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Combined treatment of heat shock and low temperature conditioning reduces chilling injury in zucchini squash

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Abstract

The severity of chilling injury in zucchini squash stored at 5°C and then transferred to 20°C was reduced by a prestorage treatment with hot water of 42°C for 30 min. The chilling injury was further reduced when zucchini squash were preconditioned at 15°C for 2 days after hot water treatment but before the 5°C storage. Zucchini squash stored at 15°C did not develop any symptoms of chilling injury. However, weight loss was most severe in zucchini squash stored at 15°C. Squash kept at 5°C had the least weight loss during the 2-week storage. Weight losses were comparable in squash treated and not treated with hot water. Analysis of polyamines in zucchini squash showed that putrescine increased with time during storage at 5°C. The putrescine level in hot water treated samples was low initially but increased rapidly during storage at 5°C. Temperature conditioned squash showed a similar rate of increase in putrescine as those treated with hot water. Spermidine and spermine levels decreased in all samples during storage at 5°C. However, temperature conditioning as well as hot water treatments maintained higher levels of spermidine and spermine in the skin of zucchini squash.

Key words: Chilling injury; Heat shock; Temperature conditioning; Squash

1. Introduction

Temperature prior to cold storage significantly affects the susceptibility of sensitive species to chilling injury (Saltveit, 1991). Many chilling-sensitive commodities can be conditioned by exposure to temperatures slightly above the critical chilling range to increase their chilling tolerance (Hatton, 1990). This type of low temperature conditioning has been reported to reduce chilling injury either by delaying the onset of the injury or by suppressing the development of the injury symptoms

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in several fruits and vegetables (Wang, 1993). Prestorage conditioning at 10°C for 5 days was effective in reducing chilling injury in sweet peppers kept at a temperature below 7°C (McColloch, 1962). Cucumbers can also be conditioned at 15°C before storage at 6.5°C to prevent chilling injury (Nakamura et al., 1985). Exposure of papaya fruit for 4 days at 12.5°C prior to storage at 2°C reduced chilling damage (Chen and Paull, 1986). Hatton and Cubbedge (1982) found that preconditioning of grapefruit at 10° or 15°C for 7 days reduced chilling injury at 0° or 1°C.

Another type of prestorage temperature manipulation which has been demonstrated to lessen chilling injury is heat treatment. Heat treatment has been used as a means to reduce pitting in grapefruit stored at 2° or 4.5°C as early as 1936 by Brooks and McColloch. Subsequently, this type of high temperature conditioning has been shown to prevent chilling injury in other horticultural commodities. Warming of cucumber fruit for 24 h at 36° to 40°C considerably reduced chilling injury during storage at 5°C (Hirose, 1985). Conditioning mature green tomatoes for 3 days at the same temperatures prevented the development of chilling injury in 2°C storage (Lurie and Klein, 1991). More recently, the use of hot water immersion or vapor heat treatment has been demonstrated to increase the chilling tolerance of cucumbers and mangos (McCollum and McDonald, 1993; McCollum et al., 1993).

Although various biochemical and physiological alterations have been associated with temperature conditioning or hardening treatments, the mechanism for these changes is not clear. Low temperature conditioning has been shown to increase polyamine levels and S-adenosylmethionine decarboxylase activity (Kramer and Wang, 1990). The increase in polyamine biosynthesis has been proposed to enhance the stabilization of membranes and reduce free radical damage. The present study was undertaken to determine the relationship of heat treatment and changes in polyamine levels, and the effect of a combination of treatment including heat shock and low temperature conditioning on chilling tolerance in zucchini squash.

2. Materials and methods

Plant materials and treatments

Zucchini squash (*Cucurbita pepo* L., cv. Elite) used for this study were freshly harvested from a local farm near Beltsville, Maryland, USA. Samples were selected for their uniformity of size (16–22 cm in length) and randomly divided into five lots. The first group was placed in storage at 5°C as a chilled control. The second group was placed in storage at 15°C as a nonchilled control. The third group was preconditioned at 15°C for the first 2 days of storage and then moved to 5°C for the remainder of the study. The fourth group was immersed in hot water (42°C dist. H_2O) for 30 min as the heat treatment. Following the treatment, the squash were placed on Kraft paper and allowed to dry before storage at 5°C. The fifth group was treated with procedures similar to the fourth group except that the squash were conditioned at 15°C for 2 days after the heat treatment but before 5°C storage.

Chilling injury evaluation

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 5 h after transfer of squash from storage chambers to room temperature (20°C) by rating on a scale of 1 to 5, with 1 = no abnormality, 2 = trace, 3 = slight, 4 = moderate, and 5 = severe chilling injury.

