Food Bioprocess Technol (2014) 7:909–914
DOI 10.1007/s11947-013-1165-x

COMMUNICATION

Enhancement of Chilling Stress Tolerance of Tomato Fruit by Postharvest Brassinolide Treatment

Morteza Soleimani Aghdam · Nayer Mohammadkhani

Received: 21 March 2013 / Accepted: 23 July 2013 / Published online: 1 August 2013

Abstract Tomatoes, originally a tropical fruit, cannot easily be stored at low temperatures, due to the risk of chilling injury (CI). To develop an effective technique to reduce CI, the effects of treatment with 0, 3, and 6 μM brassinolide (BR) on chilling injury, electrolyte leakage (EL), contents of malondialdehyde (MDA) and proline, and activities of phospholipase D (PLD) and lipoxygenase (LOX) were investigated in tomato fruit stored at 1 °C for 21 days. Treatment with BR, especially at 6 μM, significantly alleviated chilling injury, reduced EL and MDA content, and increased proline content. Also, fruit treated with BR exhibited significantly lower PLD and LOX activities as compared with the control fruit. These results suggest that PLD and LOX are associated with the induction of CI in tomato fruit. BR might reduce CI by inhibiting PLD and LOX activities and by enhancing membrane integrity.

Keywords Brassinolide · Tomato · Chilling injury · Postharvest

Introduction

Tomato fruit (*Solanum lycopersicum*) is of prime importance owing to its qualities for human nutrition and its economic value. In order to extend its commercial life, it is usually harvested at unripe mature stages and stored at low temperatures (Hong and Gross 2006; Re et al. 2012). Storage of tomatoes, originally a tropical fruit, at low temperature is limited by the risk of chilling injury (CI) (Bourne 2006). The cell membrane is the primary cell structure affected by CI (Rui et al. 2010). Cell membrane phase transition from a flexible liquid crystalline to a solid gel structure that occurs at chilling temperature increments the risk of loss of cell membrane functionality and its controlled semipermeability (Lyons 1973). Increase of phospholipase D (PLD) and lipoxygenase (LOX) activities, enzymes that are responsible for the degradation of unsaturated fatty acids, reduced cell membrane integrity and therefore augmented the impact of CI (Pinhero et al. 1998). Reduction of PLD and LOX activities in cucumber and tomato fruit subjected to heat and salicylic acid treatments, respectively, led to an increase of CI resistance by means of improving cell membrane integrity and diminishing lipid peroxidation (Mao et al. 2007a, b; Aghdam et al. 2012a). Maintenance of membrane integrity at low temperature has been reported to be important for the resistance to CI (Wonsheree et al. 2009).

Brassinosteroids (BRs) are a group of naturally occurring plant steroids that are important for plant growth and development and response to biotic and abiotic stresses (Bajguz and Hayat 2009), such as cold stress (Fariduddin et al. 2011), salt (Ozdemir et al. 2004), pathogen infection (Nakashita et al. 2003), oxidative damage (Cao et al. 2005), and heat stress (Ogweno et al. 2008). The potential of BRs for enhancing chilling resistance of fruits and vegetables has also been evaluated. Li et al. (2012) reported that treatment of mango fruit with brassinolide (BR) mitigated CI impact, a phenomenon associated with the reduction of electrolyte leakage. This BR treatment of mango fruit led to an increase of unsaturated fatty acid—linoleic and linolenic—contents. Thus, fluidity of the cell membrane in BR-treated mango fruit was enhanced due to an increase in the proportion of unsaturated fatty acids within the membrane (Li et al. 2012). Wang et al. (2012) reported that the BR treatment mitigated CI in green bell

M. S. Aghdam (✉)
Young Researchers and Elite Club, Ahar Branch, Islamic Azad University, Ahar, Iran
e-mail: m-aghdam@iau-ahar.ac.ir

