Planta (2002) 214: 895–901
DOI 10.1007/s00425-001-0698-9

ORIGINAL ARTICLE

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

Jasmonate and salicylate induce the expression of pathogenesis-related-protein genes and increase resistance to chilling injury in tomato fruit

Received: 7 May 2001 / Accepted: 19 September 2001 / Published online: 12 December 2001

Abstract Treatment of tomato (*Lycopersicon esculentum* L. cv. Beefstake) fruit with low concentrations of (0.01 mM) methyl jasmonate (MeJA) or methyl salicylate (MeSA) substantially enhanced their resistance to chilling temperature and decreased the incidence of decay during low-temperature storage. While studying the expression of pathogenesis-related (PR) protein genes, different accumulation patterns of PR-protein mRNAs in tomato fruit were observed. MeJA substantially increased the accumulation of PR-2b transcripts encoding intracellular β-1,3-glucanase and enhanced the mRNA levels of PR-2a and PR-3b encoding extracellular β-1,3-glucanase and intracellular chitinase, respectively. MeSA substantially increased accumulation of PR-2b and PR-3a mRNAs and slightly increased PR-3b mRNA accumulation. Chilling temperature did not appreciably enhance the accumulation of PR-protein mRNAs in untreated fruit. However, the accumulation of PR-3b mRNAs in MeSA-treated fruit was enhanced following low-temperature storage. Transcript abundance of catalase genes also was investigated in different pretreated tomatoes. The accumulation of *cat1* mRNA was increased substantially by MeJA, while it was reduced by MeSA treatment. These results suggest that the pre-treatment of tomato fruit with MeSA or MeJA induces the synthesis of some stress proteins, such as PR proteins, which leads to increased chilling tolerance and resistance to pathogens, thereby decreasing the incidence of decay.

Keywords Catalase · Chilling injury · *Lycopersicon* · Methyl jasmonate · Methyl salicylate · Pathogenesis-related protein

C.-K. Ding · C.Y. Wang (✉) · K.C. Gross · D.L. Smith
Produce Quality and Safety Laboratory,
Agricultural Research Service,
U.S. Department of Agriculture,
Beltsville, MD 20705, USA
E-mail: wangc@ba.ars.usda.gov
Fax: +1-301-5045107

Abbreviations JA: jasmonic acid · MeJA: methyl jasmonate · MeSA: methyl salicylate · PR: pathogenesis-related · SA: salicylic acid

Introduction

Tropical and subtropical horticultural crops are susceptible to chilling injury when stored at low temperatures after harvest. Chilled tomato fruit lose their ability to develop full color, develop sunken areas on the fruit (blemishes), and show increased susceptibility to *Alternaria* rot and decay. This phenomenon limits storage life and leads to substantial degradation of produce quality (Wang 1993).

Plants respond to pathogen attack by activating defense mechanisms, which may act to prevent or limit damage. A defense response frequently investigated is systemic acquired resistance (SAR), which can be invoked by a specific pathogen, and leads to resistance against subsequent challenges by a wide range of pathogens (Ryals et al. 1994). SAR is associated with the activation of a large number of genes encoding various types of stress proteins, including pathogenesis-related (PR) proteins (Ward et al. 1991; Enkerli et al. 1993). Some PR proteins have been identified as chitinases and β-1,3-glucanases (Fukuda and Shinshi 1994). Chitinase genes are known to be regulated in a development- and organ-specific pattern, but stress conditions such as fungal challenge, elicitor treatment, or exposure to ethylene also induce their expression (Graham and Sticklen 1994). In the case of fungal inoculation, enhanced chitinase activity was functionally implicated in the defense response directed toward chitin as a major cell wall component of fungi (Schlumbaum et al. 1986; Mauch et al. 1988). The combination of chitinases with β-1,3-glucanases was proposed to further potentiate antifungal activity, and was shown experimentally to inhibit the growth of many pathogenic fungi (Sela-Buurlage et al. 1993). Overexpression of a variety of these PR genes in transgenic plants resulted in enhanced

resistance to different fungal pathogens (Klessig and Malamy 1994; Ryals et al. 1994). Thus, their synthesis serves as a marker for disease resistance.

