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PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES ASSOCIATED WITH CHILLING SENSITIVITY OF "FORTUNE" MANDARINS AND TEMPERATURE CONDITIONING

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Abstract

In this study, we have compared the levels of ABA in the flavedo of 'Fortune' mandarins harvested along the season with different tolerance to low temperature. We have also evaluated the effectivity of different heat conditioning treatments to reduce chilling damage and studied the effect of the most effective one (3d at 37°C) on abscisic acid (ABA), polyamines, ethylene production and phenylalanine ammonia-lyase (PAL) activity. No relationship between ABA and the susceptibility of 'Fortune' mandarins to chilling injury was observed (CI). The increased tolerance to cold storage induced by temperature conditioning appears not to be related to the induction of ABA, but could be related to the increase of putrescine. Conditioning induced a marked decrease in chilling-induced PAL activity and ethylene production, which appears to be related with the fact that the induction of ethylene and PAL activity are cold responses, rather than the cause of chilling symptoms.

Keywords

ABSCISIC ACID, CHILLING INJURY, CITRUS RETICULATA, ETHYLENE, HEAT CONDITIONING, PHENYLALANINE AMMONIA-LYASE, POLYAMINES.

1. INTRODUCTION

Chilling injury (CI) is responsible for substantial postharvest losses in a large number of horticultural crops. The development of effective methods to reduce CI requires the understanding of its physiological and molecular basis. The general objective of our research project is to study the physiological and biochemical basis associated with chilling sensitivity of citrus fruits, and specially in 'Fortune' mandarins, and with the effectivity of high temperature conditioning. We have focused our attention on the study of the changes in the hormones ABA, ethylene and in the levels of polyamines, since the synthesis of these compounds can be induced by different biotic and abiotic stresses and, therefore, these compounds may play an important role in the stress process. We have also undertaken to study the effect of low temperature storage and high temperature conditioning on changes in activities of phenolic metabolism enzymes, since these enzymes can be activated by stressful conditions and have been related with physiological disorders in plants (Hyodo et al., 1978).

In previous reports, we have shown that the difference in susceptibility to CI between the citrus cultivars 'Fortune' and 'Navelina' is more likely related to phenylalanine ammonia-lyase (PAL) activity than to other phenolic enzymes, related to darkening of plant tissues, such as polyphenol oxidase (PPO) and peroxidase (POD) (Martinez-Tellez and Lafuente, 1993). In a further study, we showed that cold storage induces an important increase in ethylene production of 'Fortune' mandarins that preceded the most important shift in the cold-induced PAL activity. The activation of this enzyme was paralleled to the development of chilling symptoms (Martínez-Tellez et al., 1994). Nevertheless, PAL activity itself was not directly related to the pitting and staining of the flavedo in 'Fortune' mandarins. It was also proposed that ethylene itself appears not be the direct cause of pitting, although could enhance the biochemical changes associated with this physiological disorder. Through the study of the changes in polyamines with cold storage, we found that the sensitivity of this cultivar to low temperature depends on the stage of development; and that associated with the reduced sensitivity to CI was a higher ability of the flavedo to increase the content of free polyamines, specially putrescine (Put) and spermidine (Spd), in response to low temperature (Gonzalez-Aguilar et al., 1994).

In this work, we compare the levels of ABA in the flavedo of 'Fortune' mandarins harvested at two season with different tolerance to low temperature. We have also compared the effectivity of different heat conditioning treatments on reducing chilling damage, and studied the effect of temperature conditioning (3d at 37°C) on ABA, polyamines, ethylene, and PAL activity, before and after exposure of the fruits to low temperature storage.

2. MATERIALS AND METHODS

"Fortune" mandarin (*Citrus reticulata*, Blanco) fruits were harvested from a commercial orchard at Sagunto, Valencia, Spain, and immediately delivered to the laboratory. Fruits were randomly divided in lots of 100 fruits. Two lots were stored, immediately after harvest, at 80-90% RH and at 2.5 or 12°C, respectively. Other lots were subjected to the different heat conditioned treatment, and then stored at the same temperatures and RH than the non-conditioned fruits. The temperature conditioning treatments assayed were: a) 3 d at 37°C and 80-90% RH; b) 3-min water dip-treatments at:b-1) 59°C, b-2) 53°C, b-3) 50°C, b-4) 47°C, b-5) 44°C; c) 6-min dip-treatment at 53°C; and d) 9-min dip-treatment at 53°C.

