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METHYL JASMONATE REDUCES CHILLING INJURY IN *CUCURBITA PEPO* THROUGH ITS REGULATION OF ABSCISIC ACID AND POLYAMINE LEVELS

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Wang C. Y. and Buta J. G. *Methyl jasmonate reduces chilling injury in* Cucurbita pepo *through its regulation of abscisic acid and polyamine levels.* Environmental and Experimental Botany **34,** 427–432, 1994.—Zucchini squash fruit (*Cucurbita pepo* L.) were pressure infiltrated (82.7 kPa for 3 min) with methyl jasmonate (MJ) in aqueous suspension and then stored at a chilling temperature of 5°C. Control fruit were treated with distilled water and handled in a similar manner. Control fruit started to show chilling injury after 4 days of storage, whereas treated fruit had a 2–4 day delay in the onset of chilling injury symptoms. The abscisic acid (ABA) level in exocarp tissue of the control fruit increased after chilling treatment. An even larger increase in ABA level was found in the MJ-treated tissue upon exposure to 5°C, indicating that MJ may stimulate ABA synthesis at chilling temperatures. The treated and control fruit had similar increases in putrescine content when exposed to chilling temperatures. Spermidine and spermine contents decreased during storage at 5°C in treated and control squash. However, the treated fruit maintained higher levels of spermidine and spermine than the control fruit throughout storage at 5°C. These results indicate that MJ may prevent chilling injury by a mechanism which involves regulation of ABA and polyamine levels.

Key words: *Cucurbita pepo*, abscisic acid, chilling injury, methyl jasmonate, polyamine.

INTRODUCTION

Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MJ), have been found to occur naturally in a wide range of higher plants.[17,27] Jamonates were first detected as fragrant constituents of essential oils in *Jasminum*[3] and *Rosmarinum*.[1] Their physiological activities as senescence-promoting substances and growth inhibitors have been reported by several investigators.[19,21,32,33] Jasmonates have been shown to affect a number of biological processes including promotion of leaf abscission, inhibition of seed germination and root growth, promotion of stomatal closure and respiration in leaves, stimulation of chlorophyll degradation, inhibition of flower bud formation, and enhancement of fruit ripening.[20,27]

Many of the physiological responses to jasmonates are similar to the effects caused by abscisic acid (ABA).[20,27] ABA is a plant hormone whose

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increased levels in tissues are associated with imposition of environmental stressors.[7,37] Since ABA has been shown to increase the chilling tolerance of several plant species,[24,34,36] it was postulated that jasmonates would induce a similar response in plants to alleviate chilling injury. In addition, some specific abundant proteins induced by jasmonates are similar to those induced by heat shock.[18,20,35] Heat treatment has been demonstrated to be effective in alleviating chilling injury.[14–16] It has also been found that treatment with MJ increases linolenic acid and decreases linoleic acid content in tomato fruit.[2] A higher ratio of linolenic acid to linoleic acid in MJ treated samples can be beneficial in attaining higher chilling tolerance.[31] Post-harvest treatments that enhance polyamine levels in the tissue also increase chilling tolerance.[11] For these reasons, this study was undertaken to determine the relation of MJ to ABA and polyamines, and to test the hypothesis that MJ reduces chilling injury through its regulation of ABA and polyamine levels at chilling temperatures.

MATERIALS AND METHODS

Plant material and chemical treatment

Zucchini squash (*Cucurbita pepo* L., cv. Elite) used in this study were picked from a farm near Beltsville, MD, U.S.A. Fruit were harvested at commercial maturity (16–22 cm in length) and were immediately treated with 0.05, 0.1, 0.5 or 1.0 mM MJ (Bedoukian Research, Inc.) in aqueous suspension. Fruit were completely immersed in the solution, and a pressure of 82.7 kPa for 3 min was used for the infiltration of MJ. This amount of pressure and treatment duration were previously found to be optimal for the infiltration of chemicals without tissue injury. Control fruit were infiltrated with distilled water in a similar manner. Following infiltration, the fruit were placed on Kraft paper and allowed to air dry before storage at 5°C.

