JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Effect of Exogenous γ-Aminobutyric Acid Treatment on Proline Accumulation and Chilling Injury in Peach Fruit after Long-Term Cold Storage

Haitao Shang,[†,‡] Shifeng Cao,[*,‡] Zhenfeng Yang,[§] Yuting Cai,[†] and Yonghua Zheng[*,†]

[†]College of Food Science and Technology, Nanjing Agricultural University, Nanjing, 210095, Jiangsu, People's Republic of China

[‡]Nanjing Research Institute for Agricultural Mechanization, Ministry of Agriculture, Nanjing 210014, Jiangsu, People's Republic of China

[§]College of Biological and Environmental Sciences, Zhejiang Wanli University, Ningbo 315100, People's Republic of China

ABSTRACT: The effect of exogenous γ-aminobutyric acid (GABA) on chilling injury of peach fruit was investigated. Freshly harvested peaches were treated with 1, 5, or 10 mM GABA at 20 °C for 10 min and then stored at 1 °C for up to 5 weeks. The results showed that all of the GABA treatments could reduce chilling injury of peach fruit with 5 mM being the most effective concentration. GABA treatment significantly enhanced the accumulation of endogenous GABA and proline, which resulted from the increased activities of glutamate decarboxylase, Δ^1-pyrroline-5-carboxylate synthetase, and ornithine δ-aminotransferase and decreased proline dehydrogenase activity. Our results revealed that GABA treatment may be a useful technique to alleviate chilling injury in cold-stored peach fruit, and the reduction in chilling by GABA may be due to the induction of endogenous GABA and proline accumulation. These data are the first evidence that exogenous GABA induced chilling tolerance in postharvest horticultural products.

KEYWORDS: γ-Aminobutyric acid, peach, chilling injury, proline

INTRODUCTION

Chilling injury is a physiological disorder that occurs in postharvest peach fruit during cold storage. The main symptoms induced by chilling in peaches include internal browning and flesh mealiness, which causes a loss in commercial quality and storage life.[1,2] Hence, the low temperature injury is a main problem when peaches are stored at low temperatures and should be handled urgently for storage and transportation.

γ-Aminobutyric acid (GABA), a nonprotein amino acid, is regarded as an endogenous signal molecule that plays an important role for regulating the stress response, plant growth, and development.[3] GABA is metabolized via a pathway called the GABA shunt that consists of three enzymes: glutamate decarboxylase (GAD), GABA transaminase, and succinic semialdehyde dehydrogenase, in which GAD is the key enzyme.[4] In plants, intracellular levels of GABA are typically low, but they can be greatly and rapidly accumulated in response to drought, salt, and low temperature stresses and involved in the defense against these stresses.[4] Recently, Song et al.[5] reported that exogenous GABA could alleviate oxidative damage caused by aluminum and proton stresses on barley seedlings. However, little information is available about the effect of exogenous GABA treatment as a factor that affects storage life and quality in postharvest horticultural products.

It has been proposed that proline accumulation can serve as an adaptive mechanism to chilling stress in higher plants.[6] The physiological effect of proline accumulation may be expressed in sustained photosynthesis and osmotic regulation and/or prevention of proteins, including enzymes, from degradation.[7] In postharvest horticultural products, the increased proline concentration associated with increased resistance to chilling during cold storage is well-known in some fruit species.[8,9] In plants, proline is synthesized from either glutamate or ornithine via Δ^1-pyrroline-5-carboxylate synthetase (P5CS) or ornithine δ-aminotransferase (OAT), respectively. Meanwhile, the metabolism and accumulation of proline also depend on its degradation, which is catalyzed by proline dehydrogenase (PDH).[10] Studying the effects of chilling stress on enzyme activities involved in proline metabolism could provide valuable information on the physiological significance of its accumulation.

