ELSEVIER

1

Plant Science 161 (2001) 1153–1159

PLANT SCIENCE

www.elsevier.com/locate/plantsci

Reduction of chilling injury and transcript accumulation of heat shock proteins in tomato fruit by methyl jasmonate and methyl salicylate

Chang-Kui Ding, Chien Yi Wang *, Kenneth C. Gross, David L. Smith

Produce Quality and Safety Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705, USA

Received 8 June 2001; received in revised form 14 August 2001; accepted 14 August 2001

Abstract

Changes in heat shock protein (HSP) gene expression induced by vapor application of methyl jasmonate (MeJA) and methyl salicylate (MeSA) in tomato fruit were investigated and compared to the well-described heat shock response. Northern hybridization experiments involving six cDNAs, encoding class I and II tomato small HSPs (sHSPs) and three members of HSP 70 family, showed that accumulation of class I and II sHSP mRNAs was increased significantly by MeJA and MeSA. When the treated fruits were transferred to low temperature, class I and II mRNA levels initially decreased, but then subsequently increased. Accumulation of HSP transcripts was also observed in non-treated fruit between 7 and 14 days at low-temperature storage, but all decreased to undetectable levels after 21 days. Following MeJA and MeSA treatments, the transcripts of HSP 70 family accumulated to higher levels than following the heat treatment. MeJA- and MeSA-treatments were clearly shown to alleviate chilling injury (CI), whereas tomato fruit stored at 5 °C without pretreatment developed typical symptoms and severe decay. These results demonstrated that MeJA and MeSA induced the accumulation of sHSP transcripts in tomato. The increased transcript abundance of HSPs, especially class II sHSPs, was correlated with protection against CI. Published by Elsevier Science Ireland Ltd.

Keywords: Heat shock protein; Gene expression; Methyl jasmonate; Methyl salicylate; Chilling injury; Tomato

1. Introduction

One of the main postharvest problems affecting tropical and subtropical commodities is their sensitivity to low temperature, resulting in chilling injury (CI). This phenomenon limits storage life and leads to significant degradation of produce quality. Apart from the visible symptoms, several biochemical and physiological processes are altered as a consequence of the direct effects of low temperature on cellular constituents [1]. Therefore, various methods have been developed to alleviate CI symptoms [2].

High-temperature stress has been found to protect a number of fruits and vegetables, including tomatoes, against CI. The protection afforded by heat shock against CI in tomato was found to persist up to 21 days at 2 °C [3]. All organisms respond to high temperatures by inducing the synthesis of a small group of evolutionarily conserved polypeptides known as heat shock proteins (HSPs). Plants synthesize numerous small HSPs (sHSPs), ranging from 15 to 40 kDa and have been divided into six classes according to their subcellular localization, cross-reactivity and amino acid sequence homology [4]. Stress 70 molecular chaperones include most HSPs and their homologues—heat shock cognate (HSC). In most organisms, the HSPs and HSCs are encoded by a multigene family and found in all of the major subcellular compartments of plant cells [5]. The expression of the stress 70 molecular chaperones in response to heat shock is well known and it appears that low-temperature exposure can also stimulate their expression. Overall, the HSPs and the heat shock responses of plants, animals and prokaryotes are highly conserved [6], suggesting that they perform a

* Corresponding author. Tel.: +1-301-504-5981; fax: +1-301-504-5107.

E-mail address: wangc@ba.ars.usda.gov (C.Y. Wang).

0168-9452/01/$ - see front matter Published by Elsevier Science Ireland Ltd.
PII: S0168-9452(01)00521-0

universally basic and essential function during high-temperature stress. Recent studies with heat-stressed tomato fruits have shown a correlation between the accumulation of sHSP and the acquisition of chilling tolerance [7,8]. The results suggest that HSPs could be involved to some extent in the ability of heat shock to increase chilling tolerance.

Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MeJA), have been found to occur naturally in a wide range of higher plants. This compound, defined as a natural plant growth regulator, was found to be active in many physiological systems. In many cases, it has an action similar to that of abscisic acid (ABA). Recent studies demonstrated that jasmonate could induce a response similar to that of ABA in plants for alleviating CI [9,10]. In addition, some specific proteins induced by JA are similar to those induced by heat shock [11]. However, there are no reports of the expression of HSP genes triggered by JA or MeJA in association with the chilling tolerance in plants.