M. S. Aghdam
e-mail: aghdamm@ut.ac.ir

N. Mohammadkhani
Department of Biology, Faculty of Science, Urmia University, Urmia, Iran

pepper which is accompanied with reducing electrolyte leakage and malondialdehyde (MDA) content. In addition, BR treatment enhanced the activity of antioxidant enzymes such as catalase, ascorbate peroxidase, and glutathione reductase and, thus, mitigated chilling stress in green bell pepper by maintaining membrane integrity. Recently, Aghdam et al. (2012b) reported that the BR treatment mitigated CI in tomato fruit, which was accompanied with the reduction of electrolyte leakage and MDA content and the increase of proline content. Aghdam et al. (2012b) showed that BR treatment significantly enhanced phenylalanine ammonia lyase enzyme activity which led to total phenol accumulation. The objective of this study was to determine the effects of BR on MDA and proline content, electrolyte leakage, and PLD and LOX enzyme activities and their relation to CI in tomato fruit.

Materials and Methods

Fruit and Treatment

Tomato fruits (*S. lycopersicum* L. Newton) were harvested at mature green stage in July 2011 from a greenhouse in Ahar, Iran. About 1,500 fruits were manually picked and immediately transported to the laboratory. Those with defects were discarded, and the remaining 1,350 fruits were selected. They were divided into five lots of 270 fruit each for the following treatments: control (0) and BR at 1, 3, 6, or 9 μM. Each treatment was done in triplicate consisting of 90 fruits each. For BR treatments, the fruits were immersed in 10 L of fresh BR solution for 5 min and in distilled water as a control. Fruits were allowed to completely dry at room temperature before storage at 1±0.5 °C and 85–90 % RH for 3 weeks. Fifteen fruits per replicate of each treatment were removed immediately from cold storage after 7, 14, or 21 days for analyses of electrolyte leakage, PLD and LOX enzymes activities, MDA, and proline contents. Mesocarp from the sampled fruit equator area was cut into small pieces, frozen in liquid nitrogen, and stored at −80 °C. For CI evaluation, 15 fruits per replicate of each treatment were sampled weekly from cold storage and held at 25 °C for 3 days. Each treatment was replicated three times.

Chilling Injury

CI of the fruit was evaluated at 25 °C for 3 days after 7, 14, or 21 days of cold storage period. The fruits were returned to ambient temperature (25 °C) for the development of CI symptoms. Symptoms were manifested as surface pitting according to the method of Ding et al. (2002). The severity of the symptoms was assessed visually in a four-stage scale, where 0=no pitting; 1=pitting covering <25 % of the fruit surface; 2=pitting covering 25 to 50 % of the surface; 3=pitting covering 50 to 75 % of the surface; and 4=pitting covering >75 % of the surface. The average extent of cold damage was expressed as the CI index, which was calculated using the following formula:

$$\text{CI index} = \sum \left[(\text{CI scale}) \times (\text{number of fruit at the CI scale})\right] / \left(4 \times \text{total number of fruit}\right).$$

Electrolyte Leakage, Malondialdehyde, and Proline Content

Electrolyte leakage (EL) was measured using the method of Jiang et al. (2001). A 3-mm-thick mesocarp tissue was excised from equator part of five fruits. Disks were put into aqueous 0.1 M mannitol under constant shaking. The conductivity of the solution (L1) was measured with a conductivity meter. Solutions were boiled for 10 min and then cooled to 20 °C. The conductivity of tissues (L2) was measured. The percentage of electrolyte leakage was calculated using the following formula: %Electrolyte leakage=(L1/L2)×100. MDA content was measured by the thiobarbituric acid method described by Hodges et al. (1999). Absorbance at 532 nm was recorded and corrected for nonspecific absorbance at 600 nm. MDA content was expressed as micromole per gram fresh mass (FM). Proline content was measured using the acid ninhydrin method described by Shan et al. (2007). Proline in tissues was extracted with 30 mL L^{-1} sulfosalicylic acid by shaking at 100 °C for 10 min. The extract was mixed with an equal volume of glacial acetic acid and acid ninhydrin reagent and boiled for 30 min. After cooling, the reaction mix was partitioned against toluene, and the absorbance of the organic phase was recorded at 520 nm. Proline content was expressed as micrograms of proline per gram FM.