Therefore, this study was undertaken to evaluate (a) the effects of a postharvest GABA treatment on chilling injury and quality maintenance of peaches during storage at 1 °C and (b) the effects of GABA on proline content and its metabolism and (c) to elucidate the underlying mechanism by which GABA alleviated the damage caused by chilling.

MATERIALS AND METHODS

Fruit and GABA Treatment. Peach fruits (*Prunus persica* Batsch cv. Baifeng) were hand-harvested at 6.73 kg/cm^2 firmness and 11.08% total soluble solids (TSS) from a commercial orchard in Nanjing, China, on July 2, 2010, and were immediately transported to our laboratory. Fruits were selected for uniformity without any damage and randomly divided into four groups. The first three groups were immersed into solutions of 1, 5, or 10 mM GABA, respectively, for 10 min, whereas the fourth group of fruit was soaked in sterile deionized water for 10 min and considered as the control. All fruits were then air-dried for approximately

Received: November 17, 2010
Accepted: January 9, 2011
Revised: January 7, 2011
Published: February 2, 2011

30 min and stored at 1 °C and 85−95% relative humidity (RH). Samples were collected from five fruits after 3 or 5 weeks of storage at 1 °C for measurements for levels of GABA and proline and activities of GAD, P5CS, OAT, and PDH. Another sample of 10 fruits was removed after 3 or 5 weeks of storage at 1 °C and held at 20 °C for 3 days to simulate shelf conditions for chilling injury and quality evaluation. There were three replicates consisting of 15 peaches per replicate, and the experiment was conducted twice with similar results.

Chilling Injury Evaluation. Internal browning is used to evaluate the development of chilling injury in peach fruit. The degree of chilling injury was visually assessed on the mesocarp surface following a double cut parallel to the axial diameter. The extent of flesh browning was divided into four classes: 0, no browning; 1, browning covering <25% of the cut surface; 2, browning covering ≥25% but <50% of cut surface; and 3, browning covering ≥50% . The chilling injury index was calculated using the following formula:

$$\text{chilling injury index} = \sum(\text{browning level} \times \text{number of fruit at the browning level})/(3 \times \text{total number of fruit in the treatment}) \times 100$$

GABA Determination. The GABA concentration in fruit pulp was estimated by the method of Zhang and Bown.[11] GABA was determined on the basis of the increase in A_{340} after 30 min following supplier recommendations for commercially available of GABase (Aldrich, Chemical Co., Milwaukee, WI), a spectrophotometric-coupled enzyme assay system for GABA. The resulting values were compared with a standard curve constructed using known amounts of GABA and expressed as μg GABA/g fresh weight (FW).

Proline Measurement. The proline content was measured using the acid ninhydrin method described by Zhao et al.[9] Proline in tissues was extracted with 3% (v/v) sulfosalicylic acid at 100 °C for 10 min with shaking. The extract was mixed with an equal volume of glacial acetic acid and acid ninhydrin reagent and boiled for 30 min. After it was cooled, the reaction mix was partitioned against toluene, and the absorbance of the organic phase was recorded at 520 nm. The resulting values were compared with a standard curve constructed using known amounts of proline and expressed as μg proline/g FW.

GAD Assay. GAD enzyme extraction was carried out in a buffer containing 0.1 M Tris-HCl (pH 9.1), 10% (v/v) glycerol, 1 mM dithiothreitol (DTT), 5 mM ethylenediaminetetraacetic acid (EDTA), 0.5 mM pyridoxal phosphate (PLP), and 1 mM phenylmethylsulfonyl fluoride. Two grams of fresh tissue was added to the 5 mL of precooled extraction buffer and homogenized at 4 °C. The homogenate was filtered through Miracloth (Calbiochem) and then centrifuged at 24500*g* at 4 °C for 30 min. The supernatant was used for GAD determination. The GAD activity was determined using the method of Deewatthanawong et al.[12] The enzyme activity was assayed by incubating crude extract at 30 °C for 60 min in 400 μL of assay mixture containing 0.1 M potassium phosphate buffer (pH 5.8), 40 μM PLP, and 3 mM glutamate. The reaction was stopped by adding 0.1 mL of 0.5 M hydrochloric acid. The amount of GABA in samples was determined as described above. The enzyme activity was calculated as GABA produced. One unit of GAD activity was defined as the amount of enzyme catalyzing the formation of 1 μg GABA per hour.