Salicylic acid (SA) is a natural signaling molecule, mediating resistance in response to avirulent pathogens. In mammals, prior treatment with moderate levels of SA potentiates the induction of HSP 70 in response to heat stress [12]. In plants, SA is endogenously synthesized, playing an essential role in thermogenesis and in the defense against pathogen attack [13]. Despite a proposed evolutionary relationship between HSP and pathogenesis-related (PR) proteins, little attention has been paid to the induction of HSP by SA in plants. If SA potentiates the heat shock response in plants, as in mammals, SA pretreatment could enhance SA-related defense gene activation and potentially protect plants from temperature stress. However, the effect of SA on HSP expression in plants has not been investigated.

HSPs are also induced by other stresses such as cold and drought [14–16]. These HSPs are part of a group of proteins induced by environmental stresses either to protect the plant from damage or to help repair the damage caused by the stress. Similarly, pre-exposure of cells to low concentration of H_2O_2, UV, cold, drought, or salinity stresses often allows the development of tolerance towards a lethal concentration of that same reagent [17]. Some researchers dealing with stress physiology in plants have hypothesized that there is a common denominator for the mechanism of inducing tolerance with various stress treatments, e.g. when stress treatments are applied at mild levels that do not cause appreciable damage. According to this hypothesis, plants respond with similar defense systems to a wide range of stresses such as osmotic, chemical pollutants, oxidative, salinity, cold or heat, UV, low oxygen, pathogen infection, and wounding.

In order to determine whether the mechanism of alleviating CI involves the expression of plant HSP genes, this study was undertaken to investigate the relationships of MeJA, MeSA and heat treatments to the expression of plant HSP genes. It is important to determine which members of the HSP family respond to protect against CI in the future search for 'stress-labile' proteins or processes in chilling sensitive commodities.

2. Materials and methods

2.1. Postharvest treatments

Tomato fruit (*Lycopersicon esculentum* L. cv Beefstake) were harvested at the mature green stage in Florida and then immediately shipped to Maryland. Fruit were not gassed with ethylene prior to or during shipment. Breaker stage fruit were selected and divided into four lots of 160 fruit each. Ninety fruit from each lot were used for CI and decay evaluation. The remaining fruit were used for RNA isolation. Fruit at the breaker stage of ripening were defined as those that showed incipient yellow coloration at the blossom end of the fruit. In all experiments, control fruit were placed at 5 °C storage. During heat treatment, fruit were first held in a heating chamber at 38 °C for 48 h and then placed at 5 °C. For MeJA and MeSA vapor treatments, fruit were placed in 200-l airtight containers, together with MeJA or MeSA spotted onto filter paper at the final concentration of 0.01 mM, and incubated for 16 h at 23 °C. After these treatments the containers were opened, ventilated, and the fruit stored at 5 °C. After 4 weeks at 5 °C, the fruit were transferred to 20 °C and the development of CI and decay was measured.

2.2. Chilling injury and decay evaluation

Evaluation of CI was performed at the end of the 4-week cold storage and after an additional 1 day at 20 °C. Tomato fruit CI symptoms were manifested as surface pitting. The severity of CI symptoms was assessed visually according to a four-stage scale, as follows: 0 = no pitting; 1 = a few scattered pits; 2 = pitting covering up to 5% of the fruit surface; 3 = extensive pitting covering >5 but $<25\%$ of the fruit surface, and 4 = extensive pitting covering $>25\%$ of the fruit surface. The CI index was calculated, according to the severity of symptoms and the number of fruit.

The total number of fruit manifesting decay symptoms (mainly *Alternaria* rot) was determined in each treatment at the end of the 4-week cold storage period and after an additional 3 days at 20 °C, and expressed as the decay percentage. All experiments were repeated at least three times with similar and consistent results, and the representative data are presented.

2.3. RNA isolation

Total RNA was extracted according to the method of Chang et al. [18]. To extract RNA from tomato fruit pericarp, tissue was first frozen and ground in liquid N_2. Five volumes of extraction buffer (2% [w/v] CTAB, 100 mM Tris–HCl [pH 8.0], 25 mM EDTA, 2.0 M NaCl, 0.5 g/l spermidine and 2% β-mercaptoethanol) were added and warmed at 65 °C for 5 min. Then, the solution was extracted twice with an equal volume of chloroform, and separated at 10 000 rpm. A quarter volume of 10 M LiCl was added to the supernatant and mixed. The RNA was precipitated overnight at 4 °C and harvested by centrifugation at 10 000 rpm for 20 min. The pellet was dissolved in 500 μl of SSTE solution (1.0 M NaCl, 0.5% SDS, 10 mM Tris–HCl [pH 8.0], 1 mM EDTA [pH 8.0]), and extracted twice with equal volumes of chloroform. Two volumes of ethanol were added to the supernatant and precipitated at −70 °C for 30 min. The solution was centrifuged for 20 min to pellet the RNA, and the pellet was resuspended in autoclaved diethylprocarbonate-treated water.

