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Amelioration of postharvest chilling injury in anthurium cut flowers by γ-aminobutyric acid (GABA) treatments

Morteza Soleimani Aghdam, Roohangiz Naderi*, Mohammad Ali Askari Sarcheshmeh, Mesbah Babalar

Department of Horticultural Science, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

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ABSTRACT

The optimum temperature storage of anthurium flowers is 12.5–20 °C because they are very sensitive to chilling injury (CI). CI is associated with the loss of membrane integrity which can be aligned to phenolic oxidation due to polyphenol oxidase (PPO) activity, the enzyme responsible for tissue browning. The increment of phenylalanine ammonia-lyase (PAL) activity, the enzyme responsible for phenols accumulation, in response to chilling stress has been considered as defense mechanism to chilling stress. In this study, the effects of 0, 1, 5, 10, 15 and 20 mM γ-aminobutyric acid (GABA) treatment applied by preharvest spraying or postharvest stem-end dipping (15 min at 20 °C) on CI of anthurium flowers (cv. Sirion) stored at 4 °C for 21 days was investigated. CI symptoms were accompanied by spathe browning and increase in electrolyte leakage as well as malondialdehyde (MDA) content. GABA treatment at 1 and 5 mM by pre and postharvest treatment, respectively, delayed spathe browning and increases in electrolyte leakage and MDA accumulation. The GABA treated anthurium cut flowers exhibited significantly higher PAL enzyme activity, associated with lower PPO activity. Higher PAL enzyme activity in anthurium cut flowers treated with GABA coincided with higher total phenol accumulation and higher DPPH scavenging activity than control flowers during storage at 4 °C for 21 days. Also, proline content in anthurium cut flowers treated with GABA was significantly higher than control flowers during storage. These results suggest that GABA treatment can be used as a useful technology for enhancing tolerance of anthurium cut flowers to postharvest chilling injury by increasing total phenol and proline accumulation and decreasing MDA content, and thus maintaining membrane integrity.

1. Introduction

Low temperature storage is widely used as a postharvest treatment to delay senescence in vegetables and ornamentals, and ripening in fruits, and thereby maintaining their postharvest quality. However, tropical and subtropical crops such as anthurium (*Anthurium andraeanum* L.) flowers are sensitive to chilling injury (CI), a physiopathy affecting these crops when subjected to temperatures below 12 °C but above the freezing point (Aghdam and Bodbodak, 2013). The recommended optimum temperature for storage of anthurium cut flowers is 12.5–20 °C (Promyou et al., 2012). The main symptom of CI in anthurium flowers under low temperature storage is spadix wilting and spathe browning (Paull, 1987; Promyou et al., 2012). There is a tremendous need to apply economical and convenient techniques to reduce CI while prolonging maximal shelf life of anthurium cut flowers, in order to potentiate their possibilities of long distances transport, a producers demand for their introduction in the global markets (Aghdam et al., 2013). Promyou and Ketsa (2014) suggested that CI in anthurium flowers stored at 4 °C is associated with increase in the membrane lipid peroxidation and loss of membrane semi-permeability, which triggers anthurium flower senescence leading to short vase life. Recently, Promyou et al. (2012) reported that postharvest treatment with salicylic acid (2 mM for 15 min) alleviated CI in anthurium cut flowers, an effect associated with decreasing electrolyte leakage, MDA content and lipoxygenase (LOX) activity, and increasing catalase (CAT) and superoxide dismutase (SOD) activities, which led to a diminution of spathe browning and fresh weight loss, two detrimental effects of CI on anthurium cut flowers.

When horticultural crops are stored under chilling temperature: (1) PAL activity increases due to the CI effect, inducing increase of total phenols (TP) that accumulates in vacuoles; (2) a membrane selective permeability loss occurs; (3) PPO activity

* Corresponding author. Fax: +98 426 223 4857.
E-mail address: rnaderi@ut.ac.ir (R. Naderi).

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increases in cytoplasm that is responsible for browning; (4) phenols accumulated in vacuoles leak to cytoplasm due to loss of vacuole membrane (tonoplast) selective permeability and contribute to browning incidence, an effect influenced by PAL activity (Sevillano et al., 2009). PAL as a key enzyme in the phenylpropanoid pathway catalyzing the conversion of phenylalanine to trans-cinnamic acid. PAL connects primary metabolism (shikimic acid pathway) with secondary metabolism (phenylpropanoids pathway) (Dixon and Paiva, 1995). Meng et al. (2009) reported that the phenolics have dual function, firstly phenolics can be oxidized by PPO, which leads to browning, as the main CI symptom in horticultural crops and secondly phenols, which accumulate in horticultural crops in response to chilling stress, have antioxidant capacity. Chen et al. (2008) reported that heat pretreatment (38 °C for 2 days) mitigated CI in banana fruit during storage at 8 °C. Mitigation of CI was determined by a reduction of electrolyte leakage and MDA content and heat treatment increased PAL gene expression and enzyme activity, increasing TP content. It was suggested that higher TP might enhance CI tolerance in banana fruit. Aghdam et al. (2012) reported that the brassinosteroids (BRs) 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. (2012) showed that BRs treatment enhanced PAL enzyme activity which led to total phenol accumulation.

