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Elevated growing temperatures during the day improve the postharvest chilling tolerance of greenhouse-grown cucumber (*Cucumis sativus*) fruit

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Abstract

Growing cucumber fruit in a greenhouse at elevated temperatures during the day increased their tolerance to postharvest chilling. Fruit grown with an average day temperature of 32 ± 1 °C (high temperature) had a storage life (i.e. time to loose 7% fresh weight) of 16 days at 10 °C and did not exhibit chilling injury, while fruit grown at 27 ± 1 °C (control) developed symptoms of chilling injury (i.e. appearance of translucent, water-soaked areas in the mesocarp) after 12 days at 10 °C. The rate of fresh weight loss and the storage life (i.e. 7% water loss) of fruit from both treatments was 12 days when stored at 20 °C. Chilling-induced ion leakage from mesocarp disks was lower from high temperature grown fruit than from control fruit. During storage at 10 °C, firmness, vitamin C content, and activity of superoxide dismutase (SOD; EC 1.15.1.1), and catalase (CAT; EC 1.11.1.6) were higher in high temperature grown fruit than in control fruit. Enhanced antioxidant enzyme activity in high temperature grown fruit may have contributed to increased chilling tolerance.

Keywords: CAT; Chilling injury; Firmness; GPX; High-temperature treatment; Ion leakage; SOD; Vitamin C; Weight loss

1. Introduction

The storability and quality of horticultural crops is influenced by various preharvest factors such as growing temperature, irrigation, light conditions, maturity, mineral nutrition, and pest management (Wang, 1997). For example, superficial scald is a disorder of apples and pears that may develop after months of cold storage and is affected by temperature conditions during fruit growth (Bramlage and Weis, 1997). Chilling injury is common in many plants indigenous to the tropics and subtropics when they are exposed to nonfreezing temperatures below 10 °C (Saltveit and Morris, 1990).

Damage from exposure to chilling stress along with illumination may be mediated by reactive oxygen species (ROS), such as superoxide radicals, singlet oxygen, hydrogen peroxide, and hydroxyl processes (Wise and Naylor, 1987).

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Although the generation of ROS is a common event in growth and developmental processes, it increases in abiotic stress, such as chilling, heat, drought, pollutants, and UV radiation. ROS are highly reactive and can damage membrane lipid, protein and nucleic acids, thus disrupting the homeostasis of the organism (Scandalios 1993). Plants have both enzymatic and non-enzymatic antioxidant systems to prevent or alleviate the damage from ROS. Several enzymes can efficiently detoxify ROS. The superoxide radicle ($O_2^{\bullet}$) is dismutated to H_2O_2 by superoxide dismutase (SOD; EC 1.15.1.1), and catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.11), and guaiacol peroxidase (GPX; EC 1.11.1.7) metabolize H_2O_2 to H_2O. APX requires reduced ascorbate and GPX requires a phenolic compound like guaiacol to function. Glutathione reductase (GR; EC 1.6.4.2) functions in the regeneration of reduced ascorbate after it is converted to monodehydroascorbate by APX.

Chilled cucumbers exhibit reduced storage life characterized by accelerated water loss, surface pitting, and increased susceptibility to disease (Eaks and Morris, 1957; Ryall and Lipton, 1979). A significant increase in ion leakage from mesocarp tissue excised from cucumber fruit exposed to chilling temperatures is a qualitative indicator of chilling injury (Cabrera and Saltveit, 1990). The rate of chilling-induced ion leakage from excised cucumber cotyledons is significantly reduced by exposure to 37 °C before chilling (Lafuente et al., 1991). Hirose (1985) reported that pre-storage heating of cucumber fruit to 36–40 °C for 24 h increased tolerance to subsequent chilling. Kang and Saltveit (2001) reported that elevated levels of SOD and CAT are correlated with the development of heat shock-induced chilling tolerance of cucumber seedling radicles.

Research reported in this paper was done to clarify the effects of daily growing temperatures in plastic greenhouses on the quality, activity of antioxidant enzymes, and chilling tolerance of cucumber fruit.

