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Effect of Abscisic Acid on Chilling Injury of Zucchini Squash

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Abstract. The endogenous levels of abscisic acid (ABA) in zucchini squash were increased by temperature conditioning at 10°C for 2 days. This temperature conditioning treatment reduced the severity of chilling injury in the squash during subsequent storage at 2.5°C. The ABA levels remained higher in treated squash than in untreated samples throughout the storage. Direct treatments of squash with ABA at 0.5 and 1.0 mM before storage at 2.5°C increased ABA levels in the tissue and were also effective in reducing chilling injury.

Certain fruits and vegetables are injured by low but nonfreezing temperatures (Hardenburg et al. 1986). Injuries by these chilling temperatures result in a loss of quality manifested as surface lesions, water-soaking, internal discoloration, uneven or incomplete ripening, and decay (Saltveit and Morris 1990). Thus, chilling injury limits the storage life of many horticultural commodities. The development of methods to alleviate chilling injury is needed to reduce postharvest losses of these commodities.

The influence of plant growth regulators on the resistance of plants to low temperature injury, including chilling and freezing injury, has been reported (Carter and Brenner 1985, Irving 1969, Rikin et al. 1983). An increase in resistance to low temperatures has been associated with an increase in the ABA/gibberellic acid (GA) ratio (Waldman et al. 1975) or a decrease in GA content (Irving and Lanphear 1968, Reid et al. 1974). Treatment with auxin reduced chilling-induced leaf wilting of sorghum seedlings (Tajima and Shimizu 1977). Applications of benzyladenine, GA, and 2,4-dichlorophenoxyacetic acid significantly altered the susceptibility of grapefruit to chilling injury (Ismail and Grierson 1977). The exposure of "Honey Dew" muskmelons to ethylene at 20°C for 24 h before storage at 2.5°C for 2.5 weeks significantly reduced the incidence of chilling injury (Lipton and Aharoni 1979).

The beneficial effects of ABA treatment in reducing chilling injury have been shown in grapefruit (Kawada et al. 1979), tomato seedlings (King et al. 1982), cotton seedlings (Rikin et al. 1979, 1980), cucumber cotyledons and plants (Sasson and Bramlage 1981, Semeniuk et al. 1986), and one cultivar of coleus (Semeniuk et al. 1986). There is increasing evidence that high endogenous levels of ABA are related to increased chilling tolerance. The protection of rice seedlings and corn leaves against chilling injury by mefluidide was found to be mediated through its effect on ABA levels (Tseng and Li 1984, Tseng et al. 1986, Zhang et al. 1986). Mefluidide triggered an increase in endogenous ABA content in maize leaves when the plants were grown in a nonchilling environment with adequate water supply (Tseng et al. 1986). It was suggested that this increase in endogenous ABA before chilling may be an essential step in activating a protective mechanism against chilling injury during low temperature exposure (Tseng and Li 1984). This study was initiated to determine if ABA is involved in the reduction of chilling injury in zucchini squash treated with temperature conditioning, and if direct treatment of ABA would reduce chilling injury.

Materials and Methods

Plant Materials and Treatments

Zucchini squash (*Cucurbita pepo* L. cv. Ambassador) were freshly harvested from a local farm near Beltsville, MD. Samples were selected for the uniformity of size (18–22 cm in length) and randomly divided into two groups. The first group was placed in storage at 2.5°C as a control. The second group was preconditioned at 10°C for the first 2 days of storage, and then moved to 2.5°C for the remainder of the study. Relative humidity of the storage rooms was maintained at 95%. Three squash were removed from each storage group every 2 days for evaluation of

chilling injury and analysis of endogenous ABA levels. The degree of chilling injury, as judged by the extent of surface pitting, was rated on a scale of 0 to 4: 0 = no abnormality, 1 = trace, 2 = slight, 3 = moderate, and 4 = severe chilling injury. Squash with a chilling injury index >3 were considered not marketable. In another experiment, freshly harvested zucchini squash were treated with exogenous ABA before storage at 2.5°C. The squash were pressure infiltrated with ABA by immersing them in 0.5 or 1 mM ABA [(±)-*cis-trans* isomer from Sigma Chemical Co.] solutions and subjecting them to 0.7 kg cm^{-2} of air pressure for 3 min. The control fruit were similarly infiltrated with distilled water.

