Accumulation of Pal Transcript and Pal Activity as Affected by Heat-Conditioning and Low-Temperature Storage and Its Relation to Chilling Sensitivity in Mandarin Fruits

M. T. Sanchez-Ballesta,[†] L. Zacarias,[†] A. Granell,[‡] and M. T. Lafuente*,[†]

Instituto de Agroquímica y Tecnología de Alimentos, CSIC, Apdo Correos 73, Burjassot, 46100 Valencia, Spain, and Instituto de Biología Molecular y Celular de Plantas, CSIC-UPV, Camino de Vera s/n, 46022 Valencia, Spain

The effects of different periods of heating at 37 °C on phenylalanine ammonia-lyase (PAL) and how this relates to chilling tolerance was investigated in fruits of the chilling-sensitive Fortune mandarin. All effective heat-conditioning treatments caused an early and transient increase in PAL mRNA and PAL activity. Conditioning fruits at 37 °C for 1 or 2 days prevented the manifestation of chilling symptoms but not the accumulation of PAL mRNA and PAL activity observed in untreated fruits. In fruits conditioned for 3 days, cold-induced damage and PAL activity were also suppressed but not the accumulation of PAL transcript upon subsequent storage at 2 °C. Storage of 3-day-heated fruits at a nonchilling temperature (12 °C) induced an early and transient increase in both PAL mRNA and PAL activity. High levels of PAL transcript and PAL activity were detected in freshly harvested fruits of a chilling-resistant mandarin (Hernandina) that decreased upon cold storage at 2 °C in heat-treated and nontreated fruits. These results indicate that sensitivity of mandarins to chilling correlates with low constitutive levels of PAL mRNA and PAL activity and with the inducibility of both upon exposure to low temperatures.

Keywords: *Chilling injury; citrus; cold storage; heat-conditioning; heat-shock; phenylalanine ammonia-lyase; wounding*

INTRODUCTION

Fortune mandarin (*Citrus clementina* Hort. Ex Tanaka × *Citrus reticulata*, Blanco) is a late ripening citrus cultivar that is greatly appreciated for the quality of its fruit but unfortunately is very sensitive to chilling injury (CI). Pitting, necrosis, and staining of the flavedo tissue (the outer pigmented portion of the peel) of this citrus cultivar occur during postharvest storage when temperatures fall below 5 °C (Martínez-Telléz and Lafuente, 1993). The physiological and molecular mechanisms underlying this disorder are little known, as are the factors that determine the sensitivity to chilling, which itself may change during the season (Lafuente et al., 1997) and from season to season (Holland et al., 1999).

Phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) has been found to be associated with postharvest disorders induced after prolonged storage at low temperature (Martínez-Telléz and Lafuente, 1997). This enzyme catalyzes the first reaction of the biosynthesis of a large class of natural plant products based on the phenylpropanoid backbone (Hahlbrock and Sheell, 1989; Dixon and Pavia, 1995). PAL was one of the first plant "defense genes" identified, and both PAL mRNA levels and PAL activity accumulate in response to different external stimuli such as wounding, light, low temperature, fungal elicitors, and pathogens (Lawton and Lamb, 1987; Dixon and Lamb, 1990; Christie et al., 1994; Diallinas and Kanellis, 1994). With regard to citrus fruits, the induction of PAL activity is concomitant with the development of chilling symptoms in Fortune mandarin (Martínez-Téllez and Lafuente, 1993; Sanchez-Ballesta et al., 2000), and it has also been related to the peel damage induced by γ radiation in Shamouti oranges (Riov et al., 1968) and to the healing process occurring after mechanical wounding in Valencia oranges (Ismail and Brown, 1979).

Prestorage heat treatments may increase chilling tolerance in different plant commodities during postharvest storage, and this ability has been found to be contingent on the presence of heat-shock proteins (HSPs) (Lafuente at el., 1991; Lurie and Klein, 1991; Woolf et al., 1995; Sabehat et al., 1996). Heat-shock (HS) causes a transient but profound alteration in gene expression in plants, resulting in the induction of HSPs and the suppression of the synthesis of some of the normally expressed proteins (Ho and Sachs, 1989). In most cases, the molecular effects of HS have been studied in plants shortly after a brief exposure to high temperature, and very little is known about the long term effects of prolonged exposures at high and low temperatures such as those used for extending fruit storage life. Only a few studies have been conducted in fruit, and they indicate that concomitant with the increase in abundance of HS mRNAs, transcripts corresponding to fruit ripening genes disappear (Picton and Grierson, 1988; Lurie et al., 1996). In previous papers, we have shown that the conditioning of Fortune mandarin fruits at 37 °C for 3 days prevented cold-induced damage (Gonzalez-Aguilar at al., 1998) and caused an increase in the activities of catalase, ascorbate peroxi-

* To whom correspondence should be addressed (telephone 34-96-3900022; fax 34-96-3636301; e-mail postco@iata.csic.es).
[†] IATA-CSIC.
[‡] IBMCP-CSIC-UPV.