2. Materials and methods

2.1. Plant material

Cucumber (*Cucumis sativus* L. var. Summer Delight) seeds were sown on 2 March 1995 and seedlings were transplanted on 28 April. Cucumber fruits were harvested 9–11 days after pollination from 20 June to 30 June, when the number of leaves on each plant exceeded 13. Storage life was determined as the time it took the fruit to loose 7% fresh weight, or to develop the chilling injury symptoms of translucent, water-soaked areas in the mesocarp. This was by cutting the fruit in half transversely and visually examining the exposed mesocarp tissue.

2.2. Temperature treatments

The control temperature treatment had a maximum day temperature of 35 ± 1 °C and an average day temperature of 27 ± 1 °C, while the high temperature treatment had a maximum day temperature of 39 ± 1 °C and an average day temperature of 32 ± 1 °C from 10 June to 30 June. Yield and fruit size were the same in both treatments. Those temperatures were maintained by controlling ventilation of the plastic greenhouse (Fig. 1). Treatments were applied from 11:00 to 16:00 h to avoid the early morning pollination of the fruit. Pre-harvest heat treatments could be easily and economically applied in Korea because the maximum day temperature from June to September was 36 ± 1 °C in greenhouses. Harvested cucumber fruits were enclosed in perforated films (40 μm polyethylene film with 1-cm holes every 10×10 cm), and 20 fruit (four sampling times × five replicates) were then stored at 10 °C, 70% relative humidity or 15 fruit (three sampling times × five replicates) at 20 °C, 30–50% relative humidity.

2.3. Weight loss

The fruit were weighed every 2 days and the fresh weight loss calculated as a percentage of original weight.

2.4. Ion leakage

Ion leakage was measured when symptoms of chilling injury occurred after 12 days of storage at 10 °C. Cylinders of mesocarp tissue were excised with a 10-mm diameter stainless steel cork borer from the upper part (3 cm from the stem end) and lower part (3 cm from the blossom end) of five fruit. Mesocarp disks (4 mm thick) were cut from the mid-portion of each cylinder with a stainless steel razor blade. Four disks from each treatment were put into 10 ml of aqueous 0.3 M mannitol and shaken at 100 cycles per min for 3 h, and then boiled for 5 min. The conductivity of the solution ($\mu S\ cm^{-1}$) was periodically measured with a Suntex SC-12 (Tokyo, Japan) conductivity meter. Ion leakage was calculated as the percentage of the total, boiled value (Lafuente et al., 1991).

2.5. Carbon dioxide and ethylene production

Gas samples were removed after 1 h from a closed 1.2 l jar containing five fruit (approximately 200 g) held at 20 °C. Carbon dioxide and ethylene production was measured by injecting gas samples into a gas chromatograph (Hewlett Packard 5890 II) using an active carbon column at 180 °C and a TCD detector at 240 °C (injector temperature: 180 °C) for carbon dioxide and an active alumina column at 110 °C and FID detector at 150 °C (injector temperature: 110 °C) for ethylene.

2.6. Firmness, vitamin C content, and the ratio of fresh to dry weight

Firmness, vitamin C content, and the ratio of fresh to dry weight were measured every 4 days from five fruit. Firmness was measured twice per fruit; at the upper (3 cm from the stem end) and lower (3 cm from the blossom end) end of the fruit with an Instron firmness tester fitted with a 9-mm probe (Model 1101, Instron Ltd. England). The 2,6-dichlorophenolindophenol method was used to assay vitamin C content by spectrofluorometer (SFM-25, Kontron). A wavelength of 350–430 nm was used to determine total vitamin C content (AOAC, 1995). Dry weight was measured by drying 50 g of sliced fruit (3 mm thick slices with peel) at 83 °C for 5 days and then at 102 °C for 1 day. The dry weight ratio was calculated as percentage of fresh weight.