2.4. Northern analysis

Total RNA (15 μg) was size-fractionated in a 1.2% formaldehyde-denaturing agarose gel [19] and blotted onto a Hybond-N^+ membrane (Amersham), fixed by incubating for 2 h at 80 °C. Following electrophoresis, the formaldehyde gel was briefly stained with ethidium bromide for 20 min, washed and photographed before blotting for preliminary assessment of equal loading. After prehybridization at 42 °C for 4 h, the blots were hybridized for 16 h at 42 °C with ^{32}P-labeled cDNA probes in hybridization buffer containing 50% formamide, 0.2% SDS, 5 × SSC (1 × SSC: 0.015 M NaCl and 1.5 mM sodium citrate, pH 7.0), 5 × Denhardt's solution (1 × Denhardt's solution: 0.02% [w/v] BSA, 0.02% [w/v] Ficoll, and 0.02% [w/v] PVP), and 100 μg/ml salmon sperm DNA. Following hybridization, blots were washed twice with 0.1 × SSC and 0.1% × SDS at 58 °C (HSC 70-1, -2 and -3) or 65 °C (HSP 17.4, 17.6 and 17.7) for 15 min. For all RNA gel-blot experiments, the blots were exposed to BioMax MS (Eastman Kodak, Rochester, NY) film using an intensifying screen for 22–24 h at −80 °C. The same blot re-probed with 26S ribosomal DNA was used as a loading control [20].

Clones for tomato cytosolic class II sHSP 17.4 (accession no. AF090115), 17.6 (accession no. U72396) and cytosolic class I sHSP 17.7 (accession no. AF123255) were obtained from Dr D.R. Dilley and Dina Kadyrjanova (Michigan State University, USA). The three heterologous cDNA probes from spinach, corresponding to cytosolic HSC 70-1 (accession no. L26243), ER HSC 70-2 (accession no. L23551) and cytosolic HSC 70-3 (accession no. AF033852), were obtained from Dr C.L. Guy (University of Florida, USA).

3. Results

3.1. Effects of different treatments on chilling injury and decay in tomato fruit

Chilling injury in tomato fruit is manifested through a number of symptoms. Chilled fruits lose their ability to develop full color, develop sunken areas on the fruit (blemishes), and show increased susceptibility to *Alternaria* rot and decay. As can be seen in Table 1, non-treated control fruits that were stored for 4 weeks at 5 °C showed a very high incidence of decay (100%) and developed severe symptoms of CI, whereas 0.01 mM MeJA and 0.01 mM MeSA vapor treatment for 16 h before moving them to 5 °C prevented the development of CI symptoms and significantly decreased decay. Heat treatment also reduced the incidence of decay and alleviated CI symptoms (Table 1). Overall, all these treatments provided effective protection against CI and decay.

3.2. Accumulation pattern of HSP mRNAs during tomato fruit ripening

Expression of *tom66* and *tom111* HSP genes has been described as being ripening-related [7,21]. Some reports also indicate that fruit at different developmental stages have different sensitivities to chilling temperatures [2]. We tested whether HSP genes are expressed during fruit ripening. Class II sHSP 17.4 mRNA was detected at all stages, but accumulated to the higher levels in the later stages of ripening, while class II HSP 17.6 mRNA

Table 1
The effects of different treatments on resistance to chilling stress and decay in tomato fruit

Treatment	Chilling injury (index)	Decay (% of fruit)
Control	3.55 ± 0.95	100
0.01 mM MeJA, 16 h	1.28 ± 0.25	23.5 ± 5.6
0.01 mM MeSA, 16 h	1.42 ± 0.34	19.8 ± 4.2
38 °C-heat, 2 days	1.31 ± 0.14	25.3 ± 3.4

Fruit underwent various treatments before being stored at 5 °C for 28 days. Control fruit were placed at 5 °C immediately after being sorted. The chilling injury (from 0 to 4) was measured after transferring the fruit from 5 to 20 °C for 1 day and the decay was measured after transferring the fruit from 5 to 20 °C for 3 days. The experiment was repeated three times with 30 fruit for each treatment, and SD (±) is indicated.

