene biosynthesis and antioxidant enzymes were evaluated. Hot water dip (HWD) of fruit at 42°C for 15 min, delayed the onset of chilling injury (CI) symptoms for approximately 20 days at 1°C. Prestorage conditioning of fruit at 37°C for 10 h, HWD at 53°C for 2 min and HWD at 42°C for 15 min reduced the CI index, as compared with untreated fruit after 15 and 30 days at 1°C (plus 3 days of shelf life at 20°C). The ACC oxidase activity increased in parallel to the CI index in conditioned and untreated fruit. In contrast, in HWD at 53°C for 2 min the ACC oxidase activity remained constant. However, the ACC oxidase activity in fruit treated with HWD at 42°C for 15 min was lower in all the assessments, remaining almost the same over the storage period. There was a great increase in catalase (CAT) activity in all the fruits following 15+3 days. Afterwards, a rapid decline in CAT activity occurred, and it was associated with increased chilling injury. On the other hand, ascorbate peroxidase (APX) activity had a linear increase during storage in fruit treated with HWD at 53°C for 2 min, while the other heat treated fruit (42°C for 15 min) showed a slight increase in APX activity after 15+3 days, remaining constant along the storage period. According to our results, the reduction in activity of ACC oxidase, caused by HWD treatments enhanced the resistance of fruit to CI development.

INTRODUCTION

The exposure of ‘Tahiti’ lime fruit to low but not freezing temperatures still on a tree or during storage favours chilling injury (CI) development, especially after fruit rewarming during shelf life. Typical CI symptoms in citrus fruit are peel pitting which turns from brown to dark sunken lesions with increasing incidence (Sala and Lafuente, 1999). Although this disorder affects consumer acceptability and may result in commercial losses, it does not have effects on the internal fruit quality (Sala, 1998).

Since chilling sensitive fruits often have a reduced storage life, considerable efforts have been made in order to improve the fruit tolerance to low temperature (Lurie, 1998). Recently, it was reported that postharvest heat and temperature conditioning treatments may alleviate chilling symptoms in citrus fruit during storage (Sala and Lafuente, 2000; Porat et al., 2000; Kluge et al., 2006). This acquired tolerance to chilling is in part related to the capacity of tissue to scavenge reactive oxygen species by means of antioxidant enzymes (Sala, 1998; Wang, 2003).

In chilling-sensitive plants, it has been hypothesized that oxidative stress is a major component of chilling stress (Hodges, 1997). Supporting this hypothesis Sala (1998) reported that chilling tolerant citrus cultivars have a more efficient antioxidant system than chilling sensitive ones.

It has also been suggested that ethylene could play a role in the chilling development (Ben-Amor et al., 1999). In some species ethylene may alleviate or potentiate chilling

symptoms. In cantaloupe melon expressing an antisense ACC oxidase gene, the inhibition of ethylene synthesis was related to preventing chilling damage of fruit by lowering the membrane deterioration and maintaining higher activity of CAT, SOD and POX.

In this study, the influence of heat treatments on the induction of cold tolerance in 'Tahiti' lime and their effects on ethylene biosynthesis and antioxidant enzymes were evaluated.