The estimation of CI index, the PAL activity assay and the ethylene measurements were done as previously described by Martinez-Tellez et al. (1994). Scald damage was estimated using also a visual scale from 0 to 3. Polyamines were analyzed according to González-Aguilar et al. (1994). The ABA was extracted from 1 g of flavedo with cold 80% acetone containing 0.5 g/l citric acid and 100 ml/l of the antioxidant butylated hydroxytoluene in an Omni-Mixer. Extracts were centrifuged at 3000g, and aliquotes of the supernatant were diluted in serial dilutions in ice-cold TBS (6.05g Tris, 0.2 mg MgCl2, and 8.8 g NaCl per l, at pH 7.8). Three samples were analyzed for each sample dilution, that were within the linear range of the ABA standard curve, by the indirect ELISA proposed by Walker-Simmons (1987).

3. RESULTS

The effectivity of the different temperature conditioning treatments assayed is compared in Fig. 1 and Fig. 2. The data shown in Fig. 1 and Fig. 2 correspond to fruits harvested in January 13th and February 28th, respectively. The susceptibility to CI of fruits harvested in January was higher than that of fruits harvested in February. In both experiments, however, the most effective conditioning treatment was the pre-storage of the fruits during 3 days at 37°C. The chilling index of the fruits exposed to the 3-min dip-treatment at 47° C and then stored at 2.5°C during 3 weeks, was similar to that of the 3 d cured fruits at 37°C (Fig.2). However, for longer storage periods the hot-dip treatment was less effective than the curing one. The effectivity of the 3-min dip-treatments at 44 or 50 °C to reduce chilling damage was low. These treatments, however, did not induce scald damage after storage of the fruits at 2.5 or 12°C for 10 weeks (Fig.2). Conditioning at 53°C during 3 min also reduced initially CI; but this treatment induced scald damage after long cold storage periods (data not shown). However, no scald damage occurred neither after the hot-dip treatment nor after the storage, of the dipped fruits, at 12°C. The degree of scald damage, after cold storage, increased with the dipping time (6 or 9 min) or with the temperature of the conditioning treatment (59°C). Thus, the most severe chilling and scald index was found in fruits treated during 9 min at 53° C or 3 min at 59 °C (Fig. 1). It is important to point out, that rot decay in heat conditioned fruits after storage at 12 or 2.5 °C was not relevant (data not shown).

To study the possible role of ABA on chilling sensitivity of 'Fortune' mandarins two approaches were used: we evaluated the changes in ABA levels in flavedo with storage temperature, as well as, the endogenous levels in flavedo of fruits harvested at different dates, in which fruits showed very different sensitivity to CI.

Changes in free ABA levels depend on storage temperature. No relationship between bound ABA and storage temperature was found, although after 25 d storage, the levels of bound ABA were about twice those of the recently harvested fruits (non stored fruits) (Fig. 3). The free ABA content remained nearly constant in fruits stored, for up to 25 days, at temperatures that caused CI (2.5°C and 5 °C). At 12 °C , however, the ABA content increased about 3-fold after 25 d storage. As we previously reported (Martínez-Tellez and Lafuente, 1993), the severity of CI, after 25 days storage, was higher in fruits kept at 2.5°C than in fruits kept at 5°C. However, the endogenous levels of free ABA was similar at both temperatures (Fig 3).

Changes in chilling susceptibility and endogenous levels of free and bound ABA with harvest date are shown in Table 1). The maximum susceptibility corresponded to fruits harvested at the beginning of January . At this date, the CI index of the fruits stored 14 d at 2.5°C was 1.7; whereas the CI index of fruits harvested early (4/12/1992) or later (11/2/93) on the season were 0.53 and 1.3, respectively. On the contrary, free and bound ABA increased with maturation. The lowest ABA levels corresponded to the most tolerant fruits (Table 1).