P5CS and PDH. Five grams of flesh tissue was ground with in 50 mM Tris-HCl buffer (pH 7.4) containing 7 mM $MgCl_2$, 0.6 M KCl, 3 mM EDTA, 1 mM DTT, and 5% (w/v) insoluble polyvinylpyrrolidone. Homogenates were filtered through two layers of Miracloth (Calbiochem), and the filtrate was centrifuged at 39000*g* for 20 min. The supernatants were desalted on a sephadex G-25 column (Pharmacia Biochem PD-10, Uppsala, Sweden) and eluted with 50 mM Tris-HCl (pH 7.4) containing 10% (v/v) glycerol. The P5CS activity was measured as describe by López-Carrión et al.[13] The reaction mixture contained 100 mM Tris-HCl (pH 7.2), 25 mM $MgCl_2$, 75 mM sodium glutamate, 5 mM ATP, and the enzyme extract. The reaction was initiated by the addition of 0.4 mM NADPH. The PDH activity was assayed by the reduction of NAD^+ at 340 nm.[14] The reaction mixture contained 0.15 M Na_2CO_3−HCl buffer (pH 10.3) containing 2.67 mM proline and 10 mM NAD^+. One unit of all of the three enzymes activities was defined as the amount of enzyme causing a decrease of 0.001 in absorbance per minute at 340 nm.

OAT Assay. Five grams of flesh tissue was ground in 100 mM potassium phosphate buffer (pH 7.9) with 1 mM EDTA, 15% (v/v) glycerol, and 10 mM 2-mercaptoethanol. The extract was centrifuged at 15000*g* for 15 min, and the supernatant was treated with 60% $(NH_4)_2SO_4$ for 45 min.[14] OAT was assayed following Sánchez et al.[14] The reaction mixture contained 0.2 M Tris-HCl buffer (pH 7.8) containing 46.8 mM ornithine, 12.5 mM α-ketoglutarate, and 0.125 mM NADH. One unit of OAT activity was defined as the amount of enzyme causing a decrease of 0.001 in absorbance per minute at 340 nm.

Protein Determination. The protein content in the enzyme extracts was estimated using the Bradford[15] method, using bovine serum albumin as a standard. The specific activity of the enzymes was expressed as units per milligram protein.

Quality Parameters. The firmness of 10 fruits from each replicate was measured using a FT327 firmness tester (Facchini FG, Alfonsine, Italy) fitted with a 5 mm diameter probe. The extractable juice content was estimated from the weight loss from placental tissue plugs in response to low-speed centrifugation. Four plugs (7 mm wide, 10 mm thick) were placed over sterile cotton rod in a 50 mL centrifuge tube and centrifuged for 10 min at 1700*g* at room temperature. The results were expressed as the loss of FW of tissue plugs after centrifugation. The same 10 fruits from each replicate were then wrapped in cheesecloth and squeezed using a hand press. The resulting juice was analyzed for its TSS, titratable acidity (TA). TSS was determined at 20 °C using a portable refractometer (WYT-4; Quanzhou zhongyou optical instrument Co., Ltd., Fujian, China). TA was determined by titrating 20 mL of juice to pH 8.2 using 0.1 M NaOH.