10.1021/jf991141r CCC: $19.00 © 2000 American Chemical Society
Published on Web 06/10/2000

dase, and superoxide dismutase (Sala and Lafuente, 1999). This heat treatment prevented the increase in PAL activity that normally occurs around the necrotic zones of Fortune fruits when stored at low temperatures (Martínez-Téllez and Lafuente, 1997; Sanchez-Ballesta et al., 2000). A similar effect of HS has been observed in plants exposed to stress conditions and results in the induction of PAL activity. Thus, an HS given before ultraviolet exposure prevented the ultraviolet-induced increase in PAL activity in cultured parsley cells (Walter, 1989), and it caused a reduction in PAL activity and in the corresponding accumulation of phenolic compounds in wounded lettuce segments (Loaiza-Velarde et al., 1997). The mechanism by which heat treatments have such dramatic effects on PAL and how this relates to the ability of the fruit to withstand chilling stress are not understood.

In this paper, we have examined the effect of conditioning fruits at 37 °C on the accumulation of PAL mRNA and PAL enzymatic activity and how this relates to the chilling tolerance of Fortune fruits when subsequently stored at low temperature. Furthermore, we have also characterized the PAL response in Hernandina mandarin fruits, a citrus cultivar tolerant to chilling.

MATERIAL AND METHODS

Plant Material. Fruits of the hybrid mandarin Fortune (*C. clementina* Hort. Ex Tanaka × *C. reticulata*, Blanco) and fruits of Hernandina mandarin, a spontaneous mutation of one of the Fortune parentals, Clementino Fino (*C. clementina* Hort. Ex Tanaka), were harvested from trees grown at Sagunto, Valencia, Spain. For each experiment, fruits of both cultivars were randomly sorted into two lots. The first lot was subdivided into three replicates of 20 fruits to visually estimate the CI index. The second lot, made up of 15 fruits per temperature and storage period, was used to evaluate the changes in PAL gene expression and PAL activity. Fruits of both cultivars were stored for up to 21 days at 2 °C (chilling temperature) and 80−85% RH. Flavedo tissue was excised from the fruits, cut into small pieces, frozen in liquid nitrogen, and stored at −80 °C for later analysis.

Heat-Conditioning and Storage Temperatures. Fortune mandarins were first conditioned to 37 °C and 90−95% RH for 4 and 10 h and 1, 2, and 3 days. After 1, 2, and 3 days of heat-conditioning, fruits were stored at 2 °C and 80−85% RH under the same conditions as freshly harvested fruits. Another lot of Fortune fruits that had been conditioned for 3 days was stored at a nonchilling temperature (12 °C) and 80−85% RH. Hernandina fruits were heat-conditioned at 37 °C and 90−95% RH for 3 days and then stored at 2 °C and 80−85% RH.

Wounding. To study the effect of heat treatment on wound-induced PAL mRNA levels and PAL activity, Fortune fruits were treated for 3 days at 37 °C and then wounded by pressing them with a sharp edged metal brush that performed about 9 punctures/cm^2 of 2−3 mm depth throughout the flavedo tissue. Heat-treated and nontreated fruits were kept at 20 °C for 27 h.

Estimation of CI Index. Brown pit-like depressions in the fruit are the main CI symptoms in Fortune mandarins. Fruits were visually inspected and a score assigned to estimate the degree of injury. A rating scale ranging from 0 (no injury) to 3 (severe injury), based on necrotic surface and intensity of browning, was used. The extent of injury was described as the CI index and determined using the following formula:

$$\sum[\text{pitting scale } (0-3) \times \text{number of corresponding fruits within each class}]/\text{total number of fruits}$$