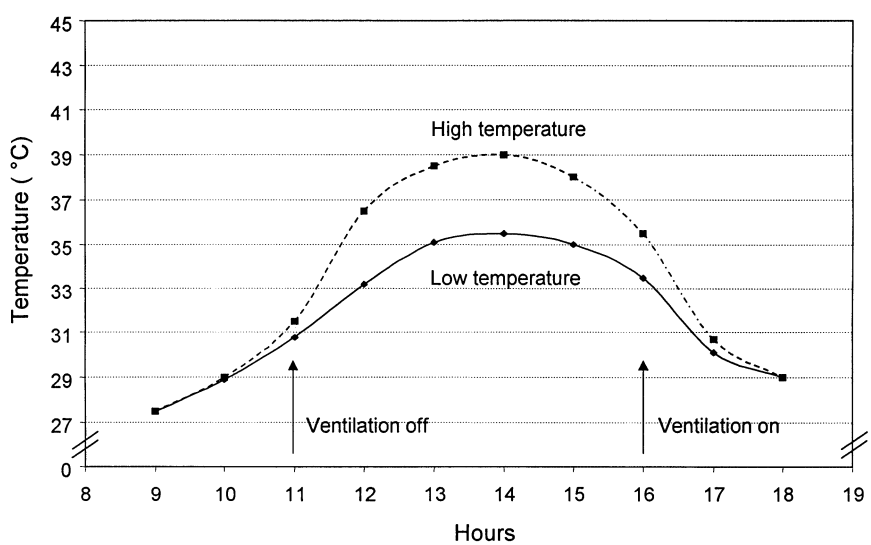

Fig. 1. Generalized temperature profile in plastic greenhouse from 10 June to 30 June.

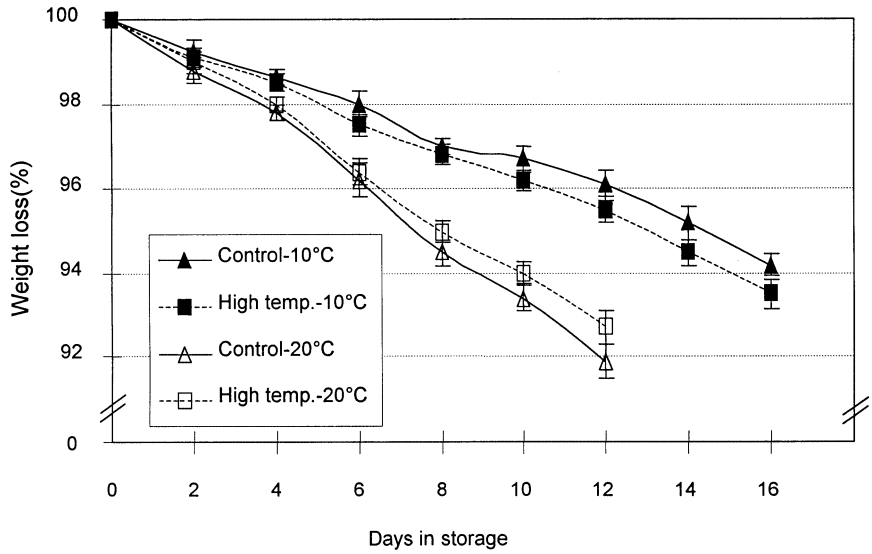

Fig. 2. Changes in fresh weight of cucumber fruit grown at control or high temperatures and stored at 10 or 20 °C. Vertical bars represent S.D. from the mean ($n = 5$).

2.7. Enzyme activity

Five cucumber fruit were diced and 5 g samples were homogenized at 4 °C in extraction buffer (50 mM Tris–HCl buffer, pH 7.5, 3 mM $MgCl_2$, and 1 mM EDTA) with mortar and pestle. The homogenate was centrifuged at $25\,000 \times g$ for 20 min and the supernatant used as the crude enzyme extract.

SOD activity was assayed by measuring its ability to inhibit the NBT photochemical reduction using the method of Dhindsa et al. (1981). The 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 2 μM riboflavin, 0.1 mM EDTA, and 0–50 μl enzyme extract. Riboflavin was added last and the tubes were shaken and placed 30 cm below a light bank consisting of two 15 W fluorescent lamps for 10 min. The absorbance by the reaction mixture was read at 560 nm.

CAT activity was assayed by measuring the rate of disappearance of hydrogen peroxide using the method of Maehly and Chance (1959). The reaction mixture for CAT contained 2.5 ml of 50 mM phosphate buffer (pH 7.4), 0.1 ml of 1% hydrogen peroxide, and 0–50 μl of an appropriately diluted enzyme extract. The decrease in hydrogen peroxide was followed as a decline in absorbance at 240 nm.