MATERIALS AND METHODS

'Tahiti' lime fruit were harvested at commercial orchard located at Mogi Mirin, State of São Paulo, Brazil. Immediately the fruits were transported to the laboratory of Postharvest Physiology and Biochemistry of the 'Luiz de Queiroz' Agricultural College. After the selection and grading, according to uniformity of peel color and size, samples were randomly divided into four treatment lots of 120 fruit each, and then fruit were subjected to following treatments. Conditioning of fruit at 37°C for 10 hours; hot water dip (HWD) at 52°C for 2 minutes; HWD at 42°C for 15 minutes. As a control fruit were immersed in water at 20°C for 3 minutes. Following the treatments, the fruit were left to dry at room temperature for about 15 minutes and subsequently were stored for 45 days at 1°C (90% RH). After each storage period (15, 30 and 45 days) fruit were removed from storage and kept at 20°C for 3 days to simulate commercial storage and marketing conditions. The degree of CI was determined according to Kluge et al. (2006). Fruit were classified into five categories, according to the surface area affected, as follows: 0 = none; 1= 1-5%; 2= >5-25%; 3= >25-50%; 4= >50%. The *in vivo* ACC oxidase activity was determined in approximately 1g of peel discs which were incubated in 4 ml of reaction medium containing 5 mM ACC, 0.4 M mannitol in 25 mM phosphate buffer, pH 6. Vials of 40 ml were sealed and incubated for 1 h at 30°C, and the ethylene produced in the headspace of the vials was determined by gas chromatography and expressed as nL g^{-1} h^{-1}. At the same time flavedo tissue was removed from samples of each treatment, frozen in liquid nitrogen and stored until the enzyme assays.

Approximately 1g of frozen flavedo tissue was pulverized in a mortar and pestle with 3 ml of cold 100mM potassium phosphate buffer, pH 7.5 containing 1 mM ethylenediamine tetraacetic acid (EDTA), 3mM dithiothreitol (DTT) and 4% polyvinyl-polypyrrolidone (PVPP) to extract CAT and APX. The homogenate was centrifuged at 10.000 g for 30 minutes at 4°C and the supernatant used to determine the activity of CAT and APX. Catalase activity was determined at 25 °C according to Azevedo et al. (1998). One unit of CAT was defined as the amount of enzyme that decomposes 1 µmol of H_2O_2 min^{-1} at 25 °C. Ascorbate peroxidase activity was determined as described by Nakano and Asada (1981). One unit of APX was defined as the amount of enzyme that oxidized 1 µmol of ascorbate per minute. Protein was determined by the method of Bradford (1976), using bovine serum albumin (BSA) as a standard.

A completely randomized design was used with four replicates of 10 fruits. All data were evaluated through ANOVA, and subsequently, when statistical significance was found, were compared by Tukey test at $P \leq 0.05$.

RESULTS AND DISCUSSION

'Tahiti' lime fruit stored at 1°C develop chilling symptoms after two weeks storage, and the incidence increased considerably with storage time, mainly in control fruit (Fig. 1). In contrast, hot water dip (HWD) of fruit at 42°C for 15 min, delayed the onset of symptoms in approximately 20 days at 1°C. Furthermore, HWD at 42°C for 15 min and HWD at 53°C for 2 min were the most effective heat treatments in reducing developments of CI symptoms as compared to other treatments (Fig. 1 and 2). The positive effect of heat treatments on induction chilling tolerance of fruits has been attributed to principle that the exposure of

plants to moderate stress causes weak oxidative stress that stimulates antioxidant system and consequently induces tolerance to subsequent stress (Wang, 2003).

All the postharvest heat treatments significantly reduced the CI index, as compared with control fruit at 15 and 30 days at 1°C plus 3 days at 20°C (Fig. 2). However, after this period only HWD fruit remaining below the maximum CI index. Upon removal from storage after each period (15, 30 and 45 days) CI symptoms became more severe. In addition, at the end of storage, while HWD of fruit maintained CI index close to 3 prestorage conditioning and control fruit showed maximum index (4). Our results are in accordance with those reported by others researchers (Porat et al., 2000; Kluge et al., 2006).

Ethylene production was not detected up to two weeks storage at 1°C (data not shown). After this period, the rate of ethylene production remains low in all the fruits. Upon removal of fruit from cold storage a stimulation of ethylene synthesis occurred (data not shown). This ethylene evolution during rewarming of fruits was correlated with the development of chilling symptoms (Ben-Amor et al., 1999) and the increased ACC oxidase activity.