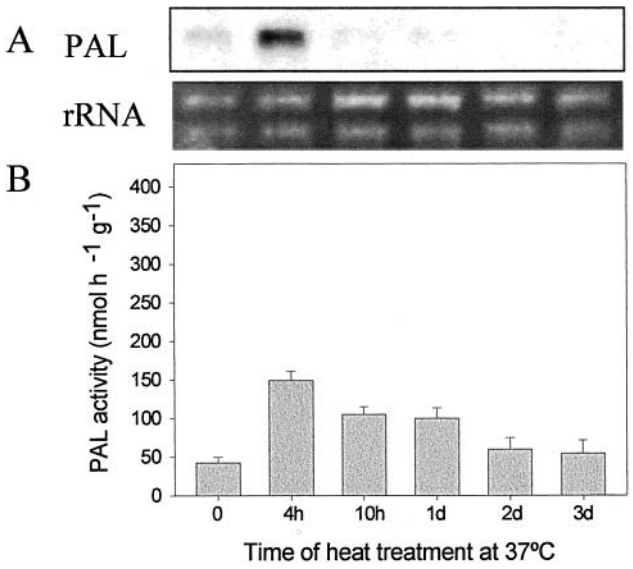

Figure 1. Time-course of accumulation of PAL mRNA (A) and activity (B) in Fortune mandarins exposed for up to 3 days at 37 °C and 90−95% RH. PAL activity values are the mean of three replicate samples ± SE. Eight micrograms of total RNA extracted from flavedo tissue was fractionated, blotted, and hybridized with FPal2 probe. Equivalence of RNA loading of the lanes was demonstrated by ethidium bromide staining.

RNA Extraction and Northern Analysis. RNA was isolated from 4 g of flavedo tissue by the method of Cathala et al. (1983). Aliquots of 8 μg of total RNA were denatured at 65 °C and fractionated on a 1.2% (w/v) agarose−formaldehyde gel. RNA loading was checked on ethidium bromide-stained gels. The RNA was transferred to a Hybond-N membrane (Amersham) for at least 15 h and cross-linked using a UV Stratalinker 800 (Stratagene, La Jolla, CA). Membranes were prehybridized and hybridized at 65 °C in 330 mM sodium phosphate buffer (pH 7.2), 1 mM EDTA, and 7% (w/v) SDS. A FPal2 cDNA isolated from a cDNA library of Fortune mandarins stored for 21 days at 2 °C was used as probe (Sanchez-Ballesta et al., 2000). Probes were prepared by random primer labeling using ^{32}P-dCTP. Membranes were washed twice for 10 min in 2× SSC (150 mM NaCl and 15 mM trisodium citrate, pH 7.0) and 0.1% (w/v) SDS (room temperature) and then twice in 0.1× SSC and 0.1% (w/v) SDS (65 °C) for 15 min. Membranes were exposed to Kodak X-Omat SX film with intensifying screens at −80 °C.

PAL Activity Assay. PAL activity was determined as previously described by Martínez-Téllez and Lafuente (1997). PAL was extracted from 0.4 g of acetone powder of flavedo tissue with 100 mM sodium borate buffer, pH 8.8, containing 20 mM β-mercaptoethanol and was partially purified by precipitation with ammonium sulfate at a final saturation of 46%. PAL activity was measured by determining the absorbance of cinnamic acid at 290 nm over a period of 2 h at 40 °C. The activity of the enzyme was expressed on a dry matter basis as nmoles of cinnamic acid h^{-1} (g of acetone powdered flavedo tissue)$^{-1}$.

RESULTS

Effect of Heat-Conditioning at 37 °C on PAL mRNA Levels and PAL Activity in Chilling-Sensitive Fortune Fruits. Treatment of Fortune fruits at 37 °C produced a transient accumulation in the levels of PAL mRNA and PAL activity. The levels of PAL transcript sharply increased in the flavedo of those fruits exposed to 37 °C for 4 h but fell to undetectable levels in those maintained for 10 h (Figure 1A). An important increase in PAL activity also occurred within 4 h, but in contrast to transcript levels, PAL activity declined very slowly thereafter (Figure 1B).