Enzyme Assessment

For PLD and LOX, 5 g of tissue was ground with 5 mL of 50 mmol L^{-1} Tris–HCl (pH 8) containing 10 mmol L^{-1} KCl, 500 mmol L^{-1} sucrose, and 0.5 mmol L^{-1} phenylmethylsulfonyl fluoride. The extracts were then homogenized and centrifuged at 12,000×*g* at 4 °C for 10 min. The supernatants were used for the enzyme assays. PLD assay was determined according to the method of Karakurt and Huber (2003). One unit of PLD was defined as the amount of enzyme that catalyzed the formation of 1 nmol D-nitrophenol h^{-1}. LOX activity was assayed using the method of Todd et al. (1990). One unit of LOX is defined as the amount of enzyme which causes an increase in absorption of 0.01 min^{-1} at 234 nm and 25 °C when linoleic acid is used as the substrate. Protein content in the enzyme extracts was estimated according to Bradford (1976), using bovine serum albumin as a standard. Enzyme activities were expressed as units (U) per milligram of protein.

Statistical Analysis

The experiment was arranged as split plots with BR treatments as the main factor using completely randomized design with three replications. Analysis of variance (ANOVA) was carried out with SPSS software. Differences between means were assessed by Duncan's multiple range tests with differences being considered significant at $P<0.05$. ANOVA showing significance of each factor and their interaction is shown in Table 1.

Results and Discussion

Chilling Injury

Slight CI symptoms appeared after 7 days at 1 plus 25 °C for 3 days in fruit from all treatment and continued to progress over time. Treatment with BR at 3 and 6 μM resulted in a lower CI index ($P<0.01$) (Fig. 1), while there was no significant effect of 1 and 9 μM BR on tomato chilling tolerance. Based on these results, 3 and 6 μM of BR were chosen for further analyses. Chilling injury is a major factor that reduces the quality and limits the storage life of tomato fruit. In this study, BR was applied and could significantly reduce postharvest CI in tomato fruit (Fig. 1). Our results were consistent with those of Li et al. (2012) and Wang et al. (2012). They reported that BR treatment mitigated CI in mango and green bell pepper, respectively.

Electrolyte Leakage, MDA, and Proline Content

If the fruits or vegetables are exposed to damaging low temperatures for too long, loss of cell membrane integrity would lead to leakage of intracellular content, which can be monitored by determination of electrolyte leakage (Sharom et al. 1994). Electrolyte leakage is a parameter to assess loss of membrane permeability and therefore may be used as an indicator of membrane integrity (Marangoni et al. 1996). Membrane leakage in fruit, as evaluated by the relative electrolyte leakage measurements, was significantly reduced in BR-treated fruit (Fig. 2; $P<0.05$). Zhao et al. (2009) have found that the correlation coefficient between CI index and electrolyte leakage in tomato fruit is high, irrespective of the differences in the chilling susceptibility between cultivars. In addition, lipid peroxidation, which is responsible for the loss of cell membrane integrity, can be evaluated by the MDA content (Wise and Naylor 1987). MDA is the end product of the peroxidation of membrane fatty acids, and the status of this compound is used as a marker of oxidative stress, since a rise in this compound is indicating damage on cell membrane integrity (Hodges et al. 1999). Treatment with BR decreased the accumulation of the lipid peroxidation product MDA ($P<0.05$; Fig. 3a), which is regarded as an indicator of the