The effect of the most effective heat conditioning treatment assayed (3d at 37°C) on chilling-induced PAL activity, ethylene, ABA, and polyamines is summarized in table 2.

The ABA, and the PAL activity levels of the flavedo, as well as, the ethylene production of the fruits, did not substantially changed with conditioning. This treatment increased, however, 2.5, 4 and 5 times the levels of the three polyamines Put, Spd, and Spm, respectively.

After holding the fruits for 25 d at 2.5°C, it was observed that conditioning affected considerably to the chilling-induced changes in PAL and ethylene production. PAL levels of the non conditioned fruits increased from 27 to 157 units with cold storage. However, no increase occurred in the conditioned fruits. After storage at 12°C, no important change in PAL activity was observed. The ethylene production of the conditioned fruits stored 25 d at 2.5°C was slightly higher than that of the nonstored ones. However, conditioning of the fruits reduced considerably the chilling-induced ethylene production from 0.11 ml/h/kg FW to 0.04 ml/h/kg FW. After storage at 2.5°or 12°C ABA levels of conditioned and non conditioned fruits were similar. High temperature conditioning induced a important increase in the polyamine content of the flavedo. After holding at 12 or 2.5°C the levels of polyamines were ,in general, similar at both temperatures, and in conditioned and non conditioned fruits. With low temperature storage, the levels of polyamines of the non conditioned fruits increased, whereas that of the conditioned ones slightly decreased. Such decrease was more important in Spd and Spm than in Put.

4. DISCUSSION

Among the different heat treatments assayed, conditioning the fruits 3 d at 37°C was the most effective to reduce CI. This high-temperature effect on protecting the fruits against chilling has also been observed in other citrus cultivars (Spalding and Reeder, 1983; Chalutz et al., 1985; McDonald, 1989). The hot dip treatment at 53°C during 3 min can be also effective to reduce CI in grapefruits (McDonald et al., 1991; Schirra and Mulas, 1993) and in 'Fortune' mandarins (Martínez et al., 1993; Schirra and Mulas, unpublished data). No phytotoxic damage induced by this treatment has been reported. In our experiments we found that, after 70 d storage at 2.5°C ,but not at 12°C, conditioned fruits presented scald damage which increased with storage at low temperature. In this work we also have shown that more drastic pre-treatment conditions , higher temperatures or longer periods of treatment, increased the scald damage. The 47°C treatment was more effective than those of 44° and 50°C. After treatment at 50°C and storage for periods longer than 10 weeks, scald damage in cold stored fruits occurred (data non shown). Although scald damage did not appear before ten weeks at cold storage, probably the heat damage induced by the 50°C treatment enhanced the severity of pitting symptoms. Further research would be conducted to better understand the mechanism involved in such difference.

Stressful temperatures can alter the internal water status and thereby ABA production (Walton, 1980), or they can directly affect ABA levels (Cooper and Ho, 1987). It has also been shown that exogenous application of ABA (Rikin et al., 1976) and pretreatments with physiological stresses, which increased the endogenous levels of ABA, increased the tolerance of plant tissues to subsequent chilling (Kawada, 1980). These result have led to the suggestions that ABA play an important role on the adaptative mechanism to cope chilling stress. From our results, such implication appear not to be the case for 'Fortune' mandarin. This is supported by the fact that the free ABA content of 'Fortune' mandarins remained nearly constant in fruits stored at 2.5° or 5°C, but increased considerably in fruits kept at 10°C. This pattern was consistent with that found in other citrus cultivar ('Navelina'), which did not show CI during cold storage (Martinez-Tellez, 1993). This result indicates, that the changes in ABA levels with temperature storage are not directly associated with the susceptibility of 'Fortune' mandarins to chilling. This result agrees with that previously found in cucumber cotyledons (Lafuente et al., 1991) and in tomato fruits (Kubik et al., 1992). In tomato seedlings, however, low temperature induced ABA accumulation (Ludford and Hillman, 1990). It is evident then, that the increase in ABA as a cold response depend on the plant and on the specific tissue. On the other hand, we found that there is not any parallelism between chilling susceptibility of this cultivar and the endogenous levels of ABA at harvest time; which further support the idea that there is no relationship between ABA and the susceptibility of this cultivar to CI.