Salicylic acid (SA) is endogenously synthesized, playing an essential role in thermogenesis and in the activation of certain plant defense responses including PR gene expression (Gaffney et al. 1993; Klessig and Malamy 1994). Pathogen attack results in an increase in cellular SA concentration, either by de novo synthesis or release from inactive conjugates (Raskin 1992). The increase in SA in tomato coincides with the induction of several defense responses (Hammond-Kosack et al. 1992). It has been demonstrated in certain plants that pre-treatment with SA can increase the activity of alternative oxidase (Rhoads and McIntosh 1992) and that this is likely to be responsible for increased heat tolerance (Ordentlich et al. 1991). It was recently reported that SA treatment of young maize plants provides protection against subsequent low-temperature stress (Janda et al. 1999). Shulaev et al. (1997) demonstrated that methyl salicylate (MeSA) could function as an airborne signal, which activates disease resistance and the expression of defense-related genes in neighboring plants and in healthy tissues of infected plants. Therefore, we propose that using SA or MeSA to treat fruits and vegetables could protect them from hypothermic stress and allow activation of SA-related defense genes, thus leading to extension of storage life.

Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MeJA), have been found to occur naturally in a wide range of higher plants. It is a final product of the enzymatic oxidation of unsaturated fatty acids, and lipoxygenase (LOX) is a pivotal enzyme in this pathway (Vick and Zimmermann 1984). Jasmonate has been shown to increase the chilling tolerance of several plant species (Wang and Buta 1994; Meir et al. 1996). In addition, postharvest decay of strawberries caused by *Botrytis cinerea* was reduced by exposure of the fruit to MeJA vapor (Moline et al. 1997). After MeJA treatment, the storage life of fresh-cut celery sticks and bell-pepper slices was extended by reduction of microbial growth and decreased physiological deterioration (Buta and Moline 1998). However, the mechanism of MeJA treatment used to protect against decay and chilling injury is unclear.

This study was undertaken to determine the physiological changes in tomato fruit treated by MeJA or MeSA during chilling-temperature storage, to investigate whether these plant regulators are involved in coordinating induction or accumulation of PR-protein mRNAs, and to test the hypothesis that those treatments might reduce chilling injury and decay through their effect on expression of PR-protein genes.

Materials and methods

Postharvest treatments

Tomato (*Lycopersicon esculentum* L. cv. Beefstake) fruit were harvested at the mature green stage in Florida and then immediately shipped to Maryland. Fruit did not receive any ethylene treatment. Breaker-stage fruit were selected and divided into lots of 80 fruit each. Fruit at the breaker stage of ripeness were defined as those that showed incipient yellow coloration at the blossom end of the fruit. Fruit were placed in 200-l airtight containers. MeJA and MeSA were spotted onto filter paper at final vapor concentrations of 0.0 (control), 0.01, 0.1 or 0.5 mM, and incubated for 16 h at 23 °C. After treatment, the containers were opened, and the fruit were stored at 5 °C for 2, 3 or 4 weeks. Fruit were transferred to 20 °C and the development of chilling injury and decay, and ripening characteristics were measured.

Evaluation of chilling injury and ripening characteristics

Chilling injury was evaluated at 20 °C for 1 day after the 2-, 3- or 4-week cold-storage periods. Symptoms of tomato fruit chilling injury were manifested as surface pitting (Wang 1993). The severity of the symptoms was assessed visually according to the following four-stage scale: 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 average extent of chilling-injury damage was expressed as a chilling-injury (CI) index, which was calculated using the following formula:

$$\text{CI index(between 0 and 4)} = \frac{\Sigma[(\text{CI level}) \times (\text{Number of fruit at the CI level})]}{\text{Total number of fruit in the treatment}}$$

Fruit decay was evaluated at 20 °C for 3 days after the 2-, 3- or 4-week cold-storage period. Decay incidence was expressed as the percentage of the total number of fruit manifesting decay symptoms (mainly *Alternaria* rot).

For determining the effect of different treatments on ripening, fruit following 3 weeks storage, were incubated in diffused light at 20 °C and were inspected daily until judged to be fully ripe by color development. A composite measure of the color of tomatoes in terms of the brightness (*L*), green-to-red component (*a*) and blue-to-yellow value (*b*) was made by Chroma meter CR-300 (Minolta, Japan). All experiments were repeated three times.