GPX activity was determined according to Upadhyaya et al. (1985). The reaction mixture contained 2.5 ml of 50 mM phosphate buffer (pH 6.1), 1 ml of 1% hydrogen peroxide, 1 ml of 1% guaiacol and 0–50 μl of the enzyme extract. The increase in absorbance at 420 nm was followed for 1 min.

3. Results and discussion

3.1. Postharvest storage life and growing temperature

The storage life at 10 °C of fruit grown under high or control day temperatures was 16 and 12 days, respectively. Control fruit exhibited severe chilling injury symptoms, such as surface pitting after 12 days of storage at 10 °C, while high temperature grown fruit did not show any chilling injury symptoms. Fresh weight loss by high and control temperature grown cucumber fruit was not significantly different. The maximum acceptable 7% weight loss (Kays, 1991) occurred after 16 days at 10 °C storage, or after 12 days at

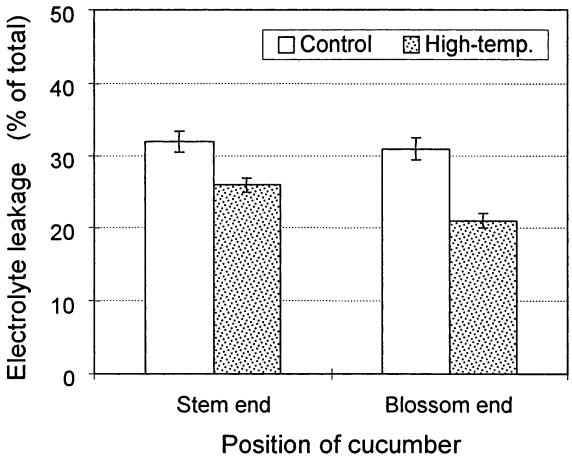

Fig. 3. Electrolyte leakage from cucumber fruit grown at control or high temperatures measured on day 12 of storage at 10 °C. Mesocarp tissue was excised from the stem or blossom end of the fruit. Vertical bars represent S.D. from the mean ($n = 5$).

20 °C storage (Fig. 2). Weight loss from fruit of both treatments was higher at 20 than at 10 °C.

Fukushima et al. (1977) found that cucumber fruit exposed to 5 °C developed pitting and/or surface depressions. A sudden rise in ion leakage accompanied the appearance of chilling injury symptoms in cucumber fruit (Tatsuma et al., 1981). We observed that chilling injury (e.g. pitting and surface depression, water-soaking of mesocarp) occurred in control fruit after 12 days at 10 °C. Ion leakage from mesocarp disks excised 3 cm from the blossom end of control fruit was significantly higher after 12 days at 10 °C, than from similar tissue excised from fruit grown at high day temperatures (Fig. 3).

Chilling-induced increases in respiration from control fruit appeared after 12 days at 10 °C, but there was no increase from chilled fruit that were grown at high day temperatures. Moreover, ethylene evolution increased suddenly after 8 days at 10 °C in control fruit, while it increased gradually after 12 days at 10 °C from fruit grown at high day temperatures (Fig. 4). McCollum et al. (1993) reported that respiration and ethylene evolution were significantly higher in chilled than in non-chilled mango fruit, even before the onset of visual symptoms of chilling injury. Hirose (1985) reported that warming cucumber fruit to 36–40 °C for 24 h before storage at 5 °C significantly reduced subsequent respiration and the appearance of surface depressions during subsequent storage. Ethylene production remained low and