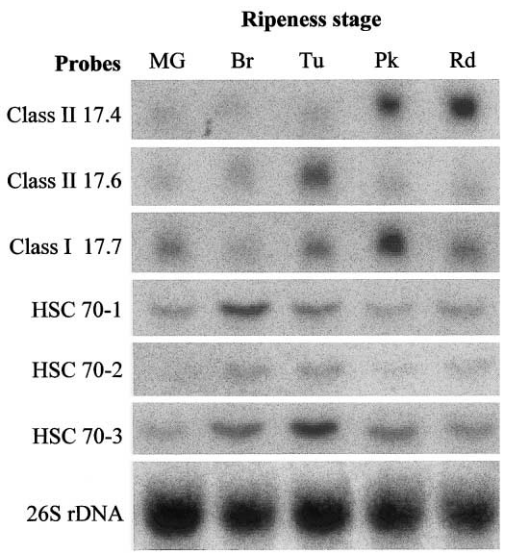

Fig. 1. Regulation of HSP gene expression during tomato fruit ripening. Total RNA (15 μg) isolated from tomato pericarp at different stages of ripeness was analyzed by RNA gel blotting and probed for the presence of transcripts from sHSPs (HSP 17.4, 17.6 and 17.7) and the HSP 70 family (HSC 70-1, 70-2 and 70-3). The ripening stage was sorted according to the surface color by mature-green (MG), breaker (Br), turning (Tu), pink (Pk) and red (Rd). A 26S ribosomal gene clone was used as a loading control for each blot and one example is shown.

accumulated to the highest level at the turning stage (Fig. 1). The class I HSP 17.7 mRNA was detected at higher level at pink stage (Fig. 1). The transcripts of the HSP 70 family were detected at all ripening stages, but the transcripts were highest at the breaker and/or turning stages (Fig. 1). These results demonstrated that the transcripts of the HSP genes were up-regulated during various stages of fruit ripening.

3.3. Accumulation pattern of HSP mRNAs in heat pretreated and untreated fruit during chilling temperature storage

To determine whether HSP transcripts accumulated during cold storage in heat-treated fruit, breaker fruit were incubated for 48 h at 38 °C and then transferred to 5 °C for up to 4 weeks. After heat treatment and during storage, RNA was extracted and analyzed for HSP transcripts. The transcripts of HSP 17.6 and HSP 17.7 were significantly increased in 38 °C treated fruit at time 0 (Fig. 2A) compared to non-heated controls (Fig. 2B) while HSP 17.4 was not affected by heat-treatment. Interestingly, the transient reduction in HSP 17.6 and 17.7 transcripts was observed at 1–3 days. Upon prolonged storage periods at 5 °C, a reinduction of transcript accumulation was observed in all the three sHSPs. Thereafter, the mRNA level of small HSPs except HSP 17.6 decreased to undetectable (HSP 17.4) or very low (HSP 17.7) levels after the storage for 21 or 28 days at 5 °C. The abundance of HSC 70-1 and 70-3 transcripts was increased by heat-treatment and was maintained at high levels throughout the storage period, while HSC 70-2 transcripts did not increase significantly by heat treatment at day 0 and at day 1, but the transcripts were significantly enhanced after 3 days of storage at 5 °C (Fig. 2A).

While heat shock is known to influence the expression of many HSPs and molecular chaperones, there is little information on the influence of low temperatures on their expression. We examined whether exposing fruits to low temperature would induce HSP mRNA accumulation. Untreated fruit at the breaker stage were stored at 5 °C for 1, 3, 7, 14, 21 and 28 days. Contrary to the responses observed during heat treatment at 38 °C, the patterns of expression in tomato fruit at 5 °C were quite variable (Fig. 2B). Most of the HSP members showed very low expression at day 0 and at day 1. But, all three sHSP mRNA levels increased between 7 and 14 days after the exposure to 5 °C. The transcripts decreased to almost undetectable levels after