γ-Aminobutyric acid (GABA), a four carbon non-protein amino acid with an amino group on the γ-carbon, is widely distributed in bacteria, plants and animals (Shelp et al., 1995; Fait et al., 2007). Abiotic stresses such as chilling, heat, drought, UV irradiation, and low O_2 cause GABA to accumulate in plants (Shelp et al., 1999). GABA is believed to function in regulation of cytosolic pH (Shelp et al., 1999), mitigation of oxidative stress (Bouche et al., 2003), induction of nitrate transport (Beuvé et al., 2004), regulation of pollen tube growth and guidance (Palanivelu et al., 2003), and cell elongation (Renault et al., 2011). GABA have anti-chilling function in horticultural crops and exogenous GABA treatment has the ability to enhance resistance to postharvest CI in fruits and vegetables such as banana (Wang et al., 2014) and peach fruit (Shang et al., 2011; Yang et al., 2011).

To our knowledge, no information exists on the effect of exogenous GABA treatment on CI in anthurium cut flowers. In this work, the effects of pre and postharvest GABA treatment on the spathe browning as CI symptoms, electrolyte leakage and MDA content as membrane integrity indicators, PAL and PPO activities associated with total phenols content, proline content and DPPH• scavenging capacity of anthurium cut flowers were evaluated. We propose that maintenance of membrane integrity associated with enhanced total antioxidant capacity and proline accumulation, and reduced PPO enzyme activity coinciding with increasing PAL enzyme activity leading to total phenolic accumulation, is proposed as possible mechanism for the mitigation impact of exogenous GABA on CI of cut flowers.

2. Materials and methods

2.1. Flowers and treatments

GABA at 1, 5, 10, 15 and 20 mM was sprayed on anthurium (*A. andraeanum* L.) flowers cv. Sirion at commercial greenhouse by using a hand-sprayer until flowers were wet to runoff. Additional flowers were also sprayed with distilled water as the control. The sprays were applied three times at 7-day intervals before harvest, when 40–50% of the true flowers on the spadix had fully opened (Promyou et al., 2012). Flowers were cut in the morning, placed in water at the growers' property and transported at 12 °C in water to the laboratory. Then, the flower stems were recut to 30 cm length, individually placed in water and stored at 4 °C (85–90% RH) for 21 days. Preharvest treatment contained 720 flowers which divided in to 6 lots of 120 flowers treated in triplicate (40 flowers per replicate): control (0) and GABA at 1, 5, 10, 15 and 20 mM. For postharvest treatment, 540 anthurium flowers cv. Sirion were harvested in the morning when 40–50% of the true flowers on the spadix had fully opened and placed in water at the growers' property and transported at 12 °C in water to the laboratory. Then, the flower stems were recut to 30 cm length and divided into 6 lots of 90 flowers for the following treatments in triplicate (30 flowers per replicate) by dipping of individual flower stems at 0 (control), 1, 5, 10, 15 and 20 mM GABA solution for 15 min at 20 °C and then removed from the GABA solution and were allowed to air-dry at room temperature and individually placed in water and stored at 4 °C (85–90% RH) for 21 days. After evaluation of chilling injury every 7 days during storage at 4 °C by determining the 10 individual flower spathes browning, electrolyte leakage and MDA content as membrane integrity indicators, PAL and PPO enzymes activity associated with total phenols content, proline content and DPPH• scavenging capacity of anthurium cut flowers were evaluated. Vase life of flowers was individually evaluated when desiccation of spadix, loss of spathe gloss, blackening or wilting of spathe were found (Paull and Goo, 1982).

2.2. Spathe browning

The browning of anthurium cut flowers spathe was assessed using two methods. The first method of browning assessment was by visualizing the total brown area on the spathes of 10 individual flowers using a scale from 1 to 5; 1 = no chilling injury; 2 = mild injury (1–20% of spathe affected); 3 = moderate injury (21–50% of spathe affected); 4 = severe injury (51–80% of spathe affected); 5 = very severe injury (81–100% of spathe affected). CI was calculated as $\sum$ (number on CI scale × number of flowers at that number on the CI scale)/total number of flower in each group, according to Promyou et al. (2012). As a second method for assessment of spathe browning, surface color of spathe was measured with a Minolta spectrophotometer (CR-400) using CIE color parameters L^* (light/dark), a^* (red/green) and b^*(yellow/blue) values and browning index calculated by formula as follow: Browning Index (BI) = [100 $(x - 0.31)$]/0.17, where $x = (a^* + 1.75L^*)/(5.645L^* + a^* - 0.3012b^*)$, according to Ding and Ling (2014).