RNA isolation and Northern analysis

Total RNA was extracted according to the method of Chang et al. (1993). Tomato fruit pericarp, tissue was ground in liquid N_2 and five volumes of extraction buffer [2% (w/v) hexadecyltrimethylammonium bromide (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. Then the solution was extracted with chloroform and precipitated using LiCl. 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. After ethanol precipitation, the pellet was resuspended in autoclaved diethylprocarbonate-treated water.

Total RNA (15 µg) was size-fractionated in a 1.2% formaldehyde-denaturing agarose gel (Sambrook et al. 1989) 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 pre-hybridization 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) polyvinylpyrrolidone (PVP)], and 100 µg/ml salmon sperm DNA. Following hybridization, the blots were washed twice with 0.1× SSC and 0.2% SDS at 65 °C 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.

Preparation of the probes

Partial cDNAs of tomato *Cat1* and *Cat2* (accession numbers M93719 and AF112368, respectively) were cloned by reverse transcription (RT)-polymerase chain reaction (PCR) using RNA from tomato fruit as template. Three micrograms of total RNA was used for RT for 1 h at 37 °C using Molony Murine Leukemia Virus reverse transcriptase (Promega, USA) and oligo (dT) as the primer. After heat inactivation and ethanol precipitation, 10% of the reaction products were used as template in the PCR using oligonucleotides corresponding to published sequences of tomato catalases. Thirty-five cycles of amplification (95 °C, 30 s; 58 °C, 30 s; 72 °C, 1.0 min) were performed using 1.25 U taq polymerase (Promega). Reaction products were gel-purified and cloned into the pCR-2.1 vector (Invitrogen). The identity of the clones was confirmed by sequence analysis. An 850-bp fragment of *cat1* was amplified using the oligonucleotides CTAATTGAGAAGCTCGCGACATTTG and ATACCAGGGACAATATGTCCAGGG as the 5′ and 3′ primers. An 860-bp fragment of *cat2* was amplified using the oligonucleotides CCATTTGGTGGAGAAACTTGCCAAC and ACACCTGGAACCACAATAGAAGGG as the 5′ and 3′ primers. Clones for tomato PR-2a (extracellular acidic β-1,3-glucanase, 451-bp fragment; Van Kan et al. 1992), PR-2b (intracellular basic β -1,3-glucanase, 383-bp fragment; Van Kan et al. 1992), PR-3a (extracellular acidic chitinase, 351-bp fragment; Danhash et al. 1993) and PR-3b (intracellular basic chitinase, 383-bp fragment; Danhash et al. 1993) were obtained from Dr. A. Schaller (Institute of Plant Sciences, ETH-Zurich, Switzerland). Probes were synthesized using a DECAprime II DNA Labeling Kit (Ambion, Austin, Texas) with [^{32}P]dATP (111 TBq/mmol) as the label.

Results

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

Chilling injury was manifested in tomato fruit by a number of symptoms. Chilled fruit lost their ability to develop full red color and showed extensive green patches or mottled yellow areas. Surface contours were uneven, suggesting that cell separation may have occurred. Severely injured fruit developed sunken areas (blemishes) and increased susceptibility to *Alternaria* rot and decay. The effects of holding fruit for various lengths of time at 5 °C are shown in Table 1. The untreated fruit underwent normal ripening at 20 °C and few visual chilling-injury symptoms were observed after 2 weeks storage at 5 °C. After 3 weeks at 5 °C, severe damage was observed on untreated fruit and fruit treated with 0.5 mM MeSA or MeJA, whereas those fruit pre-treated with 0.01 mM MeSA or MeJA were only minimally affected by low temperature. Fruit treated with 0.01 mM MeSA or MeJA maintained the same quality as before cold storage except for developing a slight yellow pigmentation. After transferring to 20 °C for 3 days, the untreated fruit and fruit treated at higher MeJA or MeSA concentrations developed a high incidence of decay. Extending storage to 4 weeks at 5 °C led to the untreated and 0.5 mM MeSA-vapor- or MeJA-vapor-treated fruit being excessively damaged with a very high decay incidence. The results indicate that a 2-week storage was the maximum that could be tolerated by untreated breaker-stage fruit. In this experiment, treatment with lower concentrations (0.01 mM) of MeSA and MeJA vapor were more effective in reducing decay and protecting against chilling injury than higher concentrations (0.1 and 0.5 mM). Fruit treated with 0.5 mM showed more harmful effects and higher decay incidence than untreated fruit.