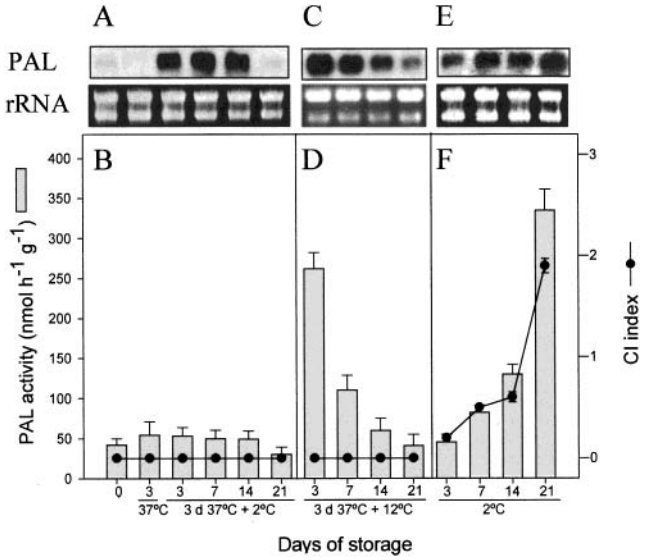

Figure 2. Effect of conditioning Fortune mandarins for 3 days at 37 °C and 90−95% RH on PAL mRNA accumulation (panels A, C, and E) and PAL activity and CI index (panels B, D, and F) in flavedo tissue of fruits stored at chilling and nonchilling temperatures. Conditioned fruits were stored at 2 °C (panels A and B) and 12 °C (panels C and D), and nonconditioned fruits were stored at 2 °C (panels E and F) for up to 21 days. PAL activity values are the mean of three replicate samples ± SE. Eight micrograms of total RNA was fractionated, blotted, and hybridized with FPal2 probe. Equivalence of RNA loading of the lanes was demonstrated by ethidium bromide staining.

The effect of different lengths of exposure to heat treatment on PAL mRNA levels and PAL activity was studied in fruits stored for up to 21 days at 12 and 2 °C. An early transitory increase in the levels of PAL mRNA, but not PAL activity, was observed when 3-day-conditioned fruits were stored at 2 °C (Figure 2, panels A and B). By comparison, a dramatic but transient increase in PAL mRNA levels, which was paralleled by the enzyme activity (Figure 2, panels C and D), was observed when conditioned fruits were stored at the nonchilling temperature (12 °C). In the latter, the highest levels of PAL transcript and enzyme activity were detected after 3 days at 12 °C, and by 21 days, mRNA accumulation and PAL activity were similar to those of freshly harvested fruits. As previously reported (Sanchez-Ballesta et al., 2000), Fortune fruits that were not heat-treated showed a later but steady accumulation of both PAL mRNA and PAL activity during storage at chilling temperature that occurred in parallel with chilling symptoms (Figure 2, panels E and F). No chilling symptoms were detected in heat-conditioned fruits (Figure 2B).

The pattern of accumulation of PAL mRNA and enzyme activity during storage at 2 °C was different in fruits conditioned for less than 3 days (Figure 3, panels A and B). PAL mRNA levels and PAL activity increased in 1- and 2-day-heat-conditioned fruits after 7 days of storage at 2 °C. After this period, the accumulation of transcript was greater in the flavedo of the 2-day-conditioned fruits, but PAL activity was about half that of those fruits conditioned for 1 day. Despite these early differences, prolonged exposure (14−21 days) at 2 °C resulted finally in fruits with similar levels of PAL activity and no chilling symptoms.

Effect of Heat Treatment on Wound-Induced PAL mRNA Levels and PAL Activity in Fortune Fruits. To investigate whether the heat-conditioning

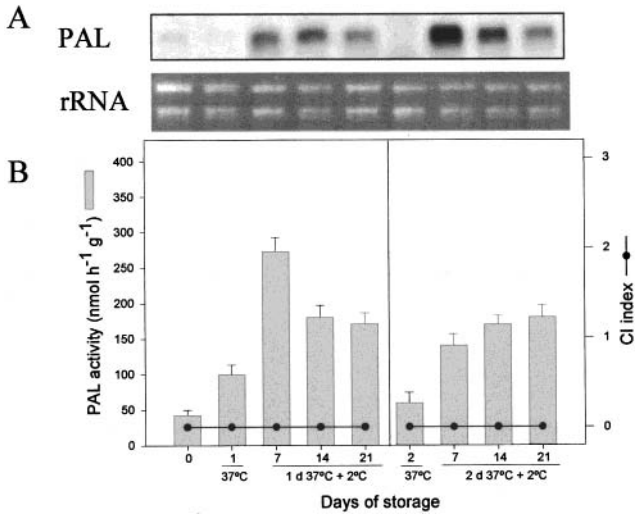

Figure 3. Effect of time of conditioning Fortune mandarins at 37 °C and 90−95% RH on PAL mRNA accumulation (A) and PAL activity and CI index (B) in the flavedo of fruits stored for up to 21 days at 2 °C. PAL activity values are the mean of three replicate samples ± SE. Eight micrograms of total RNA was fractionated, blotted, and hybridized with FPal2 probe, and equivalence of RNA loading of the lanes was demonstrated by ethidium bromide staining.