2.3. Electrolyte leakage and MDA content

Ten discs, 2 mm thickness and 15 mm diameter, were excised from the spathe with a cork borer, rinsed with 50 mL of distilled

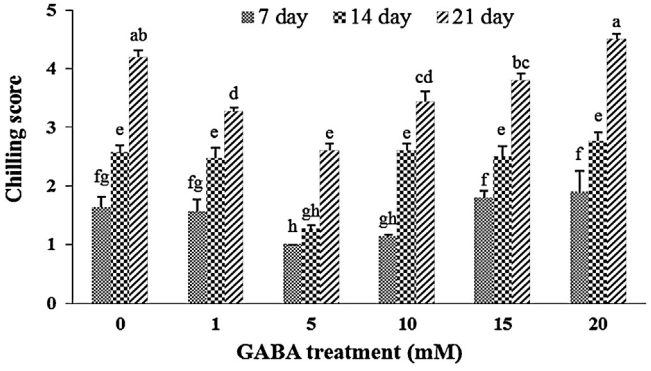

Fig. 1. Effects of postharvest GABA treatment at 0, 1, 5, 10, 15 and 20 mM on the CI score of anthurium cut flower spathes storage at 4 ± 0.5 °C for 21 days. Data shown are mean values of $n = 3$ and the error bars represent standard errors of the means. Tukey–Kramer's multiple range test at $P = 0.05$ level.

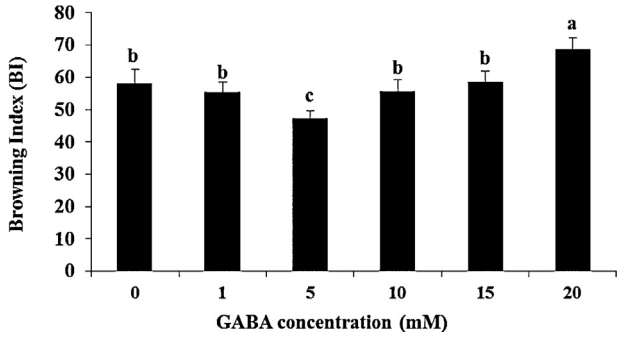

Fig. 2. Effects of postharvest GABA treatment at 0, 1, 5, 10, 15 and 20 mM on the browning index (BI) of anthurium cut flower spathes storage at 4 ± 0.5 °C for 21 days. Data shown are mean values of $n = 3$ and the error bars represent standard errors of the means. Tukey–Kramer's multiple range test at $P = 0.05$ level.

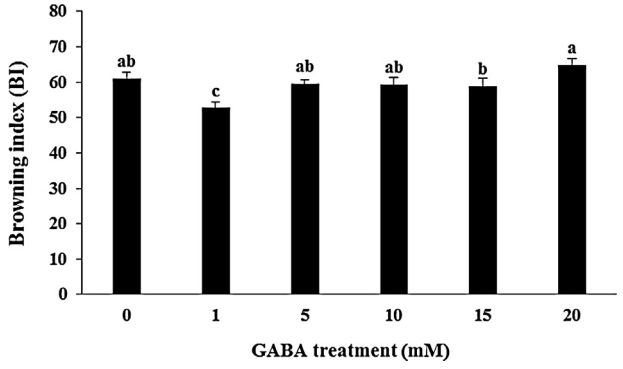

Fig. 3. Effects of preharvest GABA treatment at 0, 1, 5, 10, 15 and 20 mM on the CI score of anthurium cut flower spathes storage at 4 ± 0.5 °C for 21 days. Data shown are mean values of $n = 3$ and the error bars represent standard errors of the means. Tukey–Kramer's multiple range test at $P = 0.05$ level.

water between excisions. They were incubated in 50 mL of 0.4 M mannitol solution and shaken at 1.67 Hz, at room temperature. Initial electrolyte leakage of the solution was determined following shaking for 3 h, using a conductivity meter. Final electrolyte leakage was determined after autoclaving the material at 121 °C for 1 h to release electrolytes. The percentage of electrolyte leakage