To study the biochemical and physiological changes associated with the thermal tolerance induced by heat conditioning, we selected the 3d conditioning at 37°C, which was the most effective one. This treatment did not induce important changes in ABA levels in the flavedo of the fruit. After storage, ABA levels of conditioned and non conditioned fruits were also similar. Daie et al. (1981) reported that ABA levels of tomato plants exposed to 35 or 45°C were significantly higher than those of plants grown at the optimal temperature of 25°C. From our results, we concluded that, as occurred in cucumber cotyledons (Lafuente et al., 1991), the increased tolerance to CI induced in this cultivar by temperature conditioning appears not to be related to the induction of higher endogenous levels of ABA. On the contrary, this conditioning treatment had a substantial effect on polyamines. After conditioning, the content of Put, Spd and Spm increased in the flavedo by 2.5, 4 and 5-times, respectively. Storage of the conditioned fruits slightly affected the content of Put, but substantially reduced the content of Spd and Spm. Thus, the beneficial effect of temperature conditioning in CI appears to be related to the increase in Put. The marked effect of this treatment on the initial content of polyamines and specially Spm, which is the polyamine with the most high number of positive charges of these molecules and consequently the highest antisenescent activity, may influence the behaviour of the flavedo after storage at low temperature. Kramer and Wang (1989) found a similar variation in the levels of polyamines of zucchini squash after conditioning. In cucumber cotyledons it was also found a polyamine increase with the beneficial heat conditioning treatment (Sanchez et al.,1994).

Conditioning, also induced a marked effect on chilling-induced PAL activity and ethylene production. These results, could indicate that conditioning increases tolerance of the fruit to cold temperature, and consequently the chilling-induced PAL activity and ethylene production is lower. We could also relate this conditioning-induced decrease in PAL and in ethylene production with the decrease in chilling symptoms. In a previous work, we found that PAL activity and ethylene, themselves, may not be the direct cause of CI, although ethylene could enhance the biochemical changes associated with this physiological disorder. The whole data indicate that the induction of ethylene and PAL activity are cold responses rather than the cause of pitting.

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Table 1: Change in chilling susceptibility and endogenous levels of free and bound ABA with harvest date of "Fortune" mandarins. Fruits were stored at 2.5°C during 14 days to estimate the severity of chilling damage.

		ABA (μg/FW)	
Harvest date	CI Index	Free	Bound
04.12.1992	0.5	1.17 ± 0.05	6.0 ± 0.7
05.10.1993	1.7	1.72 ± 0.09	7.0 ± 1.4
11.02.1993	1.3	1.72 ± 0.07	11.9± 0.4

Table 2: Effect of postharvest conditioning 3d at 37°C on chilling-induced PAL activity (nmol cinnamic acid/h/g FW), ethylene production (nl/h/kg FW), ABA (mg/g FW), and polyamines (mg/g FW) in fruits of 'Fortune' mandarins. These data represent the means of three replicates ± SE.

	Conditioning		After holding for 25 d at 2.5°C		12°C	
	Before	After	NC	C	NC	C
PAL	27±9	35±12	157±5	1±1	3±2	25±7
C_2H_4	9±1	16±6	107±35	48±12	14±2	6±1
ABA	0.30±0.03	0.20±0.03	0.27±0.05	0.28±0.05	0.9±0.2	0.9±0.1
Put	249±14	712±35	514±45	600±23	570±33	500±32
Spd	10±2	43±5	21±2	16±3	31±3	22±4
Spm	2.1±0.4	11.1±2.2	2.9±0.4	2.8±1.1	1±0.2	2.6±0.4