Dipping the fruits for 2 min at 53°C or for 15 min at 45°C suppressed significantly the ACC oxidase activity in flavedo tissue (Fig. 3). In previous work, Ben-Amor et al. (1999) reported that the inhibition of ethylene synthesis in melon expressing an antisense ACC oxidase gene results in an improvement of fruit resistance to chilling. In this way, one of the effects of ethylene on development of CI may be linked with a reduction of antioxidant enzymes (Ben-Amor et al., 1999). In our work we observed that catalase (CAT) activity was higher in the flavedo tissue of conditioned (37°C for 10 h) and HWD (42°C for 15 min) limes at 15 days of storage plus three days of shelf life (Fig. 4A). Afterwards, a sharp decline in CAT activity occurred, and it was associated with increased chilling injury. On the other hand, ascorbate peroxidase (APX) activity had a linear increase during storage in fruit treated with HWD at 53°C for 2 min, while the other heat treated fruit (42°C for 15 min) showed a slight increase in APX activity after 15+3 days, remaining constant along the storage period (Fig. 4B). At the end of storage period APX activity on HWD (53°C) fruit was about two-fold higher than other fruits. These results are in part in agreement to Sala and Lafuente (1999) and Rivera et al. (2004) who suggest that the effectiveness of conditioning treatments is related to induction and subsequent maintenance of antioxidant status of tissues.

In general, we observed that the effectiveness of HWD treatments may be related to the partial inhibition of the ethylene forming enzyme and maintenance of APX activity during storage which prevents the chilling on fruits at 1°C and subsequent rewarming.

CONCLUSIONS

The reduction of *in vivo* ACC oxidase activity, caused by HWD treatments results in an increased resistance of ‘Tahiti’ lime fruit to CI development.

HWD (53°C for 2 min) protected the fruit against CI possibly due to maintenance of APX activity at increasing levels during storage.

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Figures

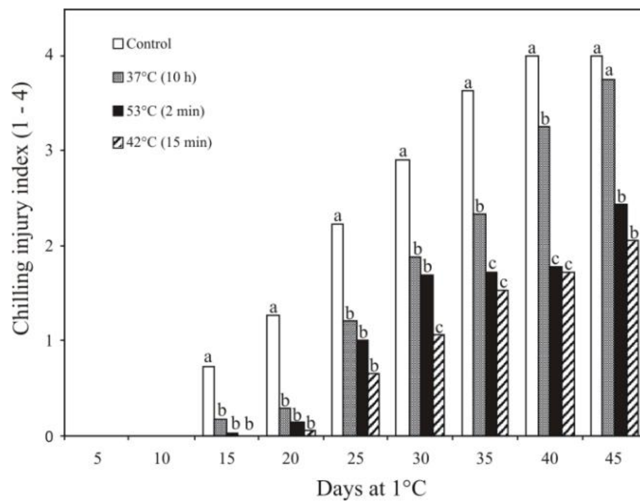

Fig. 1. Chilling injury index of Tahiti lime fruit heat treated and stored for 45 days at 1°C. Means separation by Tukey's test at P≤0.05.

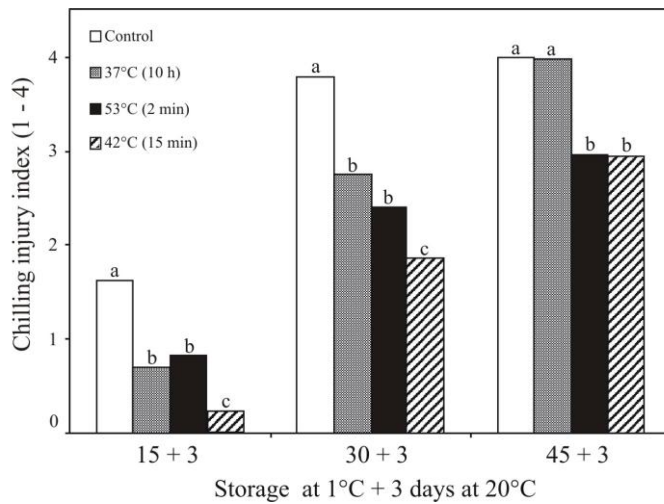

Fig. 2. Chilling injury index of Tahiti lime fruit heat treated and stored for 15, 30 and 45 days at 1°C plus 3 days at 20°C in each period. Means separation by Tukey's test at P≤0.05.