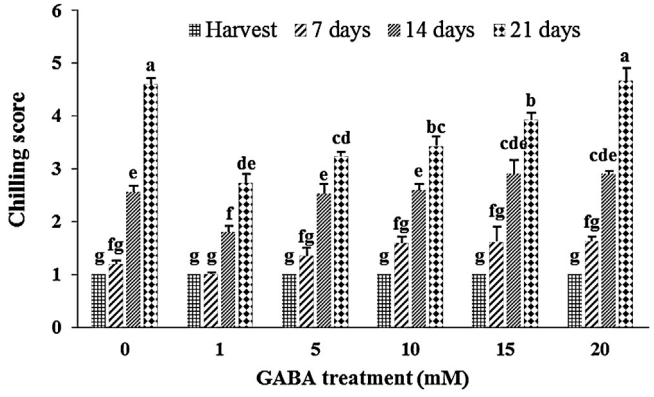

Fig. 4. Effects of preharvest postharvest GABA treatment at 0, 1, 5, 10, 15 and 20 mM on the browning index (BI) of anthurium cut flower spathes storage at 4 ± 0.5 °C for 21 days. Data shown are mean values of $n = 3$ and the error bars represent standard errors of the means. Tukey–Kramer's multiple range test $P = 0.05$ level.

was calculated using the equation (Promyou et al., 2012):

$$\text{Electrolyte leakage}(\%) = \frac{\text{Initial electrolyte leakage}}{\text{Final electrolyte leakage}} \times 100$$

MDA content was measured by the thiobarbituric acid (TBA) method described by Hodges et al. (1999). One gram of spathe was homogenized with 25 mL of 5% (w/v) trichloroacetic acid (TCA). The mixture was centrifuged for 10 min at 10000 × g. TBA reactivity was determined by adding 2.5 mL of 0.5% TBA in 15% TCA to 1.5 mL of the supernatant. The reaction solution was held for 30 min in bath containing boiling water, then cooled quickly and finally centrifuged at 12000 × g for 10 min to clarify the solution. Absorbance was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm, calculated with an extinction coefficient of 1.55 nmol L^{-1} m^{-1}. MDA content was expressed as nmol g^{-1} spathe fresh weight (FW).

2.4. Proline and total phenols content

Proline content was measured using the acid ninhydrin method described by Zhang et al. (2010). One gram of spathe were homogenized with 5 mL of 3% (v/v) sulfosalicylic acid and centrifuged at 12000 × g for 10 min. Two milliliters of glacial acetic acid and 3 mL of ninhydrin reagent were mixed with the 2 mL of supernatant and boiled for 30 min. Then 4 mL toluene was added into the reaction mixture after the solution was cooled. The absorbance of the organic phase was recorded at 520 nm. The results were compared with a standard curve of proline and concentration expressed as μmol g^{-1} spathe fresh weight (FW).

Total phenol content was determined according to the Folin–Ciocalteu procedure (Chen et al., 2008). One gram of spathe was homogenized with 8 mL of methanol and extracted for 24 h in the dark. Then the homogenate was centrifuged at 12000 × g for 20 min at 4 °C. The supernatant reacted with Folin–Ciocalteu reagent and 150 g L^{-1} Na_2CO_3. The content of total phenols was expressed as mg of gallic acid equivalent (GAE) per g of spathe fresh weight (FW).

2.5. DPPH• scavenging activity

Free radical 2,2-dipheynl-1-picrylhydrazyl (DPPH•) scavenging activity was measured according to Nakajima et al. (2004). One gram of spathe were homogenized with 8 mL of methanol and extracted for 24 h in the dark. Then the homogenate was centrifuged at 12000 × g for 20 min at 4 °C. Fifty μL of the supernatant were added to 1.0 mL of 6×10^{-5} M DPPH• in methanol. The mixture was shaken and left at room temperature for 30 min; the absorbance was measured at 515 nm. Methanol was used as the experimental control. The percent of reduction of DPPH• was calculated according to the following equation, where Abs control is the absorbance of DPPH• solution without extract.

$$\%\text{inhibition of DPPH}^{\bullet} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

2.6. PAL and PPO enzymes activity

PAL was extracted and assayed as described by Nguyen et al. (2003). One gram of spathe was homogenized in 20 mL of 50 mM borate buffer (pH 8.5) containing 5 mM 2-mercaptoethanol and 0.5 g PVPP. The homogenate was filtered through three layers of cotton cloth and then centrifuged at 18000 × g for 20 min at 4 °C. PAL activity was determined in the supernatant, 0.3 mL of which was added to a reaction mixture containing 0.7 mL of 100 mM L-

Table 1
Vase life of anthurium cut flowers treated with 0 (control), 1, 5, 10, 15 and 20 mM GABA at pre and postharvest stage storage at 4 ± 0.5 °C.[a]