For examining color development of fruit after chilling-temperature storage, breaker-stage tomatoes were exposed to 5 °C for 3 weeks and then transferred to 20 °C for ripening. As seen in Table 2, the vapor treatment with 0.01 mM MeJA or 0.01 mM MeSA, prior to 5 °C storage, was effective at alleviating chilling injury; in this treatment category fruit ripened normally and with uniform red pigmentation. However, untreated and 0.5 mM-treated fruit stored for 3 weeks at 5 °C failed to develop the normal red color with the "*a*" value only ranging from 13.50 to 17.85. These fruit ripened more slowly than fruit treated with 0.1 mM MeJA or MeSA.

Accumulation pattern of PR-protein mRNAs in differently treated tomatoes during chilling-temperature storage

While the expression of PR-protein genes has been extensively studied in plant growth, there is little information on the influence of low-temperature storage on

Table 1 Effects of MeSA and MeJA pre-treatment and low-temperature storage on chilling injury (*CI*) and incidence of decay in tomato (*Lycopersicon esculentum*) fruit. After being stored at 5 °C for 2, 3 and 4 weeks, chilling injury (from 0 to 4) was measured after transferring the fruit from 5 °C to 20 °C for 1 day and decay was measured after transferring the fruit from 5 °C to 20 °C for 3 days. The experiment was repeated three times with 30 fruit for each treatment, and SD (±) is indicated

Treatment	5 °C, 14 days		5 °C, 21 days		5 °C, 28 days	
	CI index	Decay (%)	CI index	Decay (%)	CI index	Decay (%)
Control	0.93 ± 0.12	6.7 ± 2.2	2.19 ± 0.40	51.1 ± 2.6	3.52 ± 0.20	96.7 ± 2.2
MeSA, 0.5 mM	0.78 ± 0.07	22.2 ± 3.7	2.15 ± 0.35	76.7 ± 6.7	3.33 ± 0.07	94.4 ± 7.4
MeSA, 0.1 mM	0.37 ± 0.05	11.1 ± 2.5	1.59 ± 0.17	30.0 ± 1.7	2.96 ± 0.17	80.0 ± 11.1
MeSA, 0.01 mM	0.07 ± 0.10	7.8 ± 2.1	1.13 ± 0.04	14.4 ± 0.7	1.44 ± 0.15	22.2 ± 1.8
MeJA, 0.5 mM	0.59 ± 0.05	21.1 ± 1.8	1.89 ± 0.22	45.6 ± 4.1	3.22 ± 0.44	94.4 ± 3.7
MeJA, 0.1 mM	0.26 ± 0.05	10.0 ± 0.9	1.41 ± 0.10	30.0 ± 2.2	2.70 ± 0.32	50.0 ± 6.9
MeJA, 0.01 mM	0.04 ± 0.05	6.7 ± 0.4	0.67 ± 0.07	18.9 ± 3.7	1.85 ± 0.34	24.4 ± 2.3

Table 2 Influence of MeSA and MeJA pre-treatments and low-temperature storage on ripening of tomato fruit. After being stored at 5 °C for 3 weeks, fruit were transferred to 20 °C for ripening development and were inspected daily until judged to be fully ripe. The experiment was repeated three times with 30 fruit for each treatment, and SD (±) is indicated

Treatment	Days to ripen	Fruit ripening uniformly (%)	Color (*a*)
Control	17.3 ± 4.10	33.3 ± 11.1	13.74 ± 10.80
MeSA, 0.5 mM	17.9 ± 4.30	35.0 ± 6.7	13.50 ± 8.65
MeSA, 0.1 mM	10.5 ± 3.70	71.7 ± 8.9	26.53 ± 4.29
MeSA, 0.01 mM	6.2 ± 0.76	86.7 ± 2.2	30.13 ± 2.65
MeJA, 0.5 mM	18.0 ± 4.40	66.7 ± 7.8	17.85 ± 6.24
MeJA, 0.1 mM	10.6 ± 1.72	75.0 ± 3.3	28.52 ± 4.05
MeJA, 0.01 mM	9.6 ± 0.88	88.3 ± 5.6	31.05 ± 3.21