Extraction and Analysis of ABA

The zucchini squash flesh tissues (5 g) were ground with a 30 ml solution containing 80% methanol and 10 mg 2,6-di-*t*-butyl-4-methyl phenol/L at 0°C using a Polytron homogenizer (Brinkmann Instruments). The homogenate was centrifuged at 12,000 *g* for 20 min at 0°C. The pellet was reextracted twice more with fresh solutions. The pooled extracts were evaporated under vacuum at 30°C to a 20 ml aqueous solution. An equal volume of 0.1 M phosphate buffer (pH 8.0) was added to the decanted supernatant. The pH of the solution was adjusted to 8.0 with 1 N NaOH and then the solution was partitioned three times with an equal volume of petroleum ether. The pH of the aqueous solution was then acidified to 2.8 with 6 N HCl and extracted three times with ethyl acetate. Hexane was added to the ethyl acetate fraction to make a hexane:ethyl acetate ratio of 7:3. After centrifugation at 2000 *g* for 5 min, the lower aqueous layer was discarded. The supernatant was then passed through a Baker-10 SPE 3-ml column prepacked with 500 mg of silica gel according to Powell and Maybee (1985). ABA was eluted with 3 ml of methanol:acetonitrile (1:3) and evaporated to dryness under N_2. An internal standard of 15,000 dpm [^{3}H]ABA (22.5 Ci $mmol^{-1}$, 1 Ci = 37 GBq) was used to determine losses during the purification procedure. Overall recovery was between 60 and 70%. ABA was then methylated with ethereal diazomethane for gas chromatographic analysis. Ethereal diazomethane was prepared by reacting alcoholic alkali with *N*-methyl-*N*-nitroso-N'-nitroguanidine in the presence of ether, and co-distilling the product using a hot water bath. The methylated ABA was evaporated to dryness under N_2 and then taken up with 100 µl methanol. One microliter of the derivatized samples was injected into a Hewlett Packard 5890 gas chromatograph equipped with a ^{63}Ni electron capture detector for quantification of ABA. A 12.5-m capillary column (0.2 mm i.d.) coated with 0.33 µm dimethyl silicone fluid was used. Helium at a flow rate of 1 ml/min was used as carrier gas and purified N_2 was used as make-up gas. Chromatograph temperatures were as follows: injector 250°C, detector 300°C, and column 180–200°C programmed at 2°C/min. A Pye Unicam model 104 gas chromatograph equipped with a 0.46 m × 2.0 mm (i.d.) 3% OV 1 packed glass column and Kratos AEI MS 30 double-beam mass spectrometer was used to confirm the presence of Me-ABA (Wang et al. 1987).

Results and Discussion

Reduction of Chilling Injury by Temperature Preconditioning

Temperature preconditioning of zucchini squash at 10°C for 2 days was effective in reducing the symptoms of chilling injury (Fig. 1). Surface pitting on the skin of squash, which were not previously preconditioned, appeared after 4 days of exposure to chilling temperature of 2.5°C. The symptoms of chilling injury developed rapidly in these squash and the symptoms became very severe after 8 days. Squash which had been previously conditioned did not develop chilling injury symptoms until after 6 days at 2.5°C. The reduction of chilling injury by prestorage temperature conditioning has also been shown in a number of other fruits and vegetables (Hatton 1990). However, the mechanism of how this treatment reduces chilling injury is not fully understood.