GABA treatment (mM)	Time to browning initiation (days at 4 °C)	Vase life (days at 4 °C)
Preharvest		
0	4 b	16 c
1	8.33 a	26.33 a
5	5.33 ab	22.33 b
10	5.33 ab	21.33 b
15	5.67 ab	21.67 b
20	3.67 b	14.33 c
Significant	**	**
CV (%)	22.302	4.070
Postharvest		
0	4 bc	16 c
1	5 bc	19.67 b
5	7 a	24.37 a
10	5.33 ab	20.33 b
15	5.67 ab	18.33 b
20	3.33 c	15 c
Significant	**	**
CV (%)	13.987	5.066

Different letters indicate significant differences at significance level $P = 0.05$, using Tukey–Kramer's multiple range test
*Significance at 0.05 level.
**Significance at 0.01 level.
[a] Mean values of $n = 3$.

Table 2
Effect of postharvest GABA treatment at 5 mM on electrolyte leakage, MDA content and proline accumulation of anthurium cut flower spathes storage at 4 ± 0.5 °C for 21 days.[a]

Time (day)	Treatment	Membrane integrity		
	GABA (mM)	EL (%)	MDA (n mol g^{-1} FW)	Proline (μ mol g^{-1} FW)
0		12.46 ± 0.69	12.31 ± 0.57	1.02 ± 0.26
7	0	15.73 ± 0.50 d	16.76 ± 0.33 d	1.71 ± 0.12 d
	5	12.44 ± 0.72 d	14.56 ± 0.20 e	3.63 ± 0.17 c
14	0	32.29 ± 0.70 b	20.39 ± 0.14 b	3.76 ± 0.12 c
	5	24.63 ± 0.68 c	17.72 ± 0.09 c	7.60 ± 0.24 b
21	0	43.52 ± 1.64 a	25.63 ± 0.35 a	7.57 ± 0.19 b
	5	30.92 ± 0.64 b	19.63 ± 0.03 b	11.68 ± 0.50 a
Significant	df			
Time	2	**	**	**
Treatment	1	**	**	**
T × T	2	**	**	**
CV		6.352	2.194	6.325

Different letters indicate significant differences at significance level $P = 0.05$, using Tukey–Kramer's multiple range test.
*Significance at 0.05 level.
**Significance at 0.01 level.
[a] Mean values ± SE ($n = 3$).

phenylalanine and 3 mL of 50 mM borate buffer (pH 8.5). After incubation of the mixture at 40 °C for 1 h, the reaction was stopped by adding 0.1 mL of 5 mM HCl. PAL activity was measured at room temperature. PAL activity was calculated from the absorbance of the assay mixture at 290 nm, based on the production of cinnamic acid. PAL enzyme activity expressed as μmol of cinnamic acid mg^{-1} protein h^{-1}.

PPO was extracted and assayed using the method of by Nguyen et al. (2003). One gram of spathe was homogenized in 10 mL of phosphate buffer (0.1 M, pH 7.8) with 1 g of polyvinylpyrrolidone (PVP), and the solution was then centrifuged at 20000 × g for 15 min at 4 °C. The supernatant was collected as a crude PPO extract. The reaction mixture contained 0.1 M catechol in 0.05 M phosphate buffer (pH 6.0). Changes in the absorbance at 410 nm were measured. One unit of PPO activity was defined as a change of 0.01 at 410 nm in the absorbance per min. PPO activity expressed as μkat per mg^{-1} protein. Protein content was estimated according to Bradford (1976) using BSA as a standard.

2.7. Statistical analysis

The experiment was arranged as split plots for time on the basis of completely randomized design with three replications. Analysis of variance (ANOVA) was carried out with SPSS software. Differences between means were assessed by Tukey–Kramer's multiple range test with differences being considered significant at $P < 0.05$.

3. Results

3.1. Chilling injury symptoms and vase life

CI score increased during the whole storage at 4 °C and the increase was delayed by pre and postharvest GABA treatment ($P < 0.01$; Figs. 1 and 3). CI symptoms of anthurium cut flowers cv. Sirion were visible within 7 days of storage. Treatment with preharvest GABA at 1 mM and postharvest GABA at 5 mM resulted in a lower CI score ($P < 0.01$) as well as browning index ($P < 0.05$) (Figs. 1–4), while pre and postharvest treatment with GABA at 20 mM resulted in higher CI scores and browning indices. Thus, GABA effects on CI of anthurium cut flowers are concentration dependent. Based on these results, 1 mM GABA for preharvest and 5 mM GABA for postharvest treatment was chosen for further analyses. In this study, GABA was applied at pre and postharvest stage and could significantly reduce postharvest CI in anthurium cut flowers (Figs. 1–4). The spathe browning in control anthurium cut flowers initiate after 4 days storage at 4 °C, which show shortest vase life, 16 days. But, in anthurium cut flowers treated with 1 mM GABA at preharvest and 5 mM GABA at postharvest stage, spathe browning initiate after 8 and 7 days storage at 4 °C, which show longest vase life, 26 and 24 days, respectively (Table 1).