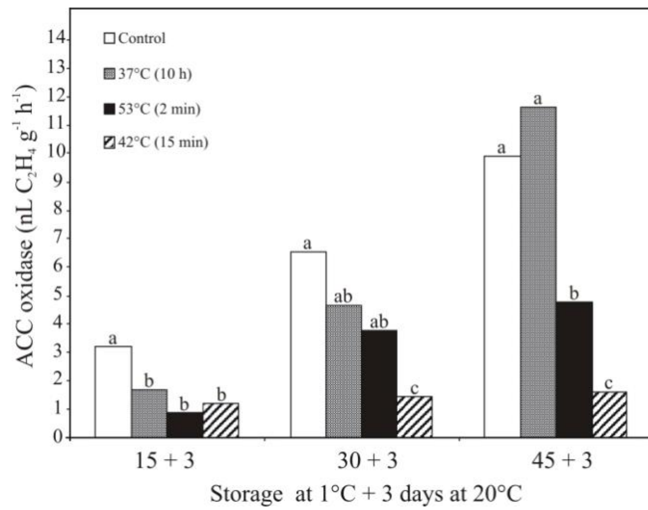

Fig. 3. *In vivo* ACC oxidase activity in Tahiti lime flavedo discs after 15, 30 and 45 days at 1°C plus 3 days at 20°C. Means separation by Tukey's test at P≤0.05.

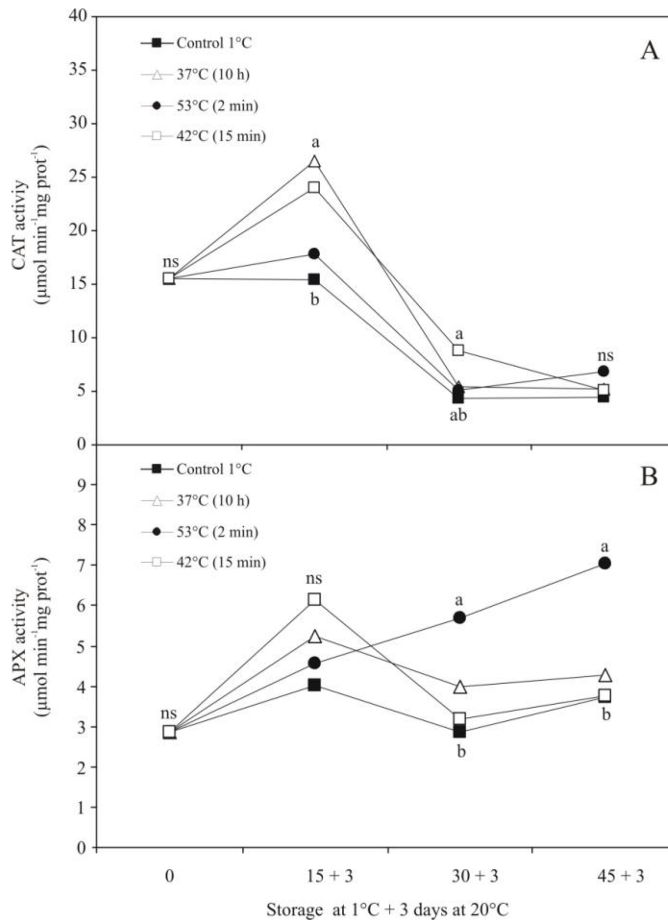

Fig. 4. Changes in the activity of catalase (A) and ascorbate peroxidase (B) in Tahiti lime flavedo after 15, 30 and 45 days at 1°C plus 3 days at 20°C. Means separation by Tukey's test at P≤0.05.