Measurement of weight loss

Five squash were marked in each treatment. The weights of these squash were recorded daily. The amount of weight loss was expressed as a percent of initial weight.

Polyamine analysis

Following the evaluation of chilling injury, three 2.0-g samples of exocarp were removed from each squash and immediately frozen. Samples were stored at −80°C prior to analysis.

Extracts for polyamine analysis were prepared by homogenizing the exocarp tissue in 15 ml 5% (v/v) perchloric acid using a Polytron homogenizer (Brinkman). 1,6-Hexanediamine (500 nmol g^{-1} fresh weight) (Sigma) was added as an internal standard. The probe was rinsed with another 15 ml aliquot of 5% perchloric acid which was then combined with the first aliquot. The homogenate was then centrifuged at 47,000 *g* for 20 min. The supernatant was removed and used for polyamine analysis.

Polyamines were analyzed using high performance liquid chromatography (HPLC) with methods similar to those of Smith and Davies (1985). Dansylation was performed by mixing 400 μl (18.5 mM in acetone) dansyl chloride (Sigma) and 150 μl saturated sodium carbonate with 200 μl of extract. After incubation overnight at room temperature, 200 μl (0.43 M) proline were added and incubation was continued for 1 h. After centrifugation for 10 min in a microcentrifuge (Beckman), the pH of the supernatant was checked and adjusted with HCl close to neutral (pH 7) as necessary. Samples (100 μl) of the supernatant were used for HPLC analysis. HPLC was performed on a system consisting of two 6000 A pumps (Waters) programmed with a 720 System Controller (Waters). Samples were injected using a Rheodyne injector onto 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, where the first number is the time (min), the second number is the % buffer A (60:40, v/v, methanol/water) and the third number is the % buffer B (100% methanol). Elution was completed in 19 min. Eluates were detected by a 1046 A 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 the comparison of peak areas with those of standards. Each data point is the average of three independent samples.

3. Results

The degree of chilling injury in zucchini squash from various treatments is shown in Fig. 1. All squash appeared normal without any symptoms of chilling injury within 2 days of exposure to 5°C. However, the differences in the severity of chilling injury among different treatments became more apparent as time progressed. Traces of pitting were detected on the skin of squash from the 5°C control group after 4 days. The symptoms of chilling injury developed rapidly in this group. Severe pitting with numerous sunken areas were observed in these squash after 12 days of exposure to 5°C. The onset of chilling injury symptoms in squash was delayed by hot water treatment. However, this treatment did not reduce the rate of chilling injury development. Temperature conditioning at 15°C for 2 days is quite effective in delaying both the onset and the development of chilling injury symptoms. The combined treatment with hot water and temperature conditioning was even more effective in reducing chilling injury. Squash from this treatment did not develop moderate or severe chilling injury until after 14 days at 5°C.

Hot water treatment did not appear to affect weight loss of the squash (Table 1). Weight losses from samples treated with hot water were comparable to those from untreated samples. Samples stored at 5°C had the least weight losses while samples kept at 15°C had significantly higher weight losses.

Fig. 2 shows the changes of putrescine levels in the skin of squash from various treatments during storage at 5°C. Putrescine increased steadily with time in storage at 5°C. A suppression in the increase of putrescine content was observed after hot water treatment. Putrescine levels in the hot water treated samples were low initially but increased very rapidly during storage at 5°C. Squash preconditioned with 15°C for 2 days and then transferred to 5°C showed a similar rate of increase in putrescine as those treated with hot water. Little difference was found in

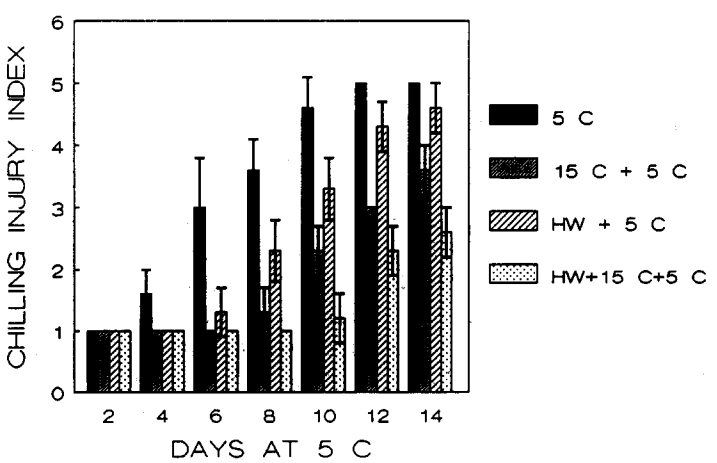

Fig. 1. Development of chilling injury in zucchini squash during storage at 5°C. Vertical bars represent SE.