Evaluation of chilling injury

Three squash were chosen at random from each treatment at 2-day intervals and examined for chilling injury. The degree of chilling injury, as judged by the extent of surface pitting, was evaluated 1 day after transferring the squash from storage chambers to room temperature by rating on a scale of 1–5, with 1 = no abnormality, 2 = trace, 3 = slight, 4 = moderate and 5 = severe chilling injury. The scoring was done blindly. After the evaluation of chilling injury, three 2.0 g samples of exocarp tissue were removed from each squash and immediately frozen. Samples were stored at −80°C prior to the analyses of abscisic acid and polyamines.

Determination of ABA

Samples of squash exocarp tissue (2.0 g) after thawing were homogenized with 100 ml of 80% acetone using a Polytron homogenizer (Brinkmann). Internal standard (1100 ng of d_3-*cis*-ABA) was added during this first stage of sample preparation.[13] The resulting extract was filtered and the acetone removed by rotary evaporation. The residue was redissolved in a minimum amount of methanol and filtered. After several washings with dichloromethane, a final volume of approximately 30 ml was obtained. The acidic hormone was partially purified by use of an Extra-Sep-NH_2 solid phase minicolumn (2.5 g 6 ml^{-1}). After prewashing the column with 50–60 ml dichloromethane, the sample was passed through under vacuum at a maximum rate of 5 ml min^{-1}, followed by successive washings with 12 ml dichloromethane, 12 ml ethyl acetate and 12 ml methanol. The acidic hormone was eluted from the column with 30 ml of 2% acetic acid in methanol and concentrated. Further purification was performed by HPLC using a Whatman Partisil 5 ODS-3 column (4.6 × 250 mm). The initial solvent system was acetonitrile:1% acetic acid (23:77) for 18 min at 1 ml min^{-1}. This was followed by a linear gradient to 100% acetonitrile in the succeeding 17 min. The zone of elution corresponding to that of the ABA standard was collected, evaporated and treated with diazomethane. The methyl ester sample of ABA was purified further by HPLC using the same chromatographic procedures with acetonitrile:1% acetic acid (10:90) for 30 min. The quantity of ABA was then determined by GC–MS–SIM by analysis of the methyl ester on a HP 5992 unit equipped with a 15 m 0.32 mm DP-1701 fused silica capillary column (J and W Scientific). Ratios of the heavy isotope ABA ion (193) compared to native ABA ion (190) were used to obtain weights of the native hormone present in the sample.

Polyamine analysis

Exocarp samples (2.0 g) of zucchini squash were homogenized in 15 ml 5% perchloric acid using a

Polytron homogenizer (Brinkmann). 1,6-Hexanediamine (500 nmol g^{-1} fresh wt.) (Sigma) was added as an internal standard. The probe was rinsed with another 15 ml aliquot of 5% perchloric acid. Aliquots were combined. The homogenate was then centrifuged at 47,000 *g* for 20 min. The supernatant was retained and used for polyamine analysis.

Polyamines were analyzed using HPLC with methods similar to those of Smith and Davies.[30] Dansylation was performed by mixing 400 μl dansyl chloride (18.5 mM in acetone) (Sigma) and 150 μl saturated sodium carbonate with 200 μl of extract. After incubation overnight at room temperature, 200 μl proline (0.43 M) were added and incubation was continued for 1 hr. After centrifugation for 10 min in a microcentrifuge (Beckman), the pH of the supernatant was checked and adjusted with HCl to neutral (pH 7). Samples (100 μl) of the supernatant were used for HPLC analysis. HPLC was performed on a system consisting of two 6000A pumps (Waters) programmed with a 720 System Controller (Waters). Samples were injected using a Rheodyne injector into a reverse phase C-18 column (Supelco 25 cm LC-18 with a Supelguard LC-18 5-μm guard column). Samples were eluted from the column at a flow rate of 1.5 ml min^{-1} with a programmed solvent gradient of 0, 100, 0; 15, 0, 100; 19, 0, 100; 19.1, 100, 0; where the first number is the time (min), the second number the percentage of buffer A (60:40, v/v, methanol:water) and the third number the percentage of buffer B (100% methanol). Elution was completed in 19 min. Eluates were detected by a 1046A programmable fluorescence detector (Hewlett Packard) using an excitation wavelength of 365 nm and an emission wavelength of 510 nm. Data were collected and analyzed using a Compaq 286 computer system equipped with a Baseline 810 Chromatography Workstation (Dynamic Solutions). Polyamines were quantified by a comparison of peak areas with those of standards. Each data point is the average of three independent samples.