Data Analysis. Experiments were performed using a completely randomized design. All statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL). The data were analyzed by one-way analysis of variance. Mean separations were performed by Duncan's multiple range tests. Differences at $p < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

Effect of Exogenous GABA Treatment on Chilling Injury. In our present study, we found that exogenous GABA treatment could effectively reduce internal browning, a typical chilling injury symptom in peach fruit, and 5 mM was the most effective concentration (Figure 1). This finding appears to be a novel observation that exogenous GABA treatment can increase chilling tolerance of peach fruit, which suggested a mechanism that GABA could promote stress-related metabolism in peach fruit. GABA has been implicated in the signaling pathway mediating induced defense responses in environmental-stressed plants.[4] Application of exogenous GABA has been reported to reduce the protein and lipid damage caused by aluminum and proton stresses in barley seedling roots.[5] Furthermore, it was reported that GABA has relaxation and immunity enhancement effects,[16] as well as antihypertensive and natriuretic effects in humans.[17] Thus, the use of exogenous GABA treatment is a safe approach to prolong storage life and enhance nutritional values of postharvest peach fruit, which could be applied for commercial purposes.

Effect of Exogenous GABA Treatment on Endogenous GABA content and GAD Activity. Many studies have demonstrated that GABA accumulation in plants responds to environmental

stresses, which play a major role against the stresses.[3,4] It was observed that GABA accumulated in postharvest horticultural products in response to high CO_2 or low O_2 and high CO_2 conditions.[12,18−20] High CO_2 treatment increased GABA content in cherimoya fruit, which was associated with the reduction of chilling injury.[21] In the present study, accumulation of GABA was also observed in peaches under the chilling stress, and exogenous GABA treatment induced a larger increase in treated peaches than the control fruit (Table 1). Therefore, the higher level of endogenous GABA accumulation may be one of the major factors that trigger the chilling resistance in peach fruit when treated with exogenous GABA. It is well-known that GAD is the key enzyme involved in GABA synthesis.[4] Deewatthanawong et al.[12] reported that CO_2 treatment induced gene expression of *GAD*2 and *GAD*3, which resulted in the higher GABA content in treated tomato fruit. In this experiment, GAD activity decreased with the GABA accumulation during the storage time, and when compared with control fruit (Table 1), the level of GABA accumulation in exogenous GABA treatment was much larger for the higher GAD activity.

Effect of Exogenous GABA Treatment on Proline Content and Activities of P5CS, OAT, and PDH. In recent years, attention on proline has been paid to the role in plant defense against abiotic and biotic stresses. A general phenomenon observed is that proline can alter its titer in response to various types of environmental stresses such as low and high temperatures, salinity, and water stress.[6,7,22−24] As compared with stress-intolerant plants, stress-tolerant plants generally have a large capacity to enhance proline biosynthesis in responses to stress.[25−27] Evidence has shown that the enhancement of proline accmulation is positively correlated with the chilling tolerance in plants. For example, rice seedlings treated with cold tolerant seed coating agents under chilling stress accumulated more proline, which may play a role in the resistance against chilling.[28] Therefore, in the present work, we have focused our attention on evaluating the effect of exogenous GABA treatment on proline accumulation in peaches. As shown in Table 1, the proline content increased in response to chilling stress during the storage, and exogenous GABA treatment enhanced the accumulation. From these results and the fact that fruit treated with GABA showed a lower sensitivity to chilling, it can be ruled out that the tolerance of peach fruit induced by GABA treatment may be partly related to the increase in proline content. A similar result was also reported by Zhang et al.,[29] who found that efficient control of chilling in cherry tomato fruit by arginine was associated with enhanced proline accumulation.