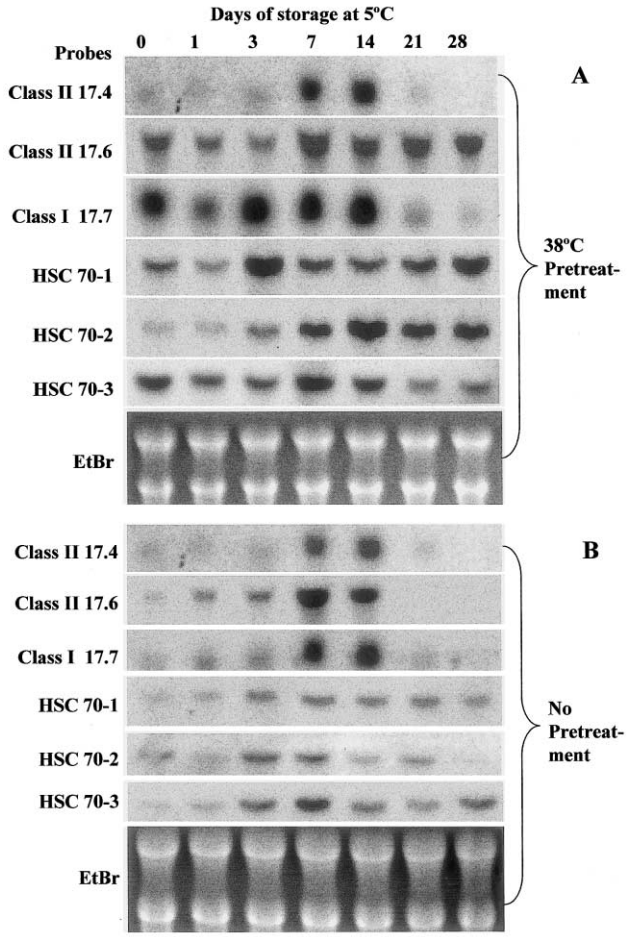

Fig. 2. HSP transcript accumulation in 38 °C pretreated (A) and untreated (B) tomato fruit during chilling temperature storage. The probes are as same as in Fig. 1. Ethidium bromide (EtBr) staining for each lane was done to confirm equal RNA loading.

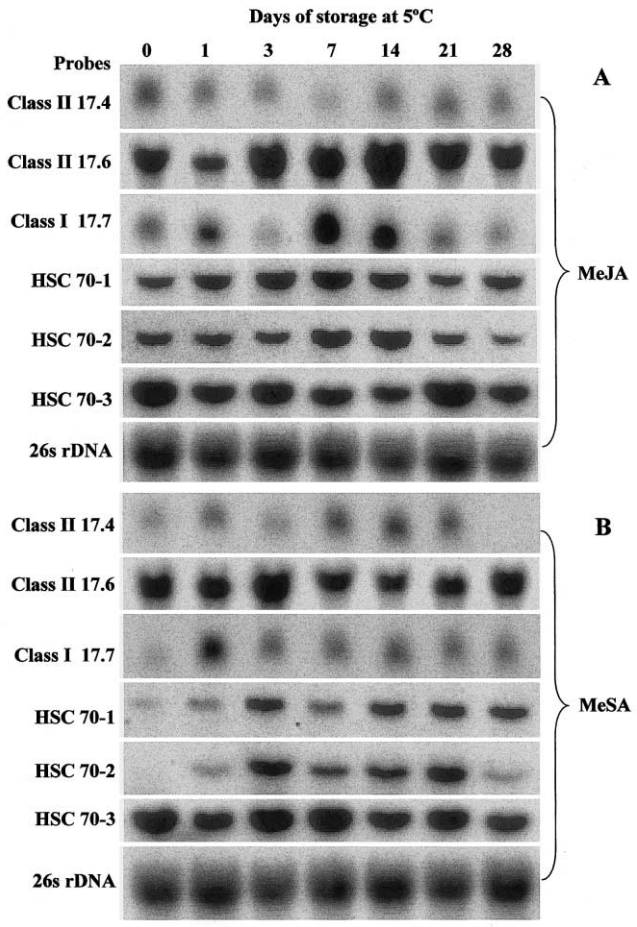

Fig. 3. Accumulation of HSP transcripts in tomato fruit pretreated with MeJA (A) and MeSA (B) during chilling temperature storage. Fruit were placed in 200-l airtight containers, together with MeJA or MeSA spotted onto filter paper at the final concentration of 0.01 mM, and incubated for 16 h at 23 °C. A 26S ribosomal gene clone was used as a loading control for each blot and one example is shown.

21 days storage at 5 °C. HSP 70 family showed increase in mRNAs after 3 days and maintained to 21 or 28 days. But the abundance was less than that of heat treatment.

3.4. Expression pattern of HSP mRNAs after treatment with MeJA and MeSA during subsequent chilling temperature storage

The response of HSP mRNA accumulation to MeJA treatment was compared with those of the control and the heat (38 °C) treatments. Significant amounts of class II sHSP transcripts were induced by MeJA at day 0 (Fig. 3A) compared with the heat treatment (Fig. 2A, time 0) and the control (Fig. 2B). The expression of class II genes (HSP 17.4 and 17.6) was higher with MeJA (Fig. 3A) than with the heat treatment (Fig. 2A). Class I (HSP 17.7) was also increased by MeJA, but was less than with heat treatment (Fig. 2A). When the treated fruit were transferred to low temperature, class II (HSP 17.6) mRNA level first decreased. However, after further exposure to low temperatures, the transcript levels of the gene were reinduced and the transcript level was more than that found at the end of the treatment. Higher sHSP mRNA levels were also observed in non-treated fruits at low temperatures, but the abundance was less compared to MeJA treated fruit. The transcripts of HSC 70-1, HSC 70-2 and HSC 70-3 were detected at high levels after 16 h of 0.01 mM MeJA treatment, especially for HSC 70-3 (Fig. 3A, time 0). During subsequent storage at 5 °C, transcripts of all HSP 70 family members was constitutive and maintained at higher levels than the control and the heat-treated fruit.