RESULTS

Inhibition of chilling injury by methyl jasmonate

Figure 1 shows the average chilling injury index of zucchini squash fruit treated with different concentrations of MJ during exposure to 5°C. Chilling injury, manifested as surface pitting, started to appear on the skin of control fruit after 4 days of exposure to 5°C and progressed rapidly with time in storage. The symptoms of chilling injury became even more pronounced after transferring the fruit from a chilling to a warmer temperature. By the 10th day of storage, most squash had developed moderate or severe pitting. In severe cases, the entire fruit was covered with numerous sunken areas. MJ treatments significantly delayed the onset of chilling injury. All of the concentrations used (0.05–1.0 mM) were effective in reducing the severity of chilling injury (Fig. 1). Surface pitting was not apparent on treated squash until 6–8 days of exposure to 5°C, and moderate or severe pitting did not develop until 14 days.

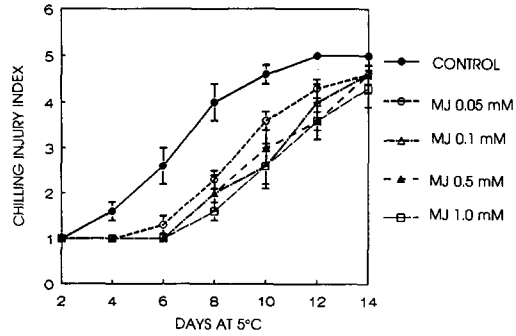

Fig. 1. Effect of methyl jasmonate (MJ) on chilling injury of zucchini squash stored at 5°C. Chilling injury was rated as 1 = no abnormality, 2 = trace, 3 = slight, 4 = moderate and 5 = severe pitting. Vertical bars represent S.E.

ABA levels as affected by methyl jasmonate and chilling temperature

ABA levels in the exocarp tissue of zucchini squash at harvest averaged 145 ng g^{-1} fresh weight After 1 day of exposure to temperatures of 5°C, ABA levels increased to 607 and 952 ng g^{-1} fresh weight in the control and MJ treated tissues, respectively (Table 1). These increases might simply be a response of warm fruit from the field (*ca* 25°C) to the cold storage temperature (5°C). After the initial stimulation, the ABA levels continued to increase under chilling stress. MJ treatment resulted in a significantly higher level of ABA in the squash exocarp tissue than the control.

Table 1. *Effect of methyl jasmonate* (MJ) *on endogenous ABA levels* (ng g^{-1} fresh weight) *in zucchini squash at chilling* (5°C) *temperatures**

	Days at 5°C		
Treatment	0	1	4
Control	145[a]	607[a]	825[a]
1.0 mM MJ	145[a]	952[b]	1004[b]

*Mean separation within columns by Duncan's multiple range test.

Values followed by different letters are significantly different at P = 0.05.

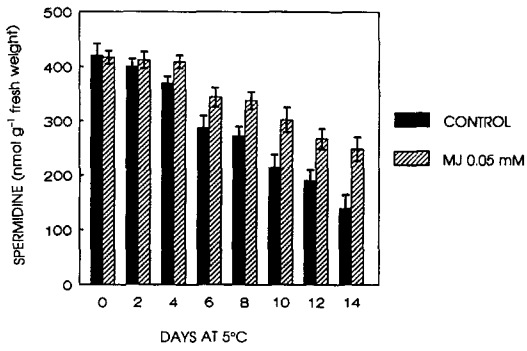

Fig. 3. Effect of methyl jasmonate (MJ) on spermidine levels in the exocarps of zucchini squash during storage at 5°C. Vertical bars represent S.E.

Polyamine content as affected by methyl jasmonate and chilling temperature

The effects of 0.05 mM MJ treatment on polyamine contents in the exocarp of zucchini squash are shown in Figs 2–4. Similar effects were found with higher concentrations of MJ, therefore, these data are not shown. Storage of zucchini squash at 5°C resulted in a steady increase in putrescine levels (Fig. 2). Treated and control fruits showed a similar increase and little difference was found in the rate of increase in putrescine between the two treatments. Spermidine levels decreased with time in storage (Fig. 3). However, the MJ-treated fruit maintained higher spermidine levels than the control samples throughout the 5°C storage. The levels of spermine in zucchini squash were low compared to those of putrescine and spermidine. Spermine declined rapidly during storage at 5°C (Fig. 4). A large decrease (30%) in spermine occurred in the control fruit after 2 days of storage. Spermine in the treated fruit also declined during storage but consistently maintained higher levels than those in the control fruit.