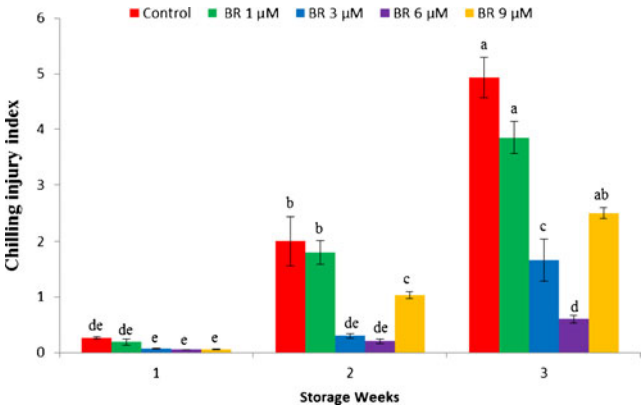

Fig. 1 Effects of brassinolide treatment at 0, 1, 3, 6, and 9 μM on chilling injury index of tomato fruit. Tomato fruits were sampled after 1 °C storage for 7, 14, and 21 days plus 3 days at 25 °C. *Different letters on bars* show significant difference between treatment based on Duncan's multiple range test at $P=0.05$ level ($n=3$)

Table 1 ANOVA for dependent variables for brassinolide (BR) treatments, storage duration, and their interactions for tomato fruit

	Treatment	Duration	Treatment × duration
Chilling injury index	–**	–**	–**
Electrolyte leakage	–*	–*	–*
MDA content	–*	NS	–*
Proline content	–**	–*	–**
PLD enzyme activity	–**	–**	–**
LOX enzyme activity	–**	NS	–**

NS nonsignificant at $P<0.05$

*$P<0.05$; **$P<0.01$ (significant)

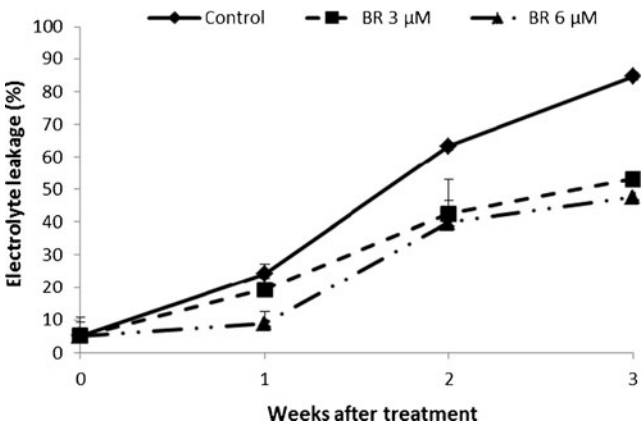

Fig. 2 Effects of brassinolide treatment at 0, 3, and 6 μM on electrolyte leakage (in percentage) of tomato fruit storage at 1 °C. Tomato fruits were sampled after 1 °C storage for 7, 14, and 21 days. *Different letters on bars* show significant difference between treatment based on Duncan's multiple range test at $P=0.05$ level ($n=3$)