Table 1
Effect of hot water treatment and low temperature conditioning on weight loss (% of initial weight) of zucchini squash during storage

Days in storage	Treatments[a]				
	1	2	3	4	5
1	0.7 ± 0.2	0.2 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.6 ± 0.2
3	4.2 ± 0.9	1.8 ± 0.3	2.5 ± 0.4	2.1 ± 0.2	3.1 ± 0.4
5	8.6 ± 1.4	2.7 ± 0.4	4.3 ± 0.6	2.9 ± 0.4	4.8 ± 0.7
7	11.5 ± 1.8	4.2 ± 0.5	5.7 ± 0.7	4.6 ± 0.5	6.1 ± 0.8
9	14.3 ± 2.4	6.5 ± 0.8	8.6 ± 0.9	7.1 ± 0.7	8.9 ± 1.0
11	16.8 ± 2.7	7.4 ± 1.3	9.7 ± 1.5	7.8 ± 1.4	10.3 ± 1.7

[a] 1 = constant 15°C storage; 2 = contant 5°C storage; 3 = 15°C 2 days + 5°C storage; 4 = hot water + 5°C storage; 5 = hot water + 15°C 2 days + 5°C storage.

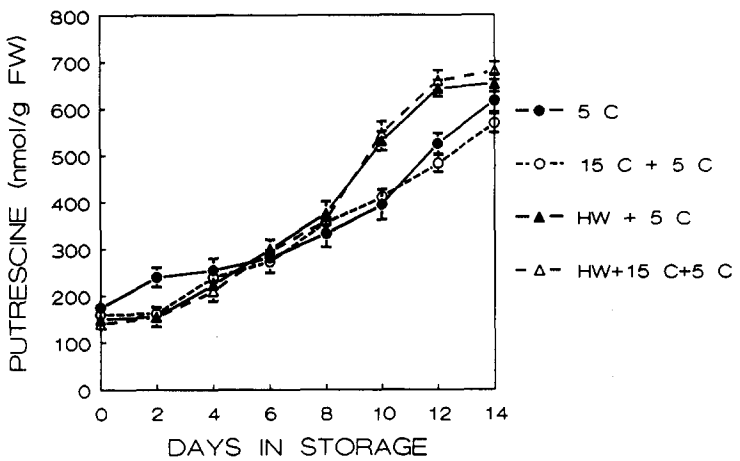

Fig. 2. Changes of putrescine content during storage in zucchini squash from various treatments. Vertical bars represent SE.

the changes of putrescine levels between those treated with hot water and then transferred directly to 5°C storage, and those preconditioned at 15°C for 2 days before the transfer.

Storage of squash at 5°C resulted in a decrease in spermidine levels over time (Fig. 3). The spermidine levels in the hot water treated squash also showed a decline during storage at 5°C. However, the levels remained higher in the hot water treated samples than in the untreated samples. Comparable changes of the spermidine levels during storage were observed in the temperature- conditioned squash and in the squash treated with a combination of hot water and temperature conditioning.

Zucchini squash contain much less spermine than putrescine or spermidine. The levels of spermine in squash also showed a similar decline as spermidine during storage at 5°C (Fig 4). Squash treated with hot water and/or temperature

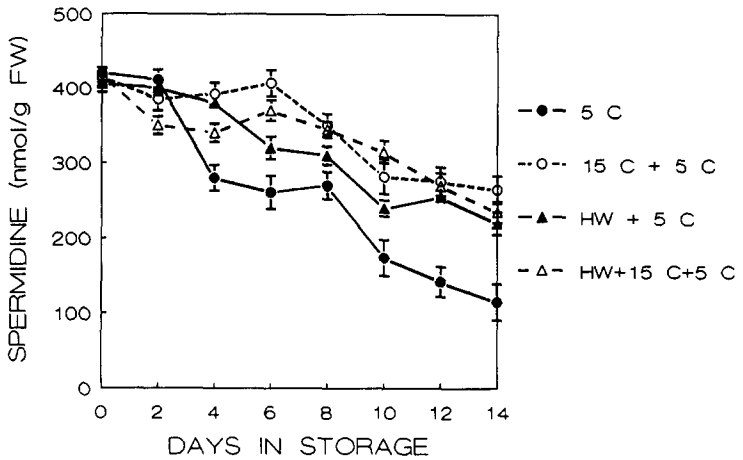

Fig. 3. Changes of spermidine content during storage in zucchini squash from various treatments. Vertical bars represent SE.