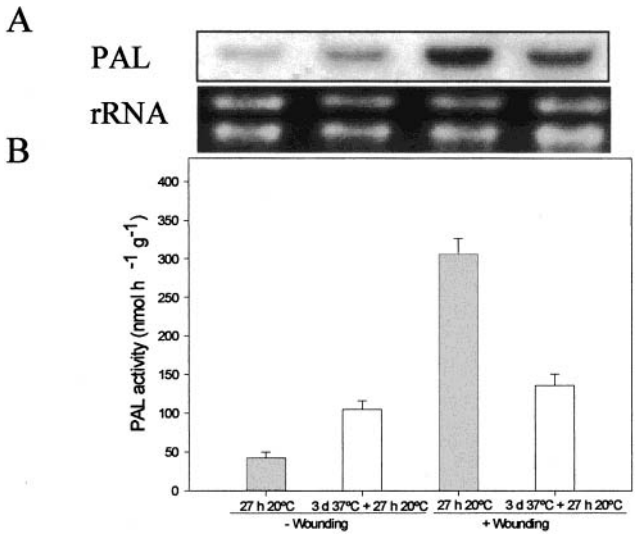

Figure 4. PAL mRNA levels (A) and PAL activity (B) in wounded and nonwounded flavedo tissue of Fortune fruits nonconditioned (■) and conditioned for 3 days at 37 °C (□) and kept 27 h at 20 °C. PAL activity values are the mean of three replicate samples ± SE. Eight micrograms of total RNA was fractionated, blotted, and hybridized with FPal2 probe. Equivalence of RNA loading of the lanes was demonstrated by ethidium bromide staining.

affected PAL mRNA levels and PAL activity induced by other stress conditions, Fortune fruits were heat-conditioned for 3 days, mechanically wounded and then stored at a nonchilling temperature. As shown in Figure 4, mechanical wounding induced the accumulation of PAL transcript (Figure 4A) and caused a 7-fold increase in PAL activity as detected after 27 h at 20 °C (Figure 4B). However, both wound-induced PAL mRNA levels and PAL activity were significantly inhibited by conditioning the fruits for 3 days at 37 °C, so that only small differences were observed between wounded and non-wounded-heated fruits.

Effect of Low-Temperature Storage and Heat-Conditioning on the Levels of PAL Transcript and

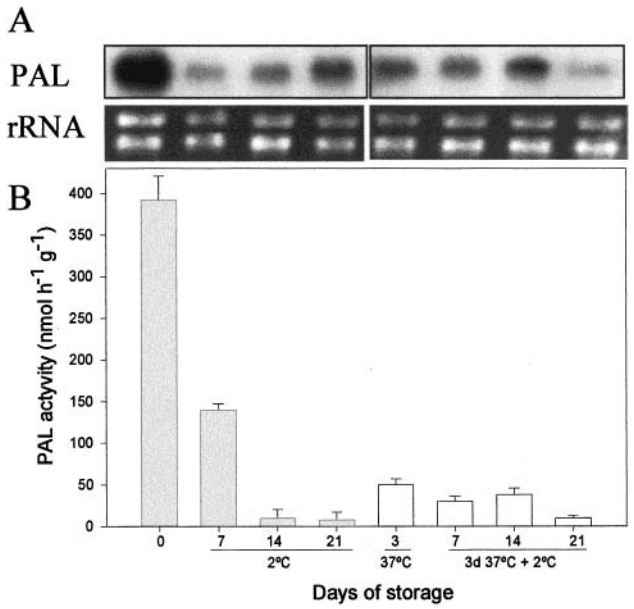

Figure 5. Changes in PAL mRNA levels (A) and PAL activity in conditioned (■) and nonconditioned (□) Hernandina fruits stored at 2 °C for up to 21 days. PAL activity values are the mean of three replicate samples ± SE. Eight micrograms of total RNA extracted from flavedo tissue was fractionated, blotted, and hybridized with FPal2 probe. Equivalence of RNA loading of the lanes was demonstrated by ethidium bromide staining.

PAL Activity in Chilling-Tolerant Hernandina Fruits. Hernandina fruits belong to the class of chilling-tolerant citrus cultivars. As shown in Figure 5, high levels of both PAL mRNA and PAL activity were consistently found in freshly harvested Hernandina fruits. The levels of PAL transcript declined sharply after 7 days of storage at 2 °C and showed little change thereafter. PAL activity, however, declined slowly for the next 21 days at 2 °C. Pretreatment of fruits at 37 °C for 3 days produced a dramatic decline in both PAL mRNA levels and PAL activity, which remained at very low levels after prolonged storage of the fruits at 2 °C (Figure 5, panels A and B).