NC: non-conditioned fruits
C: conditioned fruits

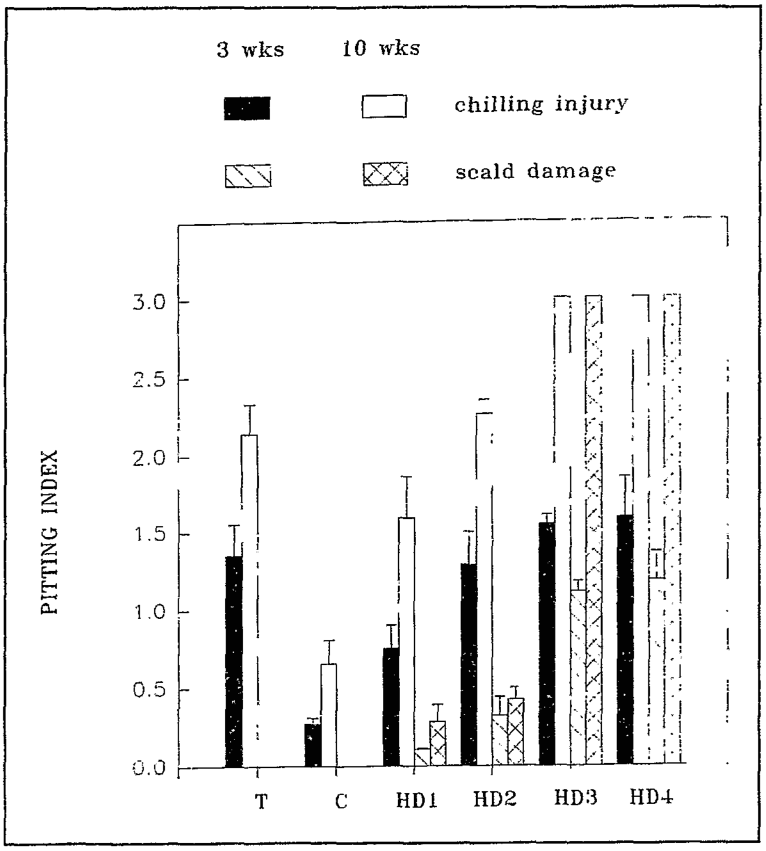

Fig 1. Effect of postharvest conditioning heat treatments on chilling and scald index in 'Fortune' mandarins stored 3 and 10 weeks at 2.5° C. The chilling and the scald index of the non- conditioned fruits (T) is compared with those of fruits exposed to the following temperature conditioning treatments: 3 d at 37°C (C); 3 min water dip-treatments at 53°C (HD1) and at 59°C (HD4); 6 min water dip-treatments at 53°C (HD2) and 9 min water-dip treatments at 53°C (HD3).