their expression. We examined whether exposing fruit to low temperature would induce accumulation of PR-protein mRNA. Breaker-stage, untreated fruit were stored at 5 °C for 1, 3, 7, 14, 21 and 28 days. RNA was extracted at each time point and subjected to Northern analysis. Before low-temperature storage, the transcripts of PR-2a (encoding extracellular β-1,3-glucanase) and PR-2b (encoding intracellular β -1,3-glucanase) were undetectable, while those of PR-3a (encoding extracellular chitinase) and PR-3b (encoding intracellular chitinase) were at very low levels (Fig. 1A, day 0). All PR transcripts were detectable in fruit after 3–14 days at 5 °C and most were abundant at day 7 or 14. PR-2b mRNA levels decreased to an undetectable level after 21 days storage at 5 °C. Levels of the other PR gene transcripts decreased after 21 days storage. Generally, the accumulation of PR-protein mRNAs in untreated fruit during cold-temperature storage was relatively low in comparison with MeSA- and MeJA-treated fruit (Fig. 1B, C).

To study whether PR-protein gene transcription is induced in fruit treated with MeSA or MeJA vapor and in subsequent cold storage, breaker-stage fruit were vapor-treated for 16 h at 23 °C in a sealed chamber and then transferred to 5 °C for up to 4 weeks. After vapor treatment and during cold storage, RNA was extracted and analyzed for PR-protein gene transcripts. Both MeSA and MeJA substantially induced accumulation of PR-2a, PR-2b and PR-3a mRNAs immediately after treatment (Fig. 1B and C, day 0) whereas they were undetectable in untreated fruit (Fig. 1A, day 0). The accumulation of PR-2b mRNA continued and was maintained at very high levels in both treated fruit throughout the cold-storage period. The transcript of PR-2a in MeJA-treated fruit continued after 1 day at 5 °C and was more pronounced than in untreated fruit during low-temperature storage (Fig. 1C). MeJA treatment also enhanced PR-3a mRNA accumulation (Fig. 1C), but less than MeSA treatment (Fig. 1B). The transcript level of PR-3b rose to a high level immediately after MeJA treatment (Fig. 1C, day 0) and remained high throughout the storage period. In comparison, PR-2b levels were affected the most by MeSA or MeJA treatments among all PR-protein gene transcripts.

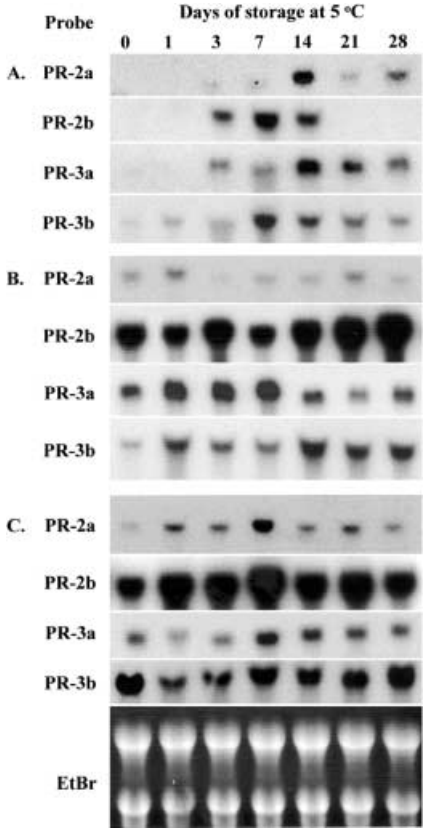

Fig. 1A–C RNA gel-blot analysis of PR genes in differently pre-treated tomatoes (*Lycopersicon esculentum*) during chilling-temperature storage. **A** Non-treated fruit; **B** 0.01 mM MeSA-treated fruit; **C** 0.01 mM MeJA-treated fruit. After treatment the fruit were stored at 5 °C for 0 (processed immediately after treatment), 1, 3, 7, 14, 21 and 28 days. Total RNA (15 μg) isolated from tomato pericarp at each time point was probed for the presence of the transcripts of intracellular PR-protein (PR-2b, PR-3b) and extracellular PR-protein (PR-2a, PR-3a) genes. Ethidium bromide (*EtBr*) staining for each lane was done to confirm equal RNA loading