The response of HSP mRNA levels to MeSA treatment was also analyzed using Northern hybridization (Fig. 3B). The small HSP 17.6 and HSC 70-3 transcript levels were significantly induced by MeSA (Fig. 3B, day 0). The transcripts of HSP 17.6 and HSC 70-3 in MeSA treated fruit were higher than in heat-treated fruit (Fig. 2A), but were similar to the MeJA treatment (Fig. 3A). The levels of HSP 17.4, HSC 70-1 and 70-3 transcripts in MeSA treated fruit were very low initially. However, after further exposure to low temperature for 3 days, expression was reinduced (Fig. 3B) and the transcript levels were higher than those found in the non-treated fruit (Fig. 2B). The transcripts of HSP 17.7 were very low after the treatment, but it increased at day 1 and maintained to 28 days.

4. Discussion

In a number of plants HSP expression is induced during specific developmental stages. Several class II sHSP mRNAs are expressed during meiosis in maize microspore [22]. Class I cytoplasmic sHSP mRNAs are expressed during somatic embryogenesis in alfalfa [23]. Class I sHSP mRNAs have also been detected in pea seeds [24]. Fray et al. [21] and Sabehat et al. [7] reported that the expression of *tom66* and *tom111* genes was increased at the late stages of fruit development. However, expression levels were lower than those in heat-treated fruit. We found that the small HSPs and HSP 70 family were up-regulated during the fruit ripening. Thus, sHSPs and the HSP 70 family may play a role in the regulation of specific developmental processes in tomato fruit under normal growth conditions.

Exposing plants to moderately high temperatures for short periods often induces chilling tolerance, which allows them to survive under chilling temperatures [25]. During moderate heat stress, HSPs are produced and are thought to contribute to the acquisition of chilling tolerance [3]. High temperatures increase the expression of small HSPs in tomato (Fig. 2A), which corresponded

to the report of Kadyrzhanova et al. [8] in which they observed 42 °C heat treatment induced HSP 17.6 expression.

A number of studies have shown the existence of cross-tolerance [26,27]. Pretreatment with a low concentration of MeJA or JA has been found to protect against CI in a number of fruits and vegetables, including zucchini squash [9] and grapefruit [10]. Plants treated with a low concentration of SA also showed increased plant chilling tolerance [28]. A broad range of plants that show this type of cross-tolerance suggests that it may be a general response. To elucidate the mechanisms involved in the acquisition of tolerance to CI by JA and SA, we studied six HSP genes that are induced by heat (Fig. 2A) and are thought to act as molecular chaperones [5]. MeJA and MeSA significantly induced the transcript accumulation of both the sHSPs and the 70 family members. The induced mRNAs accumulated to even higher levels during subsequent exposure to chilling temperature. The expression of HSP 17.6 continued for 28 days at low-temperature storage. All HSP 70 transcripts remained at significantly higher levels in MeJA- and MeSA-treated tomato fruit during the subsequent chilling period. These HSP data, together with the physiological results, suggest that MeJA- and MeSA-induced synthesis of HSPs may be involved in protecting fruit from CI. Although the mechanism is unknown, one of the possible mechanisms by which JA- and SA-treatment alleviate CI may be attributed to 'molecular chaperone' activities of HSPs [29]. Molecular chaperones are a group of intracellular proteins that control correct folding, oligomeric assembly, and transport across membranes or disposal by degradation of other conformer unstable proteins by binding and releasing these proteins. Additionally, molecular chaperones prevent incorrect interaction within and between non-native polypeptides, which result in their irreversible aggregation. Lee et al. [30] observed that recombinant HSP 18.1 and HSP 17.7, representing class I and II cytosolic sHSPs from pea, were able to enhance the refolding of the chemically denatured model substrate citrate synthase and lactate dehydrogenase and prevented their aggregation and irreversible inactivation. In vivo experiments measuring the interaction of HSP 70 and a small pea HSP 18.1 (class I) in renaturation of heat-denatured Luciferase (Luc) showed that heat-denatured Luc bound to HSP 18.1 was effectively refolded with HSP 70 [31]. They suggested that although the refolding mechanism does not require specific interactions between sHSPs and the HSP 70 system, the reaction is even more efficient with HSP 70.