3.2. Electrolyte leakage and MDA content

Electrolyte leakage of the anthurium cut flowers spathe stored at 4 °C increased during the 21 days of storage (Table 2). The electrolyte leakage of anthurium cut flowers spathe treated with 1 and 5 mM GABA at pre or postharvest stage, respectively, remained lower than that in untreated control flowers ($P < 0.01$; Tables 2 and 4,). Also, during storage at 4 °C, the MDA content in the anthurium cut flowers spathe increased (Table 2). Compared to the controls, a lower content of MDA was found in the spathe of flowers treated with 1 and 5 mM GABA at pre or postharvest stage, respectively, ($P < 0.01$; Tables 2 and 4).

3.3. Proline content

The proline content in the anthurium cut flowers spathe increased during storage at 4 °C. GABA treatment with 1 and 5 mM GABA at pre or postharvest stage, respectively, enhanced the increase in proline ($P < 0.01$; Tables 2 and 4).

3.4. Total phenols content and activities of PAL and PPO

The content of total phenols in the anthurium cut flowers spathe treated with postharvest GABA at 5 mM and without GABA treatment showed an increase during the storage at 4 °C (Table 3). Postharvest 5 mM GABA treated anthurium cut flowers spathe displayed a faster rate in the accumulation of total phenols than control flowers ($P < 0.05$; Table 3). The content of total phenols in the anthurium cut flowers spathe treated with preharvest GABA at 1 mM and without GABA treatment continually increased then

Table 3
Effect of postharvest GABA treatment at 5 mM on total phenols content, DPPH scavenging capacity, PAL and PPO enzymes activity of anthurium cut flower spathes storage at 4 ± 0.5 °C for 21 days.

Time (day)	Treatment	Phenolic metabolism			
	GABA (mM)	TP (mg GAE g^{-1} FW)	PAL (μ mol mg^{-1} protein h^{-1})	PPO (μkat mg^{-1} protein)	DPPH (%)
0		0.98 ± 0.04	72.36 ± 1.54	1.07 ± 0.18	86.41 ± 0.31
7	0	1.27 ± 0.01 d	85.35 ± 0.057 d	2.03 ± 0.11 c	88.71 ± 0.24 d
	5	1.69 ± 0.01 c	89.76 ± 4.89 d	1.37 ± 0.11 c	90.22 ± 0.29 c
14	0	1.71 ± 0.08 c	94.88 ± 3.38 cd	4.08 ± 0.17 b	88.75 ± 0.18 d
	5	2.05 ± 0.05 b	114.18 ± 2.94 b	2.48 ± 0.24 c	91.56 ± 0.26 b
21	0	1.92 ± 0.03 b	107.15 ± 5.39 bc	8.48 ± 0.21 a	90.19 ± 0.10 c
	5	2.51 ± 0.05 a	131.49 ± 5.44 a	4.40 ± 0.06 b	92.15 ± 0.52a
Significant	df				
Time	2	**	**	**	**
Treatment	1	**	*	**	**
T × T	2	*	*	**	**
CV		3.669	5.586	13.857	1.260

Different letters indicate significant differences at significance level $P = 0.05$, using Tukey–Kramer's multiple range test.
[a]Mean values ± SE ($n = 3$).
* Significance at 0.05 level
** Significance at 0.01 level.

Table 4
Effect of preharvest GABA treatment at 1 mM on electrolyte leakage, MDA content and proline accumulation of anthurium cut flower spathes storage at 4 ± 0.5 °C for 21 days.

Time (day)	Treatment	Membrane integrity		
	GABA (mM)	EL (%)	MDA (n mol g^{-1} FW)	Proline (μ mol g^{-1} FW)
Harvest	0	15.85 ± 0.57 e	13.85 ± 0.32 d	1.38 ± 0.09 f
	1	12.07 ± 0.43 e	13.64 ± 0.59 d	2.50 ± 0.12 e
7	0	20.52 ± 0.61 d	18.52 ± 0.28 c	2.19 ± 0.02 ef
	1	15.35 ± 0.66 e	17.73 ± 0.13 c	3.35 ± 0.12 d
14	0	26.33 ± 1.34 c	23.22 ± 1.11 b	4.89 ± 0.02 c
	1	22.47 ± 0.60 d	20.55 ± 0.56 bc	6.29 ± 0.27 b
21	0	40.19 ± 0.79 a	28.64 ± 0.63 a	5.59 ± 0.24 bc
	1	30.51 ± 0.58 b	23.27 ± 1.24 b	8.21 ± 0.43 a
Significant	df			
Time	3	**	**	**
Treatment	1	**	*	**
T × T	3	**	*	**
CV		5.890	6.120	8.008