Accumulation of catalase gene transcripts in response to different treatments and during chilling-temperature storage

To determine whether MeSA and MeJA directly affect catalase gene mRNA levels, we used specific probes

for *cat1* and *cat2* (accession numbers M93719 and AF112368, respectively) for Northern blot analysis. In untreated fruit, the *cat1* mRNA level was high during the first 3 days and highest at day 3. Thereafter, it decreased gradually and was maintained at a low level between 7 and 28 days of storage. Interestingly, *cat1* mRNA levels were reduced by MeSA treatment on day 0, and increased to very high levels after 3 days of storage at 5 °C; then high levels were maintained throughout the 28-day storage (Fig. 2). In MeJA-treated fruit, *cat1* mRNA accumulation was more pronounced than in MeSA-treated fruit at day 0 and day 1. The *cat1* transcripts in MeJA-treated fruit were maintained at high levels throughout the 28-day low-temperature storage (Fig. 2). The accumulation of c*at2* mRNA was very weak and there were no differences between the treatments (data not shown).

Discussion

Compounds that are able to reduce the damaging effects of various types of stress have great importance from both theoretical and practical viewpoints. In the present study, it was shown that treatment with MeJA vapor at 0.01 mM was effective in alleviating chilling injury and decreasing the incidence of decay in tomato fruit (Table 1). These results agree with the recent findings of Wang and Buta (1994) in zucchini squash and Meir et al. (1996) in grapefruits. The MeJA concentration used was similar to that found to induce physiological responses in other processes (Creelman and Mullet 1997).

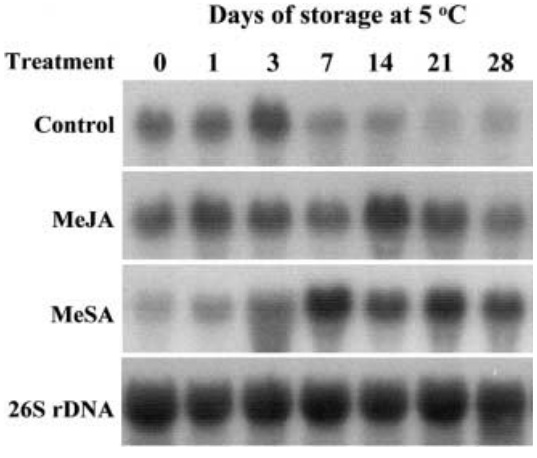

Fig. 2 Accumulation of *cat1* gene transcripts in differently pre-treated tomatoes during chilling-temperature storage. Fruit were incubated for 16 h at 23 °C with 0.01 mM MeJA or MeSA. After treatments the fruit were stored at 5 °C for 0 (processed immediately after treatment), 1, 3, 7, 14, 21 and 28 days. Total RNA (15 μg) isolated from tomato pericarp at each time point was analyzed by RNA gel blotting and probed for the presence of catalase (*cat1*) transcripts. A 26S ribosomal gene clone was used as a loading control for each blot and one example is shown (Smith and Gross 2000)

Concentrations above the optimal range were not only ineffective in reducing chilling injury, but had negative effects and increased decay incidence. Droby et al. (1999) performed in vitro experiments and indicated that neither JA nor MeJA had any direct antifungal effect on *Penicillium digitatum* spore germination or germ-tube elongation. Their results suggested that jasmonates probably reduced green-mold decay in grapefruit indirectly by enhancing the natural resistance of the fruit to *P. digitatum*.

Little information is available on SA or MeSA treatment of horticultural commodities, although there are many reports indicating that SA is an endogenous signal for the activation of certain plant defense responses and is able to enhance disease resistance (Klessig and Malamy 1994). As observed in young maize plants, 0.5 mM SA provided protection against subsequent low-temperature stress (Janda et al. 1999). Here, our results indicated that 0.01 mM MeSA vapor treatment can increase chilling tolerance and decrease decay of tomato fruit.