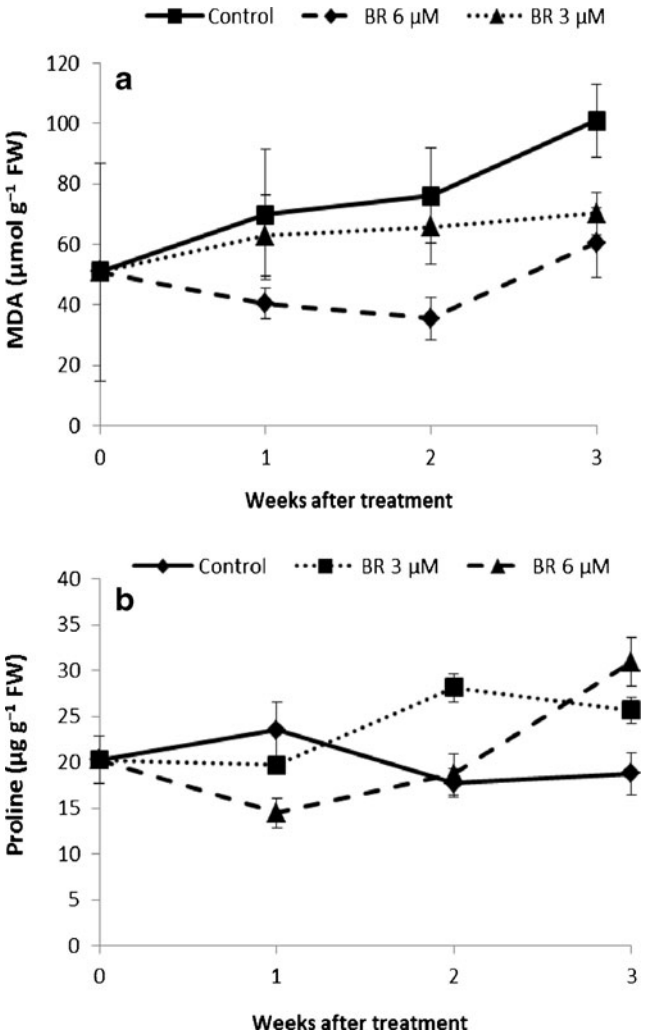

Fig. 3 Effects of brassinolide treatment at 0, 3, and 6 μM on MDA (**a**) and proline (**b**) contents of tomato fruit. Tomato fruits were sampled after 1 °C storage for 7, 14, and 21 days. *Different letters on bars* show significant difference between treatment based on Duncan's multiple range test at P=0.05 level (n=3)

loss of structural integrity in membranes, being associated with cold stress. Electrolyte leakage and MDA content, well-known physiological markers of loss of membrane semipermeability and membrane lipid peroxidation, are widely used by researchers to indirectly assess cell membrane integrity (Wise and Naylor 1987; Sharom et al. 1994). Treatment with BR resulted in a decrease in MDA content (Fig. 3a), i.e., inhibited lipid peroxidation under chilling stress, which clearly indicated that BR could strongly protect tomato fruit from oxidative damage and thus enhance chilling tolerance (Li et al. 2012; Wang et al. 2012). It was reported that treatment with BR could decrease the lipid peroxidation and MDA content induced by heat (Ogweno et al. 2008) and drought stress (Robinson and Bunce 2000). Yuan et al. (2010) reported that drought stress increased the H_2O_2 and MDA content of tomato seedlings, but this effect was significantly alleviated by the application of BRs. Mao et al. (2007a) observed that hot air treatment (37 °C for 24 h) mitigated CI in cucumber fruit, and this process was associated with a reduction of electrolyte leakage and MDA content.

Proline, a multifunctional amino acid, plays crucial roles in the osmotic regulation between the cytoplasm and vacuole, redox regulation of the NAD^+/NADH ratio, membrane stabilization, and finally in promoting reactive oxygen species scavenging systems (Sharp et al. 1990; Bohnert and Jensen 1996). Proline content in the control tomato fruit decreased with storage but increased in BR-treated fruit during storage duration. Proline concentrations were significantly higher in BR-treated fruit than in the control fruit after 21 days (P<0.01; Fig. 3b). Positive correlations between the accumulation of endogenous proline and improved cold tolerance have been found mostly in chilling-sensitive fruits (Zhao et al. 2009).