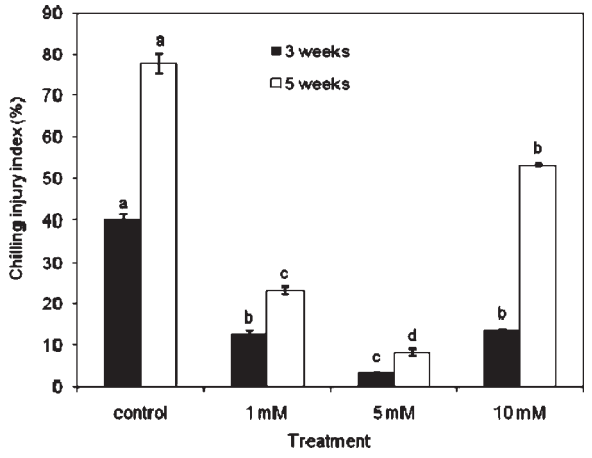

Figure 1. Effect of exogenous GABA treatment on the chilling injury index of peach fruit after 3 or 5 weeks of storage at 1 °C plus 3 days of shelf life at 20 °C. Vertical bars represent the standard errors of the means. Values with different letters for the same storage period within the same figure are significantly different according to Duncan's multiple range test at the $p = 0.05$ level.

The metabolism for proline is well established in many organisms. In higher plants, proline can be synthesized from glutamate via P5CS or from ornithine via OAT.[7] An increase in OAT activity or expression of P5CS gene along with an increase in the content of proline in plants under several environmental stresses has been reported.[25,26,30,31] Our results agreed with these authors since we found that activities of P5CS and OAT increased in peaches with the proline accumulation under chilling stress (Table 1). The enzyme PDH is reported to catalyze proline degradation.[7] In the present investigation, chilling resulted in a decrease in PDH activity in peaches (Table 1). This result suggests that inhibited proline degradation contributes to proline accumulation in peaches exposed to chilling. Ruiz et al.[30] and López-Carrión et al.[13] also reported that PDH was inhibited to a greater extent in green bean plant after the cold shock or in the Chinese cabbage under the salt stress. Furthermore, our present study also revealed that treatment with GABA increased the activities of P5CS and OAT but decreased PDH activity in peaches under chilling stress (Table 1). The increased P5CS and OAT activities could enhance the ability of proline biosynthesis, while the decreased PDH activity would contribute

Table 1. Effect of Exogenous GABA Treatment on the Contents of Endogenous GABA and Proline and Activities of GAD, P5CS, OAT, and PDH of Peach Fruit after Storage at 1 °C for 3 or 5 Weeks[a]

treatment	GABA content (μg/g FW)	GAD (U/mg protein)	proline content (μg/g FW)	P5CS (U/mg protein)	OAT (U/mg protein)	PDH (U/mg protein)
0 week	23.67 ± 0.41	50.78 ± 0.12	8.56 ± 0.06	2.98 ± 0.41	28.66 ± 0.26	32.23 ± 1.89
3 week control	30.12 ± 0.26 c	28.73 ± 0.63 b	8.95 ± 0.08 d	3.00 ± 0.42 d	37.00 ± 1.59 c	25.73 ± 2.23 a
1 mM	41.33 ± 1.46 b	53.89 ± 4.05 a	9.40 ± 0.05 c	9.38 ± 0.24 c	30.06 ± 2.60 d	17.50 ± 1.62 bc
5 mM	55.94 ± 5.47 a	59.68 ± 7.56 a	10.89 ± 0.02 a	26.11 ± 0.89 a	45.24 ± 2.39 b	14.57 ± 1.20 c
10 mM	43.79 ± 2.97 b	59.49 ± 1.49 a	9.55 ± 0.06 b	15.83 ± 0.75 b	58.28 ± 2.73 a	20.56 ± 1.22 b
5 week control	40.50 ± 2.30 c	23.71 ± 1.18 c	9.29 ± 0.02 d	7.52 ± 0.27 d	44.06 ± 1.18 b	25.23 ± 2.20 a
1 mM	55.43 ± 0.41 ab	66.09 ± 0.68 b	10.45 ± 0.01 c	13.67 ± 1.18 c	47.81 ± 1.35 b	16.25 ± 0.70 b
5 mM	61.38 ± 3.75 a	73.59 ± 1.46 a	10.40 ± 0.04 a	27.28 ± 0.09 a	57.11 ± 0.87 a	11.11 ± 5.08 b
10 mM	51.67 ± 1.56 b	61.26 ± 3.99 b	10.57 ± 0.06 b	19.41 ± 0.01 b	60.17 ± 2.36 a	19.31 ± 3.21 ab

[a] Means in a column followed by a different letter for the same storage period differ significantly at $P = 0.05$ by Duncan's multiple range tests. Data are accompanied by standard errors of the means.