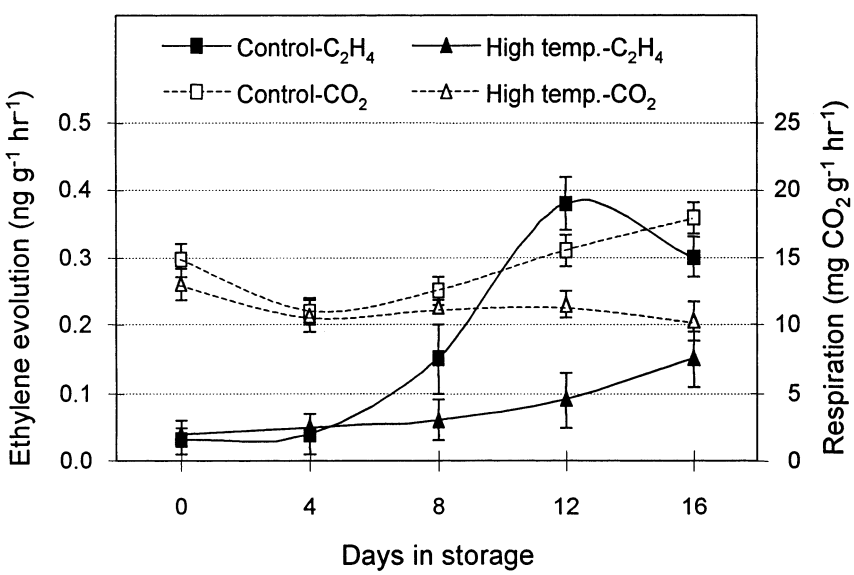

Fig. 4. Changes in ethylene (C_2H_4) evolution and respiration (CO_2) from cucumber fruit grown at control or high temperatures and then stored at 10 °C. Vertical bars represent S.D. from the mean ($n = 5$).

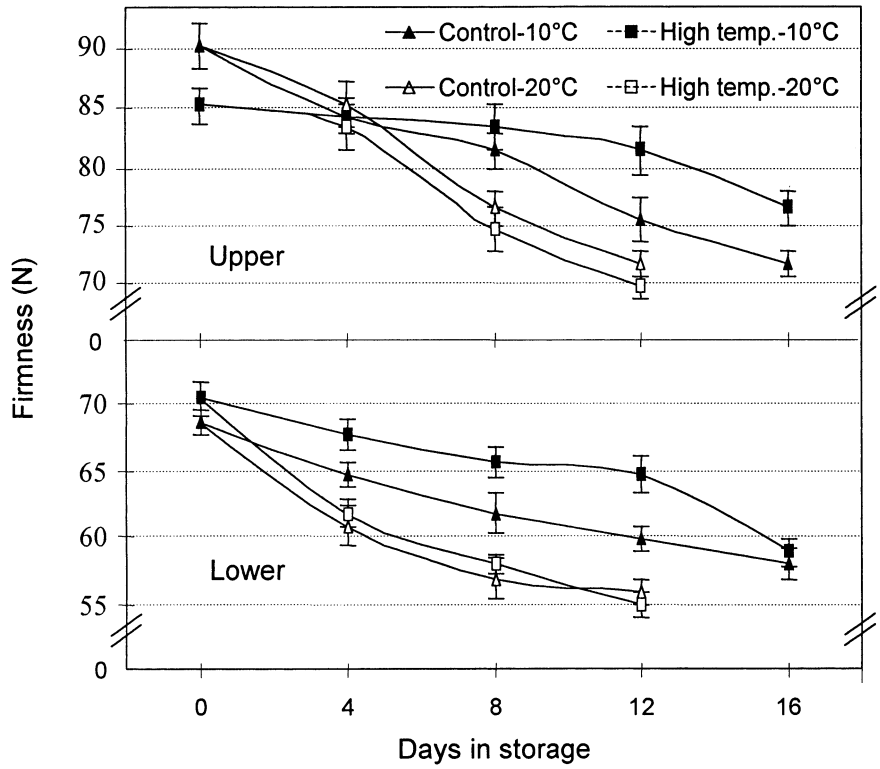

Fig. 5. Changes in firmness of cucumber fruit grown at control or high temperatures and stored at 10 or 20 °C. Vertical bars represent S.D. from the mean ($n = 10$).

stable from cucumber fruit stored at 13 °C, but it was stimulated by 2 days exposure to 2.5 °C and reached a peak after 4 days (Wang and Adams, 1980).

Loss of firmness can be reduced by storage at elevated temperatures. Fruit of apple (Klein and Lurie, 1992), pear (Maxie et al., 1974), tomato (Biggs et al., 1988), and plum (Tsuji et al., 1984) softened more slowly when held at 30–40 °C than at 20 °C. In our study, softening of fruit grown at high day temperatures was slower than in control fruit during storage at 10 °C, but this difference was not found during storage at 20 °C (Fig. 5).