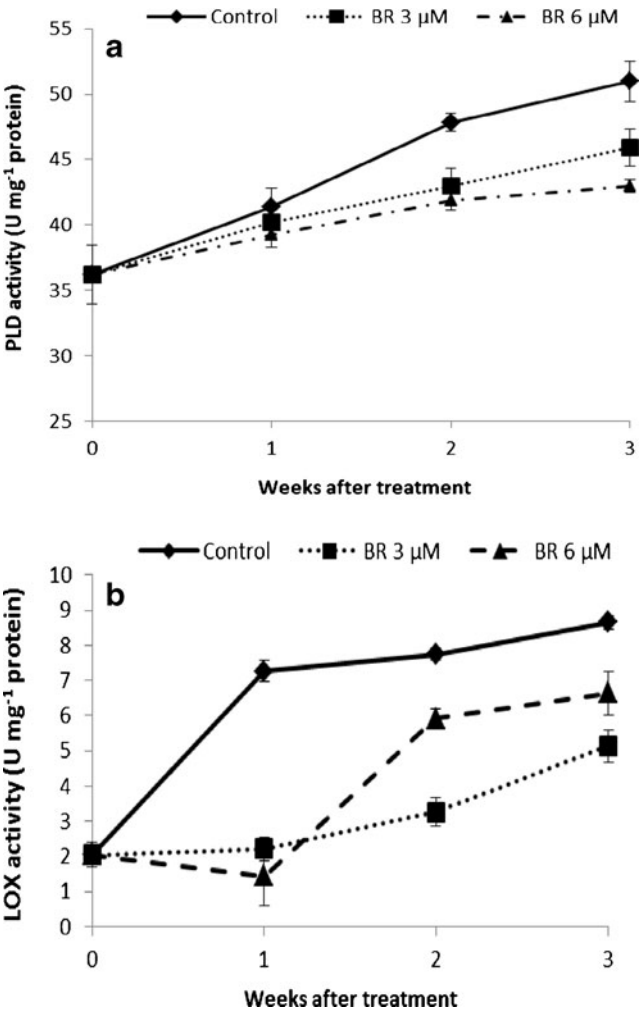

Fig. 4 Effects of brassinolide treatment at 0, 3, and 6 μM on PLD (**a**) and LOX (**b**) enzyme activity of tomato fruit. Tomato fruits were sampled after 1 °C storage for 7, 14, and 21 days. *Different letters on bars* show significant difference between treatment based on Duncan's multiple range test at P=0.05 level (n=3)

The increase in proline content of BR-treated fruit along with the amelioration of CI in this study confirms this report.

PLD and LOX Activities

PLD and LOX activities increased during storage, but BR treatment inhibited the increases in activities of both enzymes and maintained lower enzyme activities throughout storage ($P<0.01$; Fig. 4a, b). Activation of membranous lipolytic enzymes such as PLD and LOX under chilling temperature might cause irreversible membrane damage and finally the occurrence of CI (Mao et al. 2007a). In the present study, the treatment with BR significantly reduced the PLD and LOX activities and ameliorated CI in tomato fruit. Therefore, it can be concluded that the reduction of CI by BR may be attributed to the reduction of the PLD and LOX activities. Aghdam et al. (2012a) observed that PLD and LOX activities in tomato fruit increased during development of CI symptoms, a fact indicating an aggravation of membrane integrity loss. Salicylic acid treatment significantly reduced these enzymatic activities during storage at chilling temperature and led to maintenance of membrane integrity (Aghdam et al. 2012a). The authors suggested that salicylic acid treatment enhances CI tolerance in tomato fruit by raising membrane integrity upholding by means of reducing the prooxidant PLD and LOX enzymatic activities, peroxidation lipids, and loss of cell membrane semipermeability. Rui et al. (2010) reported that hot air treatment mitigated CI in loquat fruit, assessed by a reduction of electrolyte leakage and MDA content and decrease of PLD and LOX enzyme activity. They suggested that these two enzymes might be associated with the initiation of CI by being involved in membrane deterioration and signaling pathway in response to chilling stress. Lyons (1973) considered changes in membrane structure and composition as the primary event of CI by leading to a loss of permeability control and metabolic dysfunction.

Conclusion

The present study has proven beneficial effects of BR on reducing CI in tomato fruit during low-temperature storage. The development of CI in tomato fruit includes increases in PLD and LOX activities, a reduction of osmolyte (proline) content, and thus loss of membrane integrity. Applications of BR might be used in order to reduce CI in tomato fruit under low-temperature storage. Further studies must be concentrated on the elucidation of factors that might affect their efficacy such as harvest date, cultivar, production methods, and storage atmosphere.