Table 2. Effect of Exogenous GABA Treatment on Quality Parameters of Peach Fruit after 3 or 5 Weeks of Storage at 1 °C Plus 3 Days of Shelf-Life at 20 °C[a]

treatment	firmness (kg/cm^2)	extractable juice (%)	TSS (%)	TA (%)
0 week	6.73 ± 0.13	38.50 ± 0.56	11.08 ± 0.13	1.23 ± 0.02
3 week control	1.10 ± 0.38 a	52.04 ± 0.29 bc	9.83 ± 0.06 b	0.58 ± 0.01 c
1 mM	1.23 ± 0.37 a	47.18 ± 0.99 c	10.40 ± 0.17 a	0.67 ± 0.00 b
5 mM	1.53 ± 0.70 a	53.36 ± 3.17 ab	10.23 ± 0.06 a	0.88 ± 0.02 a
10 mM	1.26 ± 0.53 a	58.25 ± 1.61 a	10.13 ± 0.12 a	0.66 ± 0.00 b
5 week control	1.37 ± 0.57 a	41.15 ± 1.28 bc	9.07 ± 0.06 c	0.45 ± 0.01 c
1 mM	1.48 ± 0.59 a	38.16 ± 1.73 c	9.97 ± 0.06 a	0.62 ± 0.01 b
5 mM	1.14 ± 0.27 a	43.81 ± 1.29 ab	10.13 ± 0.12 a	0.80 ± 0.01 a
10 mM	0.86 ± 0.24 a	44.67 ± 0.02 a	9.57 ± 0.12 b	0.63 ± 0.01 b

[a] Means at the same time in a column followed by a different letter for the same storage period differ significantly at $P = 0.05$ by Duncan's multiple range tests. Data are accompanied by standard errors of the mean.

to the lower degradation of proline, which may account for the higher level of proline observed in GABA-treated fruit.

Effect of Exogenous GABA Treatment on Quality Parameters. There was no significant difference in fruit firmness of peaches among all treatments (Table 2). Fruit treated with 10 mM GABA showed significantly ($p < 0.05$) higher extractable juice as compared with the control peaches; however, GABA treatments at 1 or 5 mM had no significant effect on extractable juice (Table 2). All of the three exogenous GABA treatments maintained significantly ($p < 0.05$) higher levels of TSS in peaches. GABA at 1 or 5 mM was more effective in preserving TSS content than 10 mM GABA treatment, but no significant difference was found between 1 and 5 mM GABA treatments (Table 2). Similar to TSS, the three GABA treatments maintained higher levels of TA in peaches, and 5 mM GABA was the most effective treatment. However, there was no significant difference in TA content of peaches between 1 and 10 mM treatments (Table 2). Taking together, our results revealed that exogenous GABA treatment not only alleviated chilling injury but also maintained better quality in peach fruit. We suggested that GABA may have potential for commercialization to control postharvest chilling injury on peaches during cold storage.

In conclusion, this study showed that exogenous GABA treatment alleviated chilling injury and maintained higher fruit quality in peach fruit during cold storage. The effect of GABA treatment on alleviating chilling injury of peach may be attributed to its ability to enhance accumulation of endogenous GABA and proline contents in peaches, which was due to the increased GAD, P5CS, and OAT activities and decreased PDH activity. Thus, we found a new way to improve chilling tolerance of peach fruit during cold storage after harvest. To our knowledge, this is the first report that exogenous GABA treatment can alleviate chilling injury in postharvest horticultural product.