Tomato fruit pretreated with mild heat, or 0.01 mM of MeSA or MeJA, acquire resistance against subsequent CI. In both the cases, adaptation is related to the synthesis of stress proteins, particularly sHSPs. Our work provides the first evidence for heat shock-mediated adaptation in MeJA and MeSA treated tomato fruit against CI. Heat shock similarly induces plant tissue resistance to salt-shock [32], toxic metals [33] and oxidative injury [34]. Our results demonstrate that MeSA and MeJA target the transcription of HSPs, which may act to bind substrate proteins. The presence of HSP 70 may enhance refolding [31]. The fact that small HSPs can accumulate to over 1% of total protein in plants [35] indicates that small HSPs are likely an important reservoir for folding-competent denatured proteins, caused by extreme temperatures or other environmental stress conditions. The transcription of the class II 17.6 HSP gene was increased significantly by heat, MeJA and MeSA. The induced mRNAs were maintained at high abundance during subsequent exposure to chilling temperature for up to 28 days. The expression of HSP 17.6 is correlated with tolerance to CI. Therefore, the synthesis of HSP 17.6 can be considered as an adaptive mechanism in which cell protection may be essential. Recently, it has been demonstrated that derepression of the HS response by genetic engineering is possible and doing so increases basic thermotolerance in *Arabidopsis* [36]. It can therefore be suggested that constitutive expression of elevated levels of HSP might be an alternative strategy for protection against CI.

Acknowledgements

We thank Dr D.R. Dilley and Dina Kadyrjanova (Michigan State University, USA) for the kind gift of HSP 17.4, 17.6 and 17.7 cDNAs and Dr C.L. Guy (University of Florida, USA) for the kind gift of HSC 70-1, 70-2 and 70-3 cDNA clones.

References

[1] C.Y. Wang, Alleviation of chilling injury of horticultural crops, in: C.Y. Wang (Ed.), Chilling Injury of Horticultural Crops, CRC Press, Boca Raton, FL, 1990, pp. 281–320.
[2] C.Y. Wang, Approaches to reduce chilling injury of fruits and vegetables, Hortic. Rev. 15 (1993) 83–95.
[3] A. Sabehat, D. Weiss, S. Lurie, The correlation between heat shock protein accumulation and persistence and chilling tolerance in tomato fruit, Plant Physiol. 110 (1996) 531–537.
[4] P.R. Lafayette, R.T. Nagao, K. O'Grady, E. Vierling, J.L. Key, Molecular characterization of cDNA encoding low molecular-weight heat shock proteins of soybean, Plant Mol. Biol. 30 (1996) 159–169.
[5] Q.B. Li, D.Y. Haskell, C.L. Guy, Coordinate and non-coordinate expression of the stress 70 family and other molecular chaperones at high and low temperature in spinach and tomato, Plant Mol. Biol. 39 (1999) 21–34.
[6] D. Neumann, L. Nover, B. Parthier, R. Rieger, K.D. Scharf, R. Wollgiehn, U. zur Nieden, Heat shock and other stress response systems of plants, Biol. Zentbl. 108 (1989) 1–156.