DISCUSSION

Heat-conditioning treatments had a dramatic effect on both the induction of PAL mRNA levels and PAL activity in the flavedo tissue and on the tolerance of Fortune mandarin fruits to chilling temperature (2 °C). Sanchez-Ballesta et al. (2000) reported that the induction of PAL activity and the accumulation of PAL mRNA were restricted to damaged tissue in those fruits showing chilling symptoms. In this paper, we have shown that fruits protected from developing chilling symptoms by virtue of a heat-conditioning treatment exhibit two early increases in PAL mRNA accumulation: one during the conditioning treatment (Figure 1A) and a second immediately after transferring the fruits to chilling (2 °C) and nonchilling (12 °C) temperatures (Figure 2, panels B and C). Considering that changes in PAL activity correlate with the appearance of chilling symptoms in nonconditioned fruits kept at 2 °C (Figure 2F) (Martínez-Téllez and Lafuente, 1997; Sanchez-Ballesta et al., 1999), we propose the existence of two different mechanisms, in which PAL is induced, that may reduce fruit damage during postharvest storage: one that may reflect a demand for phenylpropanoid pathway products during exposure to low temperatures to reduce chilling symptoms development and another induced by temperature shift, which enables the fruit to better resist temperature stress.

The early transient induction of PAL at 37 °C may be part of a mechanism that confers tolerance to chilling in Fortune mandarin fruits via phenylpropanoid metabolite intermediates. This would explain why all these treatments were effective in delaying chilling symptoms for up to 21 days. The situation may not be simple however. It has been shown that, in the case of long-term cold storage (>45 days), the efficacy of the heat-conditioning increases with time of exposure at 37 °C (i.e., 3 days of heat treatment is more effective than 1 and 2 days) (Gonzalez-Aguilar et al., 1998), while the transient activation of PAL mRNA levels and PAL activity occurring within 4 h at 37 °C is the same in fruits conditioned for 1, 2, or 3 days. The observed differences in chilling tolerance may be related to the activity of other phenylpropanoid enzymes downstream from PAL or in differences in the phenylpropanoid pool generated. Support for this contention arises from the observation that the inhibition of phenylpropanoid biosynthesis by cosuppression of PAL (Maher et al., 1994) or by overexpression of the transcription factor AmMYB308 in transgenic tobacco (Tamagnone et al., 1998) led to precocious cell death, indicating that phenylpropanoid products not only play a role in necrosis but also are necessary for maintaining cellular viability (Tamagnone et al., 1998). The involvement of phenolic compounds in necrosis and cell death has also been reported in maize, in which the activity of the gene product Lls1 has been suggested to be a dioxygenase that metabolizes a phenolic mediator of cell death (Gray et al., 1997). One possibility worth considering is that the transient increases in PAL mRNA levels and PAL activity induced by the heat-conditioning treatment may contribute to the synthesis of phenolic compounds some of which are known to have antioxidant properties (Rice-Evans et al., 1997). Oxidative stress has been proposed to be involved in the chilling tolerance of citrus fruits (Sala, 1998; Sala and Lafuente, 1999).

Exposure of chilling-sensitive (Fortune) and chilling-tolerant (Hernandina) mandarin fruits for 3 days at 37 °C depleted the levels of PAL mRNA (Figures 2A and 5A). However, such depletion is not specific for PAL, as we have observed that the levels of various cold-regulated mRNAs in the flavedo also diminish with this treatment (data not shown). Lurie et al. (1996) found that in tomato fruits the levels of specific mRNAs encoding enzymes related to the ripening process such as ACC oxidase, polygalacturonase, and lycopene synthase declined dramatically after heating the fruits for 3 days at 38 °C and returned to normal after transferring the fruits to room temperature. This, however, appears not to be a general effect since heat-conditioning Fortune mandarins for 3 days at 37 °C may cause an increase in the activity of antioxidative enzymes in the flavedo tissue (Sala and Lafuente, 1999).

It should be pointed out that the molecular mechanism by which heat treatment affects the induction of PAL mRNA levels and PAL activity depends on the subsequent temperature of storage. Thus, the lack of PAL activity, but not of PAL transcript, in 3-day-heat-conditioned Fortune fruits occurred when they were transferred to a stress temperature of 2 °C but not to the nonchilling temperature (12 °C) (Figure 2, panels

B and D). This result indicates that it is the PAL activity associated with chilling symptoms that is blocked and not the ability of the fruit to increase PAL activity in response to the nonchilling temperature (12 °C). It seems therefore that effective blocking of the PAL biosynthetic machinery in Fortune fruits requires the conjunction of both extreme temperature conditions (i.e., 37 °C + 2 °C) and that this mainly affects PAL translation and/or PAL activity since PAL transcript abundance remained almost constant. It is also interesting to note that PAL activity was not blocked when fruits were conditioned for 1 or 2 days (Figure 3B).