Different letters indicate significant differences at significance level $P = 0.05$, using Tukey–Kramer's multiple range test.
[a]Mean values ± SE ($n = 3$).
* Significance at 0.05 level
** Significance at 0.01 level.

decreased during storage (Table 5). The content of total phenols in the anthurium cut flowers spathe treated with preharvest GABA at 1 mM were higher than those in control flowers ($P < 0.01$; Table 5). Also, anthurium cut flowers spathe treated with 1 and 5 mM GABA at pre and postharvest, respectively, showed the higher PAL enzyme activity ($P < 0.05$) and lower PPO enzyme activity ($P < 0.01$) during the storage at 4 °C (Tables 3 and 5).

3.5. DPPH scavenging activity

Anthurium cut flowers spathe treated with 1 and 5 mM GABA at pre and postharvest, respectively, exhibited higher DPPH scavenging activity during storage at 4 °C ($P < 0.01$; Tables 3 and 5).

4. Discussion

During storage at 4 °C for 21 days, exogenous pre and postharvest GABA treatment ameliorated the chilling injury symptoms of anthurium cut flowers indicated by the delay in CI score and browning index increase. Electrolyte leakage is an effective parameter to assess membrane permeability and therefore is used as an indicator of membrane integrity (Marangoni et al., 1996; Lyons 1973). Also, lipid peroxidation by activation of LOX, which can be evaluated by MDA, is responsible for loss of cell membrane integrity (Wise and Naylor, 1987). MDA is the end product of the peroxidation of membrane unsaturated fatty acids (unSFA) and MDA level are used as a marker of oxidative stress, and damage on cell membrane integrity (Hodges et al., 1999). A higher tolerance to chilling stress, evidenced by the slower increase of MDA content and electrolyte leakage, was observed in the spathe of anthurium cut flowers treated with GABA at pre and postharvest stage. Treatment with 1 and 5 mM GABA at pre or postharvest stage, respectively, resulted in a decrease in electrolyte leakage and MDA content, i.e., inhibited lipid peroxidation under chilling stress, which clearly indicated that GABA could strongly protect anthurium cut flowers spathe from oxidative damage and thus enhance chilling tolerance. GABA was suggested to act as an inhibitor of MDA formation during lipid peroxidation (Deng et al., 2010). Anthurium cut flowers treated with 1 and 5 mM GABA at pre and postharvest stage, respectively, showed higher chilling tolerance within longest vase life, 26 and 24 days at 4 °C, and exhibited good ability to maintain membrane integrity under low temperature, evident by the lower electrolyte leakage and MDA content. Since chilling injury reduce anthurium cut flowers vase life by stimulation of flower senescence (Promyou and Ketsa, 2014), we suggests that higher vase life in anthurium cut flower treated with GABA can be results from amelioration of chilling injury, which led to delaying flower senescence.

Oxidative stress is a common secondary stress occurring in biotic and abiotic stress, and CI is no exception to this rule. In addition to the direct effect of chilling temperatures on the membrane structure, the loss of membrane integrity is itself boosted by oxidative stress, since cold stress increases the levels of reactive oxygen species (ROS) that stimulate lipid peroxidation in cell membranes (Sevillano et al., 2009). Yang et al. (2011) showed that the treatment of peach fruit with 5 mM GABA for 10 min reduces chilling injury, which was associated with higher antioxidant enzymes activities such as SOD, CAT, ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione-S-transferase (GST), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) along with higher energy status, ATP content and adenylate

Table 5
Effect of preharvest GABA treatment at 1 mM on total phenols content, DPPH scavenging capacity, PAL and PPO enzymes activity of anthurium cut flower spathes storage at 4 ± 0.5 °C for 21 days.

Time (day)	Treatment	Phenolic Metabolism			
	GABA (mM)	TP (mg GAE g^{-1} FW)	PAL (μ mol mg^{-1} protein h^{-1})	PPO (μkat mg^{-1} protein)	DPPH (%)
Harvest	0	1.38 ± 0.04 c	95.08 ± 2.77 d	1.07 ± 0.01 ef	78.78 ± 0.28 e
	1	1.41 ± 0.03 c	109.49 ± 7.60 cd	0.70 ± 0.04 f	83.19 ± 0.08 cd
7	0	1.74 ± 0.05 b	154.64 ± 5.46 b	2.08 ± 0.06 de	84.75 ± 0.31 b
	1	1.97 ± 0.07 a	192.44 ± 3.23 a	1.53 ± 0.01 ef	86.02 ± 0.42 a
14	0	1.47 ± 0.02 c	120.57 ± 1.16 c	4.65 ± 0.06 c	83.58 ± 0.19 c
	1	1.67 ± 0.06 b	150.23 ± 0.86 b	2.62 ± 0.17 d	84.74 ± 0.14 b
21	0	1.44 ± 0.04 c	108.80 ± 0.50 cd	10.17 ± 0.25 a	82.36 ± 0.16 d
	1	1.49 ± 0.02 c	122.58 ± 1.42 c	6.00 ± 0.24 b	84.53 ± 0.25 b
Significant	df				
Time	3	**	**	**	**
Treatment	1	**	**	**	**
T × T	3	**	*	**	**
CV		2.463	5.032	11.318	1.448