3.2. Antioxidants and chilling tolerance

The growing temperature had no significant effect on the vitamin C content of cucumber fruit stored at 20 °C (Fig. 6). However, during storage at 10 °C, fruit grown at the high day temperature retained vitamin C better than control fruit. Chilling injury may involve oxidative stress that could deplete the antioxidants of the cell, including vitamin C. The dry weight ratio was lower in high temperature grown fruit than in control fruit before storage (Figs. 7 and 8) and during storage.

In general, the activities of the three-antioxidant enzymes examined in this study were higher in the high temperature grown fruit than in the controls. Fruit grown under high day temperatures had 83.5% ($\pm$15.0%) increased SOD activity, 43.5% increased ($\pm$16.3%) CAT activity, and 28.4% ($\pm$17.9%) increased GPX activity compared with control fruit. However, the increase in GPX activity was non-significant.

Among the three antioxidant enzymes, SOD eliminates the superoxide radical that is one of first produced by stressed tissue. The reaction yields hydrogen peroxide that is converted to

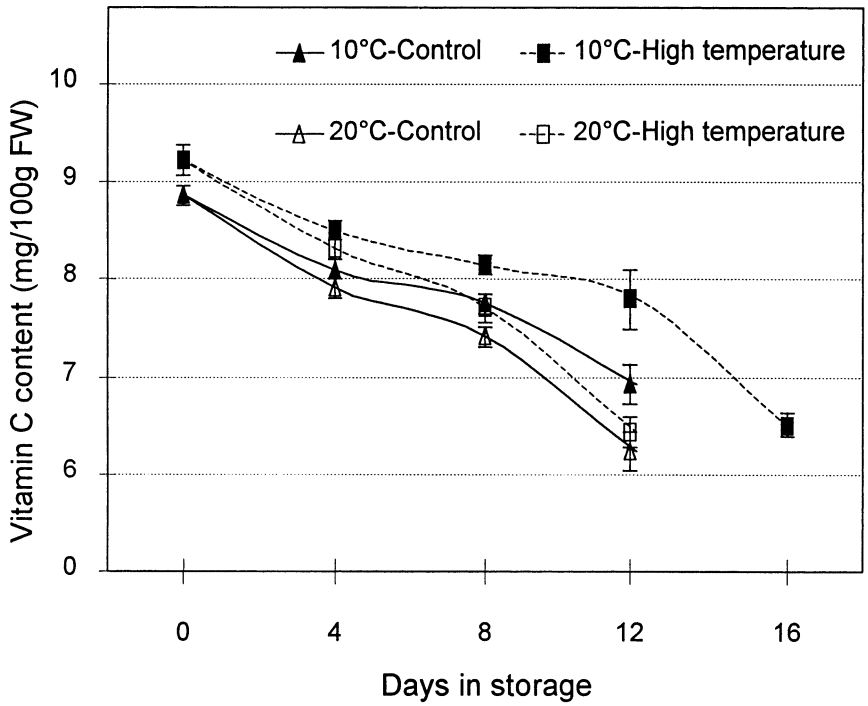

Fig. 6. Changes in vitamin C content of cucumber fruit grown at control or high temperatures during their storage at 10 or 20 °C. Vertical bars represent S.D. from the mean ($n = 5$).