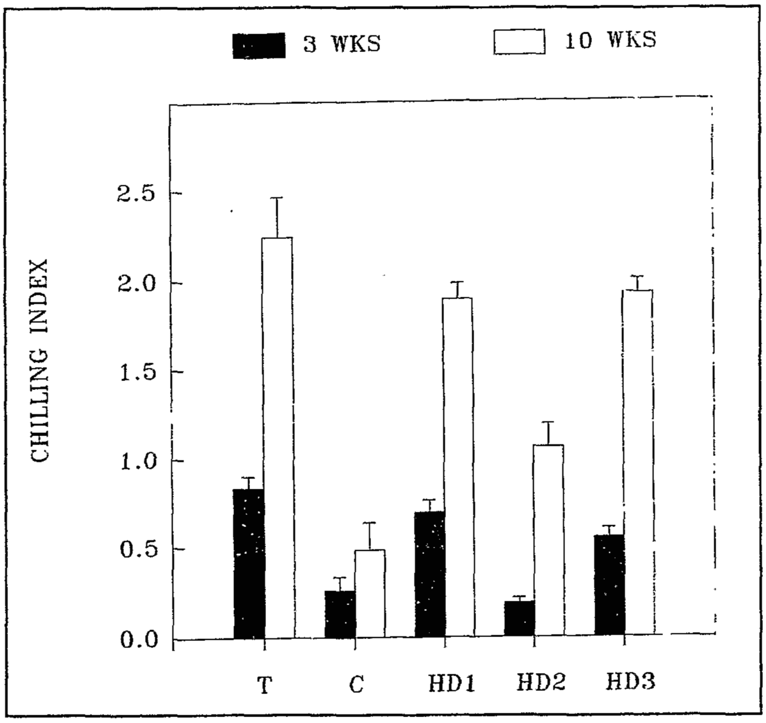

Fig 2. Effect of postharvest conditioning treatments on chilling index in 'Fortune' mandarins stored 3 and 10 weeks at 2.5 °C. The chilling index of the non conditioned fruits (T) is compared with that of fruits exposed to the following temperature conditioning treatments: 3 d at 37°C (C) and 3 min water dip-treatments at 44°C (HD1), at 47°C (HD2), and at 50°C (HD3).

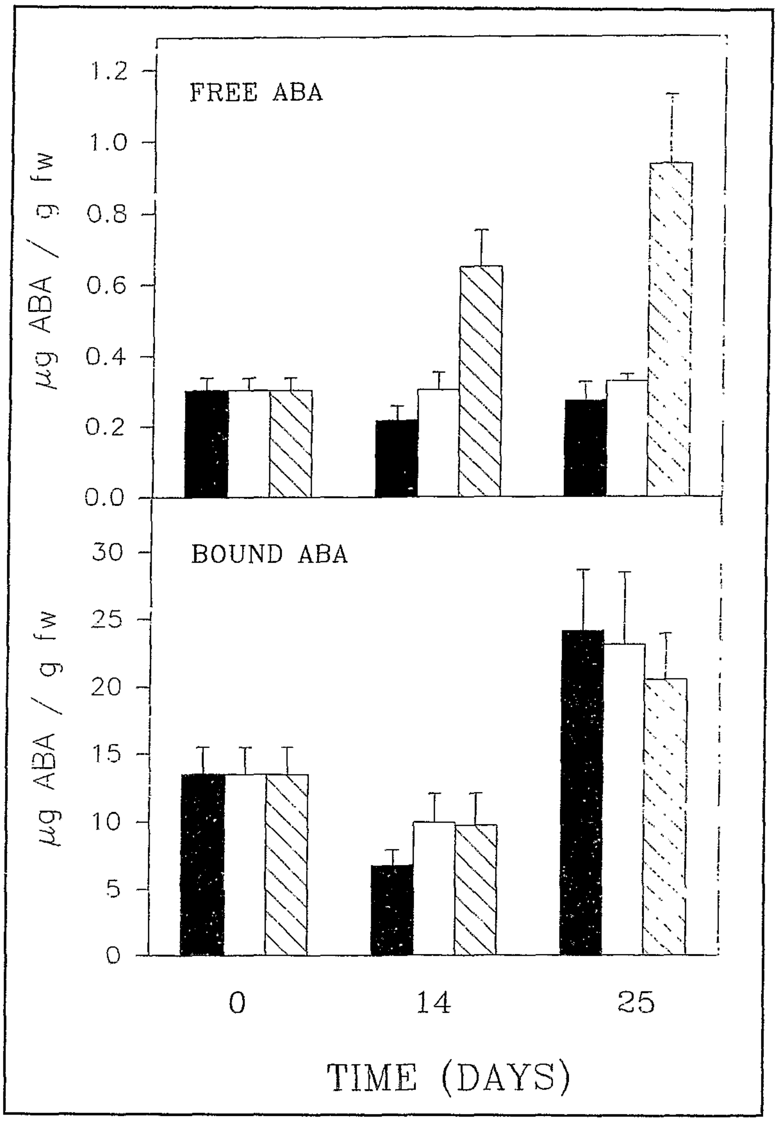

Fig 3. Changes in the endogenous levels of free and bound ABA levels in the flavedo of 'Fortune' mandarins with storage at 2.5°C (black column), 5°C (white column), and 10°C (striped column). Values are the mean of three replicates ± SE.