References

Aghdam, M.S., Asghari, M., Khorsandi, O., & Mohayeji, M. (2012a). Alleviation of postharvest chilling injury of tomato fruit by salicylic acid treatment. *Journal of Food Science and Technology*. doi:10.1007/s13197-012-0757-1.

Aghdam, M. S., Asghari, M., Farmani, B., Mohayeji, M., & Moradbeygi, H. (2012b). Impact of postharvest brassinosteroids treatment on PAL activity in tomato fruit in response to chilling stress. *Scientia Horticulturae, 144*, 116–120.

Bajguz, A., & Hayat, S. (2009). Effects of brassinosteroids on the plant responses to environmental stresses. *Plant Physiology and Biochemistry, 74*, 1–8.

Bohnert, H. J., & Jensen, R. G. (1996). Strategies for engineering water-stress tolerance in plants. *Trends in Biotechnology, 14*, 89–97.

Bourne, M. C. (2006). Selection and use of postharvest technologies as a component of the food chain. *Journal of Food Science, 69*, 43–46.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of micro-gram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Cao, S., Xu, Q., Cao, Y., Qian, K., An, K., Zhu, Y., et al. (2005). Loss-of-function mutations in *DET2* gene lead to an enhanced resistance to oxidative stress in *Arabidopsis*. *Physiologia Plantarum, 123*, 57–66.

Ding, C. K., Wang, C. Y., Gross, K. C., & Smith, D. L. (2002). Jasmonate and salicylate induce the expression of pathogenesis-related-protein genes and increase resistance to chilling injury in tomato fruit. *Planta, 214*, 895–901.

Fariduddin, Q., Yusuf, M., Chalkoo, S., Hayat, S., & Ahmad, A. (2011). 28-homobrassinolide improves growth and photosynthesis in *Cucumis sativus* L. through an enhanced antioxidant system in the presence of chilling stress. *Photosynthetica, 49*, 55–64.

Jiang, Y., Shiina, T., Nakamura, N., & Nakahara, A. (2001). Electrical conductivity evaluation of postharvest strawberry damage. *Journal of Food Science, 66*, 1392–1395.

Hodges, D. M., DeLong, J. M., Forney, C. F., & Prange, R. K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta, 207*, 604–611.

Hong, J. H., & Gross, K. C. (2006). Maintaining quality of fresh-cut tomato slices through modified atmosphere packaging and low temperature storage. *Journal of Food Science, 66*, 960–965.

Karakurt, Y., & Huber, D. J. (2003). Activities of several membrane and cell wall hydrolases, ethylene biosynthetic enzymes, and cell wall polyuronides degradation during low-temperature storage of intact and fresh-cut papaya (*Carica papaya*) fruit. *Postharvest Biology and Technology, 28*, 219–229.

Li, B., Zhang, C., Cao, B., Qin, G., Wang, W., & Tian, S. (2012). Brassinolide enhances cold stress tolerance of fruit by regulating plasma membrane proteins and lipids. *Amino Acids, 43*(6), 2469–2480. doi:10.1007/s00726-012-1327-6.

Lyons, J. M. (1973). Chilling injury in plants. *Annual Review of Plant Physiology, 24*, 445–466.

Mao, L. C., Wang, G. Z., Zhu, C. G., & Pang, H. Q. (2007a). Involvement of phospholipase D and lipoxygenase in response to chilling stress in postharvest cucumber fruits. *Plant Science, 172*, 400–405.

Mao, L., Pang, H., Wang, G., & Zhu, C. (2007b). Phospholipase D and lipoxygenase activity of cucumber fruit in response to chilling stress. *Postharvest Biology and Technology, 44*, 42–47.

Marangoni, A. G., Palma, T., & Stanley, D. W. (1996). Membrane effects in postharvest physiology. *Postharvest Biology and Technology, 7*, 193–217.