Different letters indicate significant differences at significance level $P = 0.05$, using Tukey–Kramer's multiple range test.
[a]Mean values ± SE ($n = 3$).
* Significance at 0.05 level
** Significance at 0.01 level.

energy charge (AEC). Thus, maintaining membrane integrity in anthurium cut flowers treated with GABA at pre and postharvest stage under chilling temperature, indicated by lower electrolyte leakage and MDA content, can be attributed to enhancing antioxidant system activity and energy status.

During cold storage of horticultural crops, the increased proline content led to increased resistance to CI (Shang et al., 2011; Yang et al., 2011). It has been suggested that proline acts not only as an osmoprotectant but also as a membrane stabilizer contributing to the stabilization and integrity of cellular membranes when horticultural crops stored at low temperature (Sharp et al., 1990; Bohnert and Jensen, 1996). This behavior is in agreement with our results, where GABA treatment at pre and postharvest stage increased the proline content. Thus, the rise in proline content might contribute to the stabilization and integrity of cellular membranes in anthurium cut flowers under cold stress, thereby improving the tolerance of flowers to chilling. Similar results were reported in GABA treated peach and banana fruits (Shang et al., 2011; Wang et al., 2014). GABA treatment (5 mM, 10 min) reduced CI incidence in peach fruits, and endogenous proline contents in fruits were enhanced due to increase of ornithine δ-aminotransferase (OAT) and pyrroline-5-carboxylate synthetase (P5CS) enzymes activities, which are responsible for proline biosynthesis, and decrease of proline dehydrogenase (PDH) enzyme activity, which is responsible for proline degradation (Shang et al., 2011). Recently, Wang et al., (2014) reported that the treatment of banana fruit with GABA (20 mM by vacuum infiltration for 15 min) led to mitigation of CI under storage at 7 °C for 20 days, which was associated lower electrolyte leakage and MDA content. Bananas treated with GABA had higher P5CS activity and lower PDH activity, which led to higher proline accumulation.

Accumulation of total phenols in horticultural crops under low temperature may be due to an acclimation mechanism to overcome chilling stress (Rivero et al., 2001). Phenols can delay the oxidation of lipids via inhibiting the initiation or propagation of oxidizing chain reactions (Pennycooke et al., 2005). PAL, as one of the principal enzymes involved in the biosynthesis of phenols, can be induced by chilling injury (Lafuente et al., 2004). Phenolics and PAL are thought to be involved in the alleviation of CI during cold storage. But, PPO is believed to be antagonistic to the action of PAL with respect to CI. In many horticultural crops, PPO activity is involved in tissue browning (Tomás-Barberán and Espín, 2001), a CI symptom that reduces horticultural crops marketability. In our study, GABA treatment promoted the increase of PAL activity and displayed higher content of total phenols in anthurium cut flowers during the cold storage. Higher PAL activity, associated with alleviation of CI, has been reported in banana fruit under heat treatment (Chen et al., 2008). Accumulation of total phenols in anthurium cut flowers treated with GABA at pre and postharvest stage was associated with lower PPO enzyme activity. Wang et al. (2014) reported that the treatment of banana fruit with GABA led to mitigation of CI under storage at 7 °C for 20 days, which was associated with enhanced PAL enzyme activity, which led to higher phenols accumulation. Phenols are oxidized by PPO, which led to browning (Rivero et al., 2001; Raimbault et al., 2011). The resultant browning in anthurium cut flowers spathe under chilling temperature is notable CI symptoms that affect its marketability, causing economic losses (Vela et al., 2003). Tomás-Barberán and Espín (2001) reported that PPO is involved in the oxidation of antioxidants, thereby decreasing the antioxidant status of the horticultural crops and ultimately leading to crops being susceptible to CI during cold storage. Siboza et al. (2014) reported that low temperature storage of lemon was associated with increasing lipid peroxidation along with enhancing POD and PPO enzymes activity, which led to decreasing membrane integrity and development of CI symptoms. Also, Siboza et al. (2014) reported that