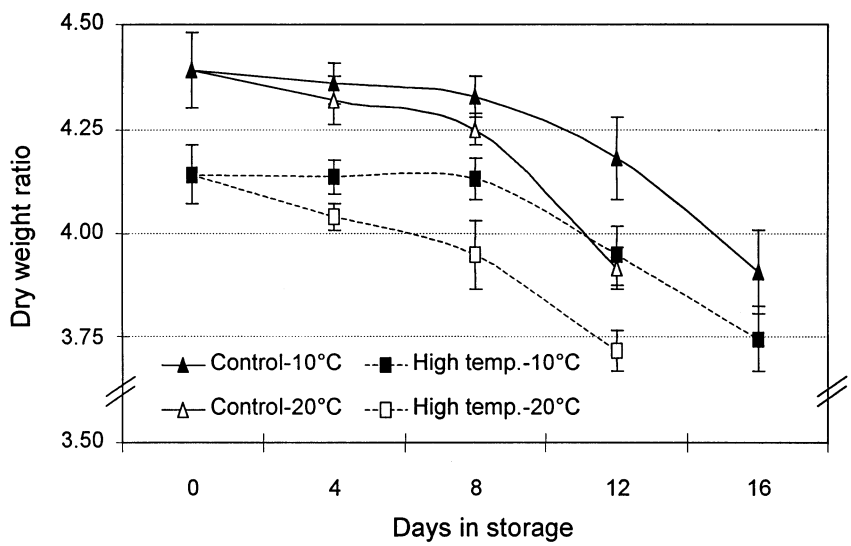

Fig. 7. Changes in the dry weight ratio of cucumber fruit grown at control or high temperatures and stored at 10 or 20 °C. Vertical bars represent S.D. from the mean ($n = 5$).

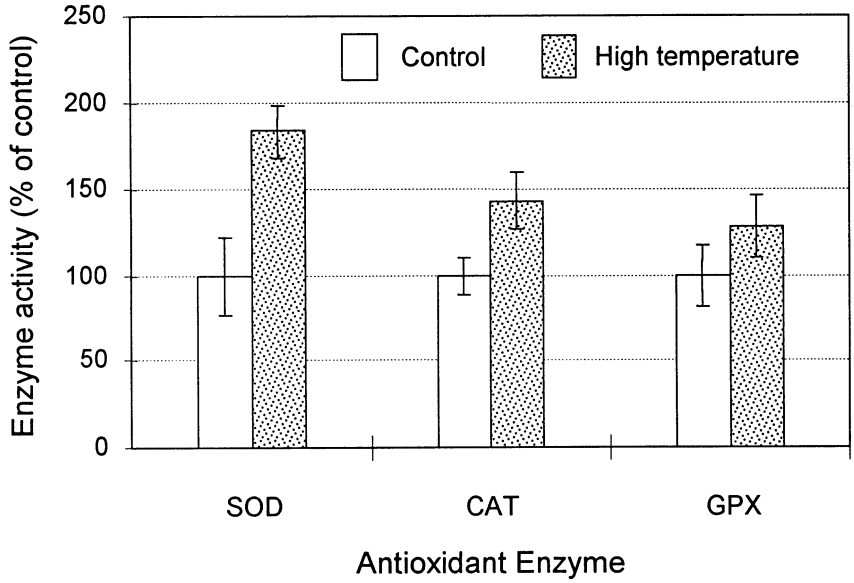

Fig. 8. Effects of growing temperature on the activity of SOD, CAT, and GPX in cucumber fruit. Vertical bars represent S.D. from the mean ($n = 5$).

oxygen and water by CAT. GPX isn't usually included in antioxidant defense system pathways (Bray et al., 2000), but this enzyme also detoxifies hydrogen peroxide. Chilling injury is reduced by heat shock, but the molecular mechanism by which heat shock confers this resistance is not known. Kang and Saltveit (2001) suggested that the heat shock induced increases in antioxidant enzyme activity might help alleviate chilling injury in their cucumber seedling radicle model system. They reported that the same heat shock treatment that induced chilling tolerance also increased the activity of SOD, CAT and APX. Under similar conditions the activity of glutathione reductase and GPX were not correlated with induced chilling tolerance. Antioxidant enzymes also appear to have a role in reducing chilling injury in temperature pre-conditioned zucchini squash (Wang, 1995), paclobutrazol-treated maize seedlings (Pinhero et al., 1997), and salicylic acid-treated maize leaves (Janda et al., 1999).

4. Conclusion

Chilling tolerance was maintained during postharvest storage at 10 °C, even though the high temperature treatment was applied to growing fruit before harvest. Cucumbers that were grown for 4 h during the day at 38–40 °C for a total of 30 h had improved storage life, and reduced chilling injury, softening, and vitamin C loss during subsequent storage at a marginal chilling temperature of 10 °C.

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