Nakashita, H., Yasuda, M., Nitta, T., Asami, T., Fujikoa, S., Arai, Y., et al. (2003). Brassinosteroids functions in a broad range of disease resistance in tobacco and rice. *The Plant Journal, 33*, 887–898.

Ogweno, J. O., Song, X. S., Shi, K., Hu, W. H., Mao, W. H., Zhou, Y. H., et al. (2008). Brassinosteroids alleviate heat-induced inhibition of photosynthesis by increasing carboxylation efficiency and enhancing antioxidant systems in *Lycopersicon esculentum*. *Plant Growth Regulation, 27*, 49–57.

Ozdemir, F., Bor, M., Demiral, T., & Turkan, I. (2004). Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidative system of rice (*Oryza sativa* L) under salinity stress. *Plant Growth Regulation, 42*, 203–211.

Pinhero, R. G., Paliyath, G., Yada, R. Y., & Murr, D. P. (1998). Modulation of phospholipase D and lipoxygenase activities during chilling. Relation to chilling tolerance of maize seedlings. *Plant Physiology and Biochemistry, 36*, 213–224.

Re, M. D., Gonzalez, C., Sdrigotti, M. A., Sorrequieta, A., Valle, E. M., & Boggio, S. B. (2012). Ripening tomato fruit after chilling storage alters protein turnover. *Journal of the Science of Food and Agriculture, 92*, 1490–1496.

Robinson, J. M., & Bunce, J. A. (2000). Influence of drought-induced water stress on soybean and spinach leaf ascorbate-dehydroascorbate level and redox status. *International Journal of Plant Science, 161*, 271–279.

Rui, H., Cao, S., Shang, H., Jin, P., Wang, K., & Zheng, Y. (2010). Effects of heat treatment on internal browning and membrane fatty acid in loquat fruit in response to chilling stress. *Journal of the Science of Food and Agriculture, 90*, 1557–1561.

Shan, D. P., Huang, J. G., Yang, Y. T., Guo, Y. H., Wu, C. A., Yang, G. D., et al. (2007). Cotton GhDREB1 increases plant tolerance to low temperature and is negatively regulated by gibberellic acid. *New Phytology, 176*, 70–81.

Sharp, R. E., Hsiao, T. C., & Silk, W. K. (1990). Growth of the maize primary root at low water potentials: II. Role of growth and deposition of hexose and potassium in osmotic adjustment. *Plant Physiology, 93*, 1337–1346.

Sharom, M., Willemot, C., & Thompson, J. E. (1994). Chilling injury induces lipid phase changes in membranes of tomato fruit. *Plant Physiology, 105*, 305–308.

Todd, J. F., Paliyath, G., & Thompson, J. E. (1990). Characteristics of a membrane associated lipoxygenase in tomato fruit. *Plant Physiology, 94*, 1225–1232.

Wang, Q., Ding, T., Gao, L., Pang, J., & Yang, N. (2012). Effect of brassinolide on chilling injury of green bell pepper in storage. *Scientia Horticulturae, 144*, 195–200.

Wise, R. R., & Naylor, A. W. (1987). Chilling-enhanced photooxidation: the peroxidative destruction of lipids during chilling injury to photosynthesis and ultrastructure. *Plant Physiology, 83*, 272–277.

Wonsheree, T., Kesta, S., & van Doorn, W. G. (2009). The relationship between chilling injury and membrane damage in lemon basil (*Ocimum citriodourum*) leaves. *Postharvest Biology and Technology, 51*, 91–96.

Yuan, G. F., Jia, C. G., Li, Z., Sun, B., Zhang, L. P., Liu, N., et al. (2010). Effect of brassinosteroids on drought resistance and abscisic acid concentration in tomato under water stress. *Scientia Horticultura, 126*, 103–108.

Zhao, D. Y., Shen, L., Fan, B., Liu, K. L., Yu, M. M., Zheng, Y., et al. (2009). Physiological and genetic properties of tomato fruit from 2 cultivars differing in chilling tolerance at cold storage. *Journal of Food Science, 74*, 348–352.