[7] A. Sabehat, L. Susan, D. Weiss, Expression of small heat-shock proteins at low temperatures: a possible role in protecting against chilling injuries, Plant Physiol. 117 (1998) 651–658.
[8] D.K. Kadyrzhanova, K.E. Vlachonasios, P. Ververidis, D.R. Dilley, Molecular cloning of a novel heat induced/chilling tolerance related cDNA in tomato fruit by use of mRNA differential display, Plant Mol. Biol. 36 (1998) 885–895.
[9] C.Y. Wang, J.G. Buta, Methyl jasmonate reduces chilling injury in *Cucurbita pepo* through its regulation of abscisic acid and polyamine levels, Environ. Exp. Bot. 34 (1994) 427–432.
[10] S. Meir, S. Philosopg-Hadas, S. Lurie, S. Droby, M. Akerman, G. Zauberman, B. Shapiro, E. Cohen, F. Yoram, Reduction of chilling injury in stored avocado, grapefruit, and bell pepper by methyl jasmonate, Can. J. Bot. 74 (1996) 870–874.
[11] R.A. Weidhase, H.M. Kramell, J. Lehmann, H.W. Liebish, W. Lerbs, B. Parthier, Methyl jasmonate-induced changes in polypeptide pattern of senescing barley leaf segments, Plant Sci. 51 (1987) 177–186.
[12] D.A. Jurivich, L. Sistonen, R.A. Kroes, R.I. Morimoto, Effect of sodium salicylate on the human heat shock response, Science 255 (1992) 1243–1245.
[13] D.F. Klessig, J. Malamy, The salicylic acid signal in plants, Plant Mol. Biol. 26 (1994) 1439–1458.
[14] J.V. Anderson, Q.B. Li, D.W. Haskell, C.L. Guy, Structural organization of the spinach endoplasmic retriculum-luminal 70-kilodalton heat shock cognate gene and expression of 70-kilodalton heat shock genes during cold acclimation, Plant Physiol. 104 (1994) 1359–1370.
[15] M.A. Coca, C. Almoguera, J. Jordano, Expression of sunflower low-molecular-weight heat-shock proteins during embryogenesis and persistence after germination: localization and possible functional implications, Plant Mol. Biol. 25 (1994) 479–492.
[16] T. Kiyosue, K. Yamaguchi-Shinozaki, K. Shinozaki, Cloning of cDNAs for genes that are early responsive to dehydration stress in *Arabidopsis thaliana* L.: identification of three ERDs as HSP cognate genes, Plant Mol. Biol. 25 (1994) 791–798.
[17] D.R. Crawford, K.J.A. Davies, Adaptive response and oxidative stress, Environ. Health Perspect. 102 (Suppl. 10) (1994) 25–28.
[18] S. Chang, J. Puryear, J. Cairney, A simple and efficient method for isolating RNA from pine trees, Plant Mol. Biol. Rep. 11 (1993) 113–116.
[19] J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: a Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
[20] D.L. Smith, K.C. Gross, A family of at least seven β-galactosidase genes is expression during tomato fruit development, Plant Physiol. 123 (2000) 1173–1183.
[21] R.G. Fray, G.W. Lycett, D. Grierson, Nucleotide sequence of a heat-shock and repining-related cDNA from tomato, Nucleic Acids Res. 18 (1990) 7148.
[22] P.S. Dietrich, R.A. Bouchard, E.S. Casey, R.M. Sinibaldi, Isolation and characterization of a small heat shock protein gene from maize, Plant Physiol. 96 (1991) 1268–1276.
[23] J. Gyorgyey, A. Gartner, K. Nemeth, Z. Magyar, H. Hirt, E. Heberle-Bors, D. Dudits, Alfalfa heat shock genes are differentially expressed during somatic embryogenesis, Plant Mol. Biol. 16 (1991) 999–1007.
[24] E. Vierling, A. Sun, Developmental expression of heat shock proteins in higher plants, in: J.H. Cherry (Ed.), Environmental Stress in Plants, Springer, Berlin, 1989, pp. 343–354.
[25] S. Lurie, J.D. Klein, Acquisition of low temperature tolerance in tomatoes by exposure to high temperature stress, J. Am. Soc. Hortic. Sci. 116 (1991) 1007–1012.
[26] S. Lurie, J.D. Klein, E. Fallik, Cross protection of one stress by another, in: J. Cherry (Ed.), Biochemical and Cellular Mechanisms of Stress Tolerance of Plants, Springer, Berlin, 1994, pp. 201–212.
[27] Y. Leshem, P. Kuiper, Is there a GAS (general adaption syndrome) response to various types of environmental stress?, Biol. Plant. 38 (1996) 1–18.
[28] T. Janda, G. Szalai, I. Tari, E. Paldi, Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (*Zea mays* L.) plants, Planta 208 (1999) 175–180.
[29] R.S. Boston, P.V. Viitanen, E. Vierling, Molecular chaperones and protein folding in plants, Plant Mol. Biol. 32 (1996) 192–222.
[30] G.J. Lee, N. Pokala, E. Vierling, Structure and in vitro molecular chaperone activity of cytosolic small heat shock proteins from pea, J. Biol. Chem. 270 (1995) 10432–10438.
[31] G.J. Lee, E. Vierling, A small heat shock protein cooperates with heat shock protein 70 systems to reactivate a heat-denatured protein, Plant Physiol. 122 (2000) 189–197.
[32] H.M. Harrington, D.M. Alm, Interaction of heat and salt shock in cultured tobacco cells, Plant Physiol. 88 (1988) 618–625.
[33] R. Wollgiehn, D. Neumann, Stress response of tomato cell cultures to toxic metals and heat shock: differences and similarities, J. Plant Physiol. 146 (1995) 736–742.
[34] N. Banzet, C. Richaud, Y. Deveaux, M. Kazmaier, J. Gagnon, C. Triantaphylides, Accumulation of small heat shock proteins, including mitochondrial HSP22, induced by oxidative stress and adaptive response in tomato cells, Plant J. 13 (1998) 519–527.
[35] E.R. Waters, G.L. Lee, E. Vierling, Evolution, structure and function of the small heat shock proteins in plants, J. Exp. Bot. 47 (1996) 325–338.
[36] J.H. Lee, A. Hubel, F. Schoffl, Derepression of the activity of genetically engineered heat shock factor causes constitutive synthesis of heat shock proteins and increased thermotolerance in transgenic *Arabidopsis*, Plant J. 8 (1995) 603–612.