Heat-conditioning of Fortune mandarins for 3 days at 37 °C also had a detrimental effect on the induction of PAL activity by the stress situation of mechanical wounding (Figure 4). In this case, the changes in PAL activity paralleled those of PAL mRNA accumulation, indicating that the mechanism by which high temperature inhibits PAL activity in response to mechanical wounding is at the transcriptional level and therefore differs from that involved in the chilling response. The interference of HS with stress-induced PAL activity is not specific for citrus fruits, since a HS treatment given before wounding decreased wound-induced PAL activity in lettuce segments (Loaiza-Velarde et al., 1997) and was able to override ultraviolet-induced PAL activity in cultured parsley (Walter, 1989).

High levels of PAL transcript and PAL activity were present in freshly harvested fruits of the chilling-tolerant cultivar (Hernandina). Maintaining the fruits at 2 °C has an inhibitory effect on PAL mRNA levels and PAL activity on heat-treated and nontreated fruits (Figure 5). In contrast, PAL mRNA levels always increased in heat-conditioned and nonconditioned fruits of the chilling-sensitive cultivar a few days after storage at 2 °C (Figure 2, panels A and E). This result indicates that accumulation of PAL transcripts in the flavedo tissue may serve as a molecular marker for chilling sensitivity in citrus fruits.

In conclusion, the accumulation of PAL mRNA during storage of citrus fruits at 2 °C may be indicative of the sensitivity of a citrus cultivar to chilling. Although the induction of PAL may be required for building protective barriers and producing metabolites that would help the fruits to resist chilling stress, additional defense mechanisms that assist in avoiding CI appear to be induced by heat treatment. The pattern of changes in PAL activity, but not in PAL mRNA levels, in the chilling-sensitive cultivar upon storage appear to be dependent on the duration of the heat treatment and the subsequent storage temperature.

ACKNOWLEDGMENT

We gratefully acknowledge the technical assistance of D. Arocas. We thank Dr. A. P. MacCabe for critically reading the manuscript.

LITERATURE CITED

Cathala, G.; Savanret, J. F.; Mendez, B.; West, B. L.; Karon, M.; Martial, J.; Baxter, J. A. Method for isolation of intact, translationally active ribonucleic acid. *DNA* **1983**, *2*, 324−335.

Christie, P. J.; Alfenito, M. R.; Walbot, V. Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: Enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. *Planta* **1994**, *194*, 541−549.

Diallinas, G.; Kanellis, A. K. A phenylalanine ammonia-lyase gene from melon fruit: cDNA cloning, sequence and expression in response to development and wounding. *Plant Mol. Biol.* **1994**, *26*, 473−479.

Dixon, R. A.; Lamb, C. J. Plant-microbial pathogen interactions. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1990**, *41*, 369−419.

Dixon, R. A.; Pavia, N. L. Stress-induced phenylpropanoid metabolism. *Plant Cell* **1995**, *7*, 1085−1097.

Gonzalez-Aguilar, G. A.; Zacarias, L.; Lafuente, M. T. Ripening affects high-temperature-induced polyamines and their changes during cold storage of hybrid Fortune mandarins. *J. Agric. Food Chem.* **1998**, *46*, 3503−3508.

Gray, J.; Close, P. S.; Briggs, S. P.; Johal, G. S. A novel suppressor of cell death in plants encoded by the Lls1 gene of maize. *Cell* **1997**, *89*, 25−31.

Hahlbrock, K.; Sheel, D. Physiology and molecular biology of phenylpropanoid metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1989**, *40*, 347−369.

Ho, T.-H. D.; Sachs, M. M. Stress-induced proteins: characterization and regulation of their synthesis. In *The Biochemistry of Plants: A Comprehensive Treatise*; Stumpf, P. K., Conn, E. E., Eds.; Academic Press: New York, 1989; Vol. 15, pp 347−377.

Holland, N.; Sala, J. M.; Menezes, H. C.; Lafuente, M. T. Carbohydrate content and metabolism as related to maturity and chilling sensitivity of Cv. Fortune mandarins. *J. Agric. Food Chem.* **1999**, *47*, 2513−2518.

Ismail, M. A.; Brown, G. E. Postharvest wound healing in citrus fruit: induction of phenylalanine ammonia-lyase in injured 'Valencia' orange flavedo. *J. Am. Soc. Hortic. Sci.* **1979**, *104*, 126−129.

Lafuente, M. T.; Belver, A.; Guye, M. G.; Salveit, M. E. Effect of temperature conditioning on chilling injury of cucumber cotyledons. Possible role of abscisic and heat shock proteins. *Plant Physiol.* **1991**, *95*, 443−449.

Lafuente, M. T.; Martinez-Tellez, M. A.; Zacarías, L. Abscisic acid in the response of Fortune mandarin to chilling. Effect of maturity and high-temperature conditioning. *J. Sci. Food and Agric.* **1997**, *73*, 494−502.