Our results show that the presence of low concentrations of MeSA or MeJA provides protection against chilling injury. When concentrations higher than 0.1 mM were applied, negative effects were observed. It is well known that some stress factors are able to reduce the effects of other stresses. For example, it was reported that drought-hardened maize plants became more chilling-tolerant (Sanchez-Diaz et al. 1993). These phenomena are probably due to mechanisms that are non-specific, but common to a wide range of stresses. A higher concentration of MeSA or MeJA (0.5 mM) could induce the PR gene expression (data not shown), but the chemical injury would directly damage the fruit tissue, which would result in higher decay and chilling injury (Table 1). Therefore, treatment of tomatoes with low concentrations of MeSA and MeJA could induce some defense-mechanism responses that indirectly provide protection against chilling damage, rather than the compounds themselves producing a direct effect.

The expression of PR genes induced by fungal pathogens has been analyzed in tobacco and tomato plants. Van Kan et al. (1992) found that intracellular basic 35-kDa β-1,3-glucanase mRNA is induced to high levels shortly after fungal penetration but not extracellular acidic 35-kDa β -1,3-glucanase expression. In our study, the intracellular basic 35-kDa β -1,3-glucanase (PR-2b) mRNA accumulates to high levels in tomato fruit in response to MeSA or MeJA and is maintained at those levels during subsequent low-temperature storage. Since intracellular proteins would only come into contact with the fungus during plant cell leakage or cell death, as occurs during a hypersensitive response, the expression of intracellular PR proteins is not harmful to the fungus when it grows exclusively in the intercellular space without penetration of tomato cells. However, in our study, besides increasing the transcript levels of all intracel-

lular PR proteins (PR-2b and PR-3b), MeSA and MeJA also affected the accumulation of extracellular PR gene transcripts. MeSA enhanced the transcript level of PR-3a but did not affect PR-2a mRNA accumulation, while conversely MeJA increased PR-2a transcripts but only slightly increased the transcripts of PR-3a.

The observation that SA can play a role in inducing PR gene transcription and protect against chilling injury most likely arises from reports that SA can inhibit catalase activity in several plant species (Chen et al. 1993). Our results showed that *cat1* expression was weakly inhibited by MeSA (Fig. 2). As a result of the inhibition of catalase by SA, the amount of H_2O_2 in cells may increase. The increase in reactive oxygen species (ROS) plays a role in the induction of defense responses such as PR-protein gene expression. The increase in *cat1* transcripts in MeSA-treated fruit after day 3 (Fig. 2) may result from H_2O_2 accumulation, which subsequently induces the transcription of catalase via a feedback mechanism. The ROS play two very different roles: exacerbating damage and signaling the activation of defense responses. To allow for these different roles, cellular levels of ROS must be tightly controlled. Our negative results on chilling tolerance when high MeSA concentrations were used may have been caused by the marked inhibition of catalase, which may have led to high H_2O_2 accumulation.

In conclusion, the results from this study show that MeSA or MeJA pre-treatment at low concentrations induces protection against chilling injury and resistance to pathogens in tomato fruit, most likely by inducing the transcription of PR-protein genes.

Acknowledgement We thank Dr. Andreas Schaller (Institute of Plant Sciences, ETH-Zurich, Switzerland) for the kind gift of PR-2a, PR2b, PR-3a and PR-3b cDNA fragments.

References

Buta JG, Moline HE (1998) Methyl jasmonate extends shelf life and reduces microbial contamination of fresh-cut celery and peppers. J Agric Food Chem 46:1253–1256

Chang S, Puryear J, Cairney J (1993) A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Rep 11:113–116

Chen Z, Silva H, Klessig DF (1993) Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science 262:1883–1886

Creelman RA, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. Annu Rev Plant Physiol Plant Mol Biol 48:355–381

Danhash N, Wagemakers CAM, Van Kan JAL, De Wit PJGM. (1993) Molecular characterization of four chitinase cDNAs obtained from *Cladosporium fulvum*-infected tomato. Plant Mol Biol 22:1017–1029

Droby S, Porat R, Cohen L, Weiss B, Shapiro B, Philosoph-Hadas S, Meir S (1999) Suppressing green mold decay in grapefruit with postharvest jasmonate application. J Am Soc Hort Sci 124:184–188

Enkerli J, Gisi U, Mosinger E (1993) Systemic acquired resistance to *Phytophthora infestans* in tomato and the role of pathogenesis-related proteins. Physiol Mol Plant Pathol 43:161–171

Fukuda Y, Shinshi H (1994) Characterization of a novel *cis*-acting element that is responsive to fungal elicitor in the promoter of a tobacco class I chitinases gene. Plant Mol Biol 24:485–493