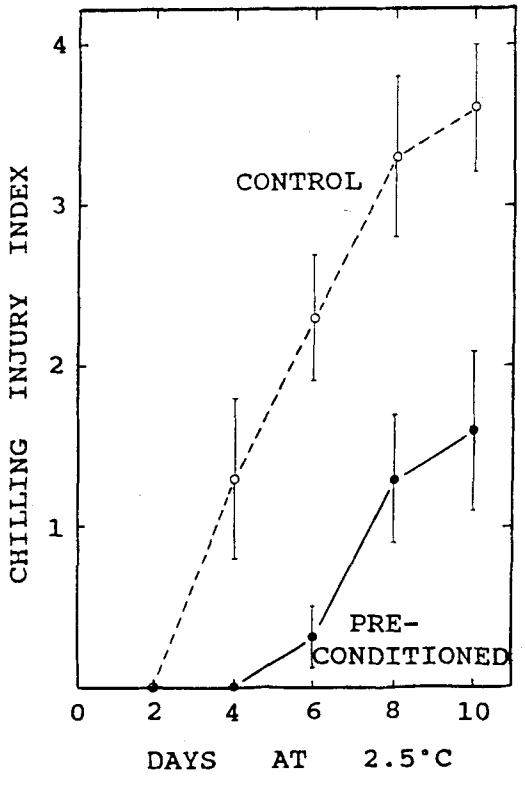

Fig. 1. Development of chilling injury in zucchini squash with time of storage at 2.5°C. Control fruit were stored continuously at 2.5°C. Preconditioned fruit were held at 10°C for 2 days then stored at 2.5°C. Bars indicate ± SE.

Effect of Temperature Preconditioning on ABA Levels

The endogenous level of ABA in zucchini squash was increased by temperature preconditioning at 10°C (Fig. 2). It is possible that the loss of moisture during 2 days at 10°C may also contribute to the

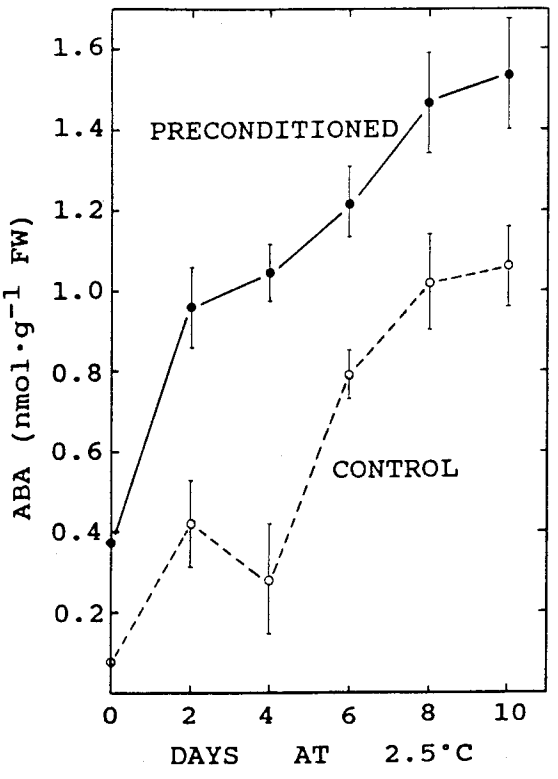

Fig. 2. Effect of preconditioning treatment on the endogenous levels of ABA in zucchini squash. Bars indicate ± SE.

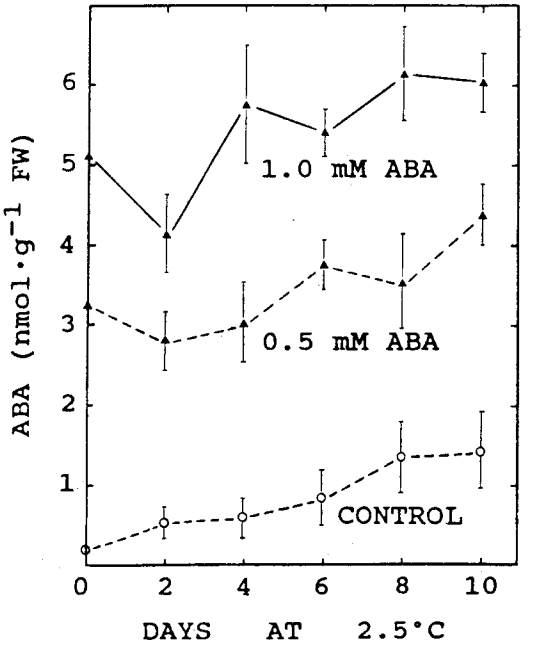

Fig. 3. ABA levels in zucchini squash during storage at 2.5°C after treatment with exogenous ABA. Bars indicate ± SE.