Lawton, M. A.; Lamb, C. J. Transcriptional activation of plant defense genes by fungal elicitor, wounding and infection. *Mol. Cell. Biol.* **1987**, *7*, 335−341.

Loaiza-Velarde, J. G.; Tomas-Barbera, F. A.; Salveit, M. E. Effect of intensity and duration of heat-shock treatments on wound-induced phenolic metabolism in iceberg lettuce. *J. Am. Soc. Hortic. Sci.* **1997**, *122*, 873−877.

Lurie, S.; Klein, J. D. Acquisition of low-temperature tolerance in tomatoes by exposure to high-temperatures stress. *J. Am. Soc. Hortic. Sci.* **1991**, *116*, 1007−1012.

Lurie, S.; Handros, A.; Fallik, E.; Shapira, R. Reversible inhibition of tomato fruit gene expression at high temperature. *Plant Physiol.* **1996**, *110*, 1207−1214.

Maher, E. A.; Bate, N. J.; Ni, W.; Elkind, Y.; Dixon, R. A.; Lamb, C. J. Increased disease susceptibility of transgenic tobacco plants with suppressed levels of performed phenylpropanoid products. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7802−7806.

Martínez-Téllez, M. A.; Lafuente, M. T. Chilling-induced changes in phenylalanine ammonia-lyase, peroxidases, and polyphenol oxidase activities in citrus flavedo tissue. *Acta Hortic.* **1993**, *343*, 257−263.

Martínez-Téllez, M. A.; Lafuente, M. T. Effect of high-temperature conditioning on ethylene, phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase activities in flavedo of Fortune mandarin fruit. *J. Plant Physiol.* **1997**, *150*, 674−678.

Picton, S.; Grierson, D. Inhibition of expression of tomato ripening genes at high temperature. *Plant Cell Environ.* **1988**, *11*, 265−272.

Rice-Evans, C. A.; Sampson, J.; Bramley, P. M.; Holloway, D. E. Why do we expect carotenoids to be antioxidants in vivo? *Free Radical Res.* **1997**, *26*, 381−98.

Riov, J.; Monselise, S. P.; Kahan, R. S. Effect of gamma radiation on phenylalanine ammonia-lyase activity and

accumulation of phenolic compounds in citrus fruit peel. *Radiat. Bot.* **1968**, *8*, 463−466.

Sabehat, A.; Weiss. D.; Lurie, S. The correlation between heat-shock protein accumulation and persistence and chilling tolerance in tomato fruit. *Plant Physiol.* **1996**, *110*, 531−537.

Sala, J. M. Involvement of oxidative stress in chilling injury in cold-stored mandarin fruits. *Postharvest Biol. Technol.* **1998**, 13255−13261.

Sala, J. M.; Lafuente, M. T. Catalase in the heat-induced chilling tolerance of cold-stored hybrid Fortune mandarin fruits. *J. Agric. Food Chem.* **1999**, *47*, 2410−2414.

Sanchez-Ballesta, M. T.; Lafuente, M. T.; Zacarias, L.; Granell, A. Phenylalanine ammonia-lyase in the response of citrus fruits to cold temperature. *Physiol. Plant.* **2000**, *108*, 382−389.

Tamagnone, L.; Merida, A.; Stacey, N.; Plaskitt, K.; Parr, A.; Chang, C. F.; Lynn, D.; Dow, J. M.; Roberts, K.; Martin, C. Inhibition of phenolic acid metabolism results in precocious cell death and altered cell morphology in leaves of transgenic tobacco plants. *Plant Cell* **1998**, *10*, 1801−1816.

Walter, M. H. The induction of phenylpropanoid biosynthesis enzymes by ultraviolet light or fungal elicitor in cultured parsley cells in overriden by heat-shock treatment. *Planta* **1989**, *177*, 1−8.

Woolf, A. B.; Watkins, C. B.; Bowen, J. H.; Lay-Yee, M.; Maindonald, J. H.; Ferguson, I. B. Reducing external chilling injury in stored 'Hass' avocados with dry heat treatments. *J. Am. Soc. Hortic. Sci.* **1995**, *120*, 1050−1056.

Received for review October 19, 1999. Accepted March 21, 2000. This work was supported by Research Grants ALI-96-0506-C03 from the Comisión Interministerial de Ciencia y Tecnología (CICYT), Spain, and FAIR-CT98-40-96 from the EU. M.T.S.-B. was supported by a fellowship from the Ministerio de Educación y Ciencia, Spain, and by an EU contract.

JF991141R