Gaffney T, Friedrich L, Vernooij B, Negrotto D, Nye G, Uknes S, ward E, Kessman H, Ryals J (1993) Requirement of salicylic acid for the induction of systemic acquired resistance. Science 261:754–756

Graham LS, Sticklen MB (1994) Plant chitinases. Can J Bot 72:1057–1083

Hammond-Kosack KE, Oliver R, Harrison K, Ashfield T, Jones J (1992) Studies on the mechanism by which tomato Cf (*Cladosporium fulvum*) resistance genes activate plant defense. In: Nester EW, Verma DPS (eds) Advances in molecular genetics of plant-microbe interactions, vol 2. Kluwer Academic Publishers, Dordrecht, pp 457–461

Janda T, Szalai G, Tari I, Paldi E (1999) Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (*Zea mays* L) plants. Planta 208:175–180

Klessig DF, Malamy J (1994) The salicylic acid signal in plants. Plant Mol Biol 26:1439–1458

Mauch F, Mauch-Mani B, Boller T (1988) Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combinations of chitinases and β-1, 3-glucanase. Plant Physiol 88:936–942

Meir S, Philosopg-Hadas S, Lurie S, Droby S, Akerman M, Zauberman G, Shapiro B, Cohen E, Yoram F (1996) Reduction of chilling injury in stored avocado, grapefruit, and bell pepper by methyl jasmonate. Can J Bot 74:870–874

Moline HE, Buta JG, Saftner RA, Maas JL (1997) Comparison of three volatile natural products for reduction of postharvest decay in strawberries. Adv Strawberry Res 16:43–48

Ordentlich A, Linzer RA, Raskin I (1991) Alternative respiration and heat evolution in plants. Plant Physiol 97:1545–1550

Raskin I (1992) Role of salicylic acid in plants. Annu Rev Plant Physiol Plant Mol Biol 43:439–463

Rhoads DM, McIntosh L (1992) Salicylic acid regulation of respiration in higher plants: alternative oxidase expression. Plant Cell 4:1131–1139

Ryals J, Uknes S, Ward E (1994) Systemic acquired resistance. Plant Physiol 104:1109–1112

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Plainview, NY

Sanchez-Diaz M, Perez de Juan J, Irigoyen JJ (1993) The effect of chilling on stomatal behavior, transpiration, water absorption and carbon dioxide exchange rate in drought-hardened and non hardened maize (*Zea mays* L). In: Wilson D, Thomas H, Pithan K (eds) Crop adaptation to cool, wet, climates. E. Guyot SA, Brussels, pp 245–250

Schlumbaum A, Mauch F, Vogeli U, Boller T (1986) Plant chitinases are potent inhibitors of fungal growth. Nature 324:365–367

Sela-Buurlage MB, Ponstein AS, Vloemans SA, Melchers LS, Van den Elzen PJM, Cornelissen BJC (1993) Only specific chitinases and β-1,3-glucanases exhibit antifungal activity. Plant Physiol 101:857–863

Shulaev V, Silverman P, Raskin I (1997) Airborne signalling by methyl salicylate in plant pathogen resistance. Nature 385:718–721

Smith DL, Gross KC (2000) A family of at least seven β-galactosidase genes is expressed during tomato fruit development. Plant Physiol 123:1173–118

Van Kan JAL, Joosten MHAJ, Wagemakers CAM, van den Berg-Velthuis GCM, de wit PJGM (1992) Differential accumulation of mRNAs encoding extracellular and intracellular PR proteins in tomato induced by virulent and avirulent races of *Cladosporium fulvum*. Plant Mol Biol 20:513–527

Vick BA, Zimmermann DC (1984) Biosynthesis of jasmonic acid by several plant species. Plant Physiol 75:458–461

Wang CY (1993) Approaches to reduce chilling injury of fruits and vegetables. Hort Rev 15:83–95

Wang CY, Buta JG (1994) Methyl jasmonate reduces chilling injury in *Cucurbita pepo* through its regulation of abscisic acid and polyamine levels. Environ Exp Bot 34:427–432

Ward ER, Uknes SJ, Williams SC, Dincher SS, Wiederhold DL, Alexander DC, Ahl-Goy P, Metraux JP, Ryals JA (1991) Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell 3:1085–1094