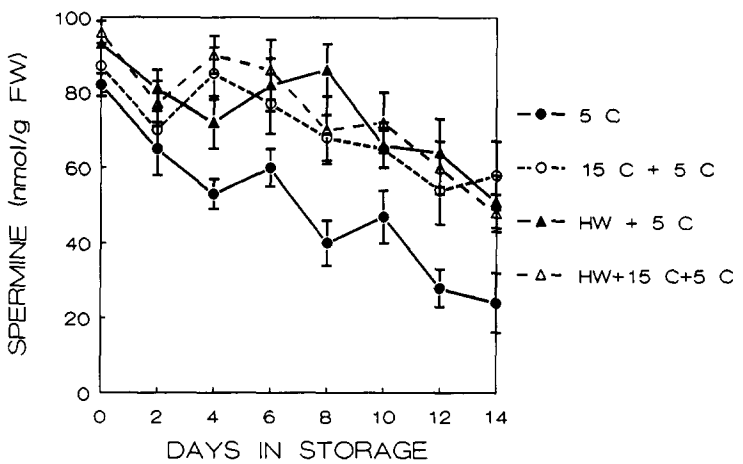

Fig. 4. Changes of spermine content during storage in zucchini squash from various treatments. Vertical bars represent SE.

conditioning also maintained higher levels of spermine than the untreated samples throughout 2 weeks of storage at 5°C.

4. Discussion

Zucchini squash are very sensitive to chilling temperatures. Symptoms of chilling injury, manifested as surface pitting on the skin, appeared after 4 days of exposure to 5°C (Fig. 1). The injury worsens with time in cold storage and affects market quality and saleability. However, the injury can be reduced by prestorage temperature

conditioning as demonstrated in this study. The present study showed that both high temperature conditioning and low temperature conditioning are effective in alleviating chilling injury in zucchini squash. The combined treatment with hot water and low temperature conditioning is even more beneficial in increasing the tolerance of zucchini squash to chilling injury.

It is apparent that temperature conditioning treatment induces an adaptive response in chilling-sensitive crops to chilling stress. Low temperature conditioning has been associated with: maintaining high levels of phospholipids in membranes; increasing the concentrations of squalene, polyamines, proline, reducing sugars, and long-chain aldehyde; and suppressing the increase of the ratio of sterols and phospholipids (Purvis and Yelenosky, 1982; Horvath et al., 1983; Kramer and Wang, 1989; Nordby and McDonald, 1991; Wang et al., 1992). All of these factors may contribute to the fluidity and flexibility of the membranes and the reduction of chilling injury.

The effect of high temperature conditioning or heat treatment is not as well understood as that of the low temperature hardening process. Even though this method has been used as early as 1936 to minimize pitting of grapefruit (Brooks and McColloch), the physiological basis of this treatment in reducing chilling injury has not been elucidated. It has been hypothesized that exposure to one type of stress may induce some factors which can protect against another type of stress (Klein and Lurie, 1991). It has also been proposed that the increased tolerance to chilling injury by high temperature treatment is related to the accumulation of heat shock proteins (Lafuente et al., 1991; Lurie and Klein, 1991). We have found that hot water treatment diminished the initial stress effect on the stimulation of putrescine levels in the tissue (Fig. 2). Accumulation of putrescine in tissues has been regarded as a general response of plants to various types of stresses (Flores, 1990). However, the initial increase in putrescine levels during chilling stress was suppressed in the hot water treated squash.

The reduction of chilling injury by both high and low temperature conditioning in zucchini squash is correlated with higher spermidine and spermine levels in the skin tissue (Figs. 3 and 4). These results are consistent with previous findings that temperature conditioning induces higher S-adenosylmethionine decarboxylase activity and enhances the biosynthesis of polyamines (Kramer and Wang, 1990). Polyamines have been shown to stabilize lipid bilayer structure and to protect membranes from peroxidation and free radical damage (Ballas et al., 1983; Drolet et al., 1986; Roberts et al., 1986). Thus, the induction of the biosynthesis of spermidine and spermine by temperature conditioning may be related to the increased resistance to chilling injury. However, the additive effect of the combined treatment of hot water and low temperature conditioning can not be explained by the changes of polyamine alone, since no difference in the amounts of spermidine and spermine was found between this treatment and other conditioning treatments. The mechanism of this combined treatment in reducing chilling injury warrant further investigation.

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