AUTHOR INFORMATION

Corresponding Author

*Tel: +86-25-84346251. Fax: +86-25-84346256. E-mail: njcaoshifeng@yahoo.com.cn (S.F.C.). Tel: +86-25-84399080. Fax: +86-25-86-25-84395618. E-mail: zhengyh@njau.edu.cn (Y.H.Z.).

Funding Sources

This study was supported by the Science and Technology Department of Zhejiang Province (No. 2009C32086) and Key Program of Science and Technology Innovative Research Team of Zhejiang Province (No. 2009R50036).

REFERENCES

(1) Brummell, D. A.; Dal Cin, V.; Crisoto, C. H.; Labavith, J. M. Cell wall metabolism during the development of chilling injury in cold-stored peach fruit: Association of mealiness with arrested disassembly of cell wall pectins. *J. Exp. Bot.* **2004**, *55*, 2041–2052.

(2) Lurie, S.; Crisosto, C. H. Chilling injury in peach and nectarine. *Postharvest Biol. Technol.* **2005**, *37*, 195–208.

(3) Shelp, B. J.; Bown, A. W.; McLean, M. D. Metabolism and functions of gamma-aminobutyric acid. *Trends Plant Sci.* **1999**, *4*, 446–452.

(4) Kinnersley, A. M.; Turano, F. J. Gamma aminobutyric acid (GABA) and plant responses to stress. *Crit. Rev. Plant Sci.* **2000**, *19*, 479–509.

(5) Song, H. M.; Xu, X. B.; Wang, H.; Wang, H. Z.; Tao, Y. Z. Exogenous γ-aminobutyric acid alleviates oxidative damage caused by aluminium and proton stresses on barley seedlings. *J Sci. Food Agric.* **2010**, *90*, 1410–1416.

(6) Hare, P. D.; Cress, W. A. Metabolic implications of stress induced proline accumulation in plants. *Plant Growth Regul.* **1997**, *21*, 79–102.

(7) Hare, P. D.; Cress, W. A.; Staden, J. V. Proline synthesis and degradation a model system for elucidating stress related signal transduction. *J. Exp. Bot.* **1999**, *50*, 413–434.

(8) Purvis, A. C. Free proline in peel of grapefruit and resistance to chilling injury during cold storage. *HortScience* **1981**, *16*, 160–161.

(9) Zhao, D. Y.; Shen, L.; Fan, B.; Liu, K. L.; Yu, M. M.; Zheng, Y.; Ding, Y.; Sheng, J. P. Physiological and genetic properties of tomato fruits from 2 cultivars differing in chilling tolerance at cold storage. *J. Food Sci.* **2009**, *74*, 348–352.

(10) Verbruggen, N.; Hermans, C. Proline accumulation in plants: A review. *Amino Acids* **2008**, *35*, 753–759.

(11) Zhang, G.; Bown, A. W. The rapid determination of γ-aminobutyric acid. *Phytochemistry* **1997**, *44*, 1007–1009.

(12) Deewatthanawonga, R.; Rowell, P.; Watkins, C. B. γ-Aminobutyric acid (GABA) metabolism in CO_2 treated tomatoes. *Postharvest Biol. Technol.* **2010**, *57*, 97–105.

(13) López-Carrión, A. I.; Castellano, R.; Rosales, M. A.; Ruiz, J. M.; Romero, L. Role of nitric oxide under saline stress: Implications on proline metabolism. *Biol. Plant* **2008**, *52*, 587–591.

(14) Sánchez, E.; López-Lefebre, L. R.; García, P. C.; Rivero, R. M.; Ruiz, J. M.; Romero, L. Proline metabolism in response to highest nitrogen dosages in green bean plants (*Phaseolus vulgaris* L. cv. Strike). *J. Plant Physiol.* **2001**, *158*, 593–598.

(15) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.

(16) Abdou, A. M.; Higashiguchi, S.; Horie, K.; Kim, M.; Hatta, H.; Yokogoshi, H. Relaxation and immunity enhancement effects of γ-aminobutyric acid (GABA) administration in humans. *Biofactors* **2006**, *26*, 201–208.