DISCUSSION

The similarities between MJ and ABA in their biological effects are well documented.[20,27,33] Both substances induce similar multiple physiological effects including growth inhibition and senescence acceleration.[20] Our study demonstrates that MJ and ABA have similar effects in reducing chilling injury. We further show that MJ can also enhance the accumulation of ABA in the exocarp tissue of zucchini squash (Table 1). ABA levels rose in both control and treated tissues upon exposure to 5°C.

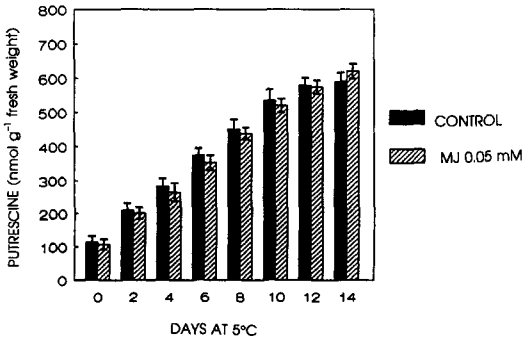

Fig. 2. Effect of methyl jasmonate (MJ) on putrescine levels in the exocarps of zucchini squash during storage at 5°C. Vertical bars represent S.E.

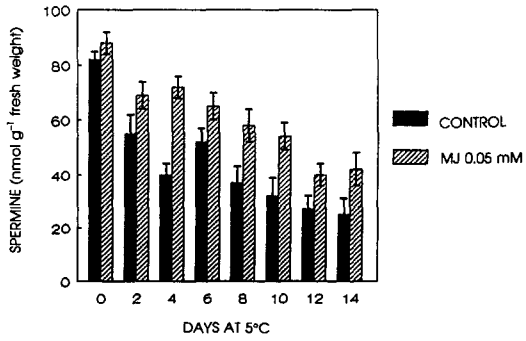

Fig. 4. Effect of methyl jasmonate (MJ) on spermine levels in the exocarps of zucchini squash during storage at 5°C. Vertical bars represent S.E.

This phenomenon is a general response of chilling sensitive tissues to the shifting of temperature from field conditions (*ca* 25°C) to cold storage (5°C).[13] MJ-treated fruit consistently had higher levels of ABA than the control fruit. It appears that MJ caused an alteration in tissue metabolism, which includes an increased synthesis of ABA at chilling temperatures. Higher levels of ABA in the MJ-treated tissue undoubtedly contributed to the higher tolerance to chilling injury. Recently, ABA was shown to induce an accumulation of 4-amino-butyrate, which may influence the tolerance of plant tissues to stress conditions.[22] Modifications of gene expression induced by ABA have been associated with an increased chilling tolerance in maize suspension-cultured cells.[37] Direct application of ABA has also been reported to reduce chilling injury in coleus,[28] seedlings of cucumber[25] and tomato,[10] grapefruit[9] and zucchini squash.[34] Changes in gene expression in response to increased ABA levels resemble the induction and accumulation of specific abundant proteins due to treatment with MJ.[20,29] MJ may act as a second messenger in plants to transduct signals and facilitate the expression of defense genes in response to various stresses.[4,8,23]

Upon exposure to chilling temperature, the putrescine level increased in the exocarp tissue of zucchini squash while spermidine and spermine levels decreased (Figs 2–4). The stimulation of putrescine formation in chilled tissue is consistent with other reports that plants tend to increase their synthesis of putrescine in response to a variety of stresses.[5] However, chilling-induced putrescine accumulation appears to originate from the ornithine decarboxylase pathway rather than the arginine decarboxylase pathway as in most other stresses.[12] Similar increases in putrescine were found in the control and the MJ-treated chilled tissues (Fig. 2). MJ treatment had little effect on the levels of putrescine throughout the 5°C storage. However, the decline of the levels of spermidine and spermine during cold storage was reduced by MJ treatment. Spermidine and spermine have been reported to be involved in reducing lipid peroxidation, inhibiting the activity of degradative enzymes, and stabilizing membrane structure.[6,11,26] The higher levels of polyamines and ABA in MJ-treated tissues appear to be effective through several types of mechanisms in alleviating the effects of chilling stress.

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