increased accumulation of ABA. The ABA level was further enhanced when these squash were exposed to 2.5°C. The ABA content continued to rise during storage. The preconditioned squash consistently had higher ABA levels than the untreated control throughout the 10-day storage period. Increases in ABA concentrations have also been reported in several chilling-sensitive species after exposure to 10°C (Daie et al. 1981). Capell and Dörffling (1989) showed that the accumulation of ABA in chilled plant tissues was preceded by low temperature-induced water stress and suggested that low temperature stress induces a rise in ABA through the development of water stress. It has been demonstrated that plants accumulate ABA during wilt or under drought conditions (Wright and Hiron 1969). There is also evidence that chilling injury is reduced by the increased levels of endogenous ABA resulting from water stress (Eamus and Wilson 1983). ABA is known to induce stomatal closure (Cummins et al. 1971, Mittleheuser and van Steveninck 1969) and reduce water loss (James and Mansfield 1970). Since wilting is one of the symptoms of chilling in some plants, the reduction of water loss should reduce the extent of chilling injury.

Effect of ABA Treatment on Chilling Injury

Pressure infiltration of 0.5 and 1 mM ABA increased ABA levels in zucchini squash (Fig. 3). These treatments also delayed the development of chilling injury symptoms (Fig. 4). Similar protection against chilling injury by ABA application has also been reported in grapefruit (Kawada et al. 1979), and seedlings of tomato, cotton, and cucumber (King et al. 1982, Rikin et al. 1979, Sasson and Bramlage 1981, Semeniuk et al. 1986). It has been suggested that the mechanism of ABA action in reducing chilling injury in plants might involve the stabilization of membranes (Markhart 1986). Flores et al. (1988) showed that terpenoid analogues of ABA reduced electrolyte leakage and the degradation of phospholipids, particularly phosphatidylcholine content. These ABA analogues also promoted proline levels and contributed to the maintenance of water status in cucumber seedlings (Flores et al. 1988). Rikin et al. (1983) reported that the depolymerization of the microtubular network was involved in the development of chilling injury in cotton seedlings, and ABA prevented chilling injury by stabilizing the microtubular network. This study showed that ABA levels in squash tissue can be augmented by temperature preconditioning treatment or by direct infiltration of exogenous ABA. Higher ABA content in zucchinni squash tissue was also correlated with reduced chilling injury. Further

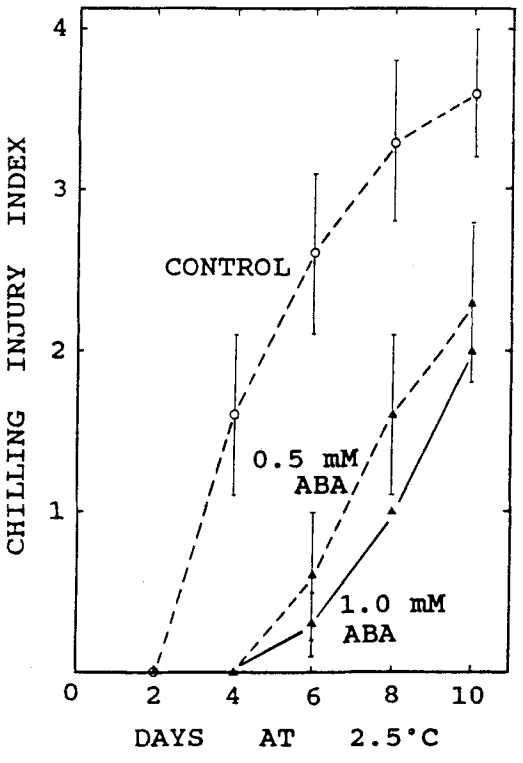

Fig. 4. Development of chilling injury during storage at 2.5°C after treatment with exogenous ABA. Bars indicate ± SE.

studies are necessary to determine if ABA treatments are effective in delaying chilling injury of other fruits and vegetables.

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