(17) Yamakoshi, J.; Fukuda, S.; Satoh, T.; Tsuji, R.; Saito, M.; Obata, A.; Matsuyama, A.; Kawasaki, T. Antihypertensive and natriuretic

effects of less-sodium soy sauce containing γ-aminobutyric acid in spontaneously hypertensive rats. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 165–173.

(18) Makino, Y.; Soga, N.; Oshita, S.; Kawagoe, Y.; Tanaka, A. Stimulation of γ-aminobutyric acid production in vine-ripe tomato (*Lycopersicon esculentum* Mill.) fruits under modified atmospheres. *J. Agric. Food Chem.* **2008**, *56*, 7189–7193.

(19) Pedreschi, R.; Franck, C.; Lammerteyn, J.; Erban, A.; Kopka, J.; Hertog, M.; Verlinden, B.; NicolaÏ, B. Metabolic profiling of 'Conference' pears under low oxygen stress. *Postharvest Biol. Technol.* **2009**, *51*, 123–130.

(20) Deewatthanawonga, R.; Nock, J. F.; Watkins, C. B. γ-Aminobutyric acid (GABA) accumulation in four strawberry cultivars in response to elevated CO_2 storage. *Postharvest Biol. Technol.* **2010**, *57*, 92–96.

(21) Merodio, C.; Muńoz, M. T.; Cura, B. D.; Buitrago, D.; Escribano, M. I. Effect of high CO_2 level on the titres of γ-aminobutyric acid, total polyamines and some pathogenesis-related proteins in cherimoya fruit stored at low temperature. *J. Exp. Bot.* **1998**, *49*, 1339–1347.

(22) Delauney, A. J.; Verma, D. P. Proline biosynthesis and osmoregulation in plants. *Plant J.* **1993**, *4*, 215–223.

(23) Zaifnejad, M.; Clark, R. B.; Sullivan, C. Y. Aluminum and water stress effects on growth and proline of sorghum. *J. Plant Physiol.* **1997**, *150*, 338–344.

(24) Ashraf, M.; Harris, P. J. C. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.* **2004**, *166*, 3–16.

(25) Lutts, S.; Majerus, V.; Kinet, J. M. NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. *Physiol. Plant* **1999**, *105*, 450–458.

(26) Lin, C. C.; Hsu, Y. T.; Kao, C. H. The effect of NaCl on proline accumulation in rice leaves. *Plant Growth Regul.* **2002**, *36*, 275–285.

(27) Kumar, S. G.; Reddy, A. M.; Sudhakar, C. NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. *Plant Sci.* **2003**, *165*, 1245–1251.

(28) Zhang, H. Q.; Zou, Y. B.; Xiao, G. C.; Xiong, Y. F. Effect and mechanism of cold tolerant seed-coating agents on the cold tolerance of early lndica rice seedlings. *Agric. Sci. China* **2007**, *6*, 792–801.

(29) Zhang, X. H.; Shen, L.; Li, F. J.; Zhang, Y. X.; Meng, D. M.; Sheng, J. P. Up-regulating arginase contributes to amelioration of chilling stress and the antioxidant system in cherry tomato fruits. *J. Sci. Food Agric.* **2010**, *90*, 2195–2202.

(30) Ruiz, J. M.; Sánchez, E.; García, P. C.; López-Lefebre, L. R.; Rivero, R. M.; Romero, L. Proline metabolism and NAD kinase activity in greenbean plants subjected to cold-shock. *Phytochemistry* **2002**, *59*, 473–478.

(31) Thippeswamy, M.; Chandraobulreddy, P.; Sinilal, B.; Kumar, M. S.; Sudhakar, C. Proline accumulation and the expression of Δ^1-pyrroline-5-carboxylate synthetase in two safflower cultivars. *Biol. Plant* **2010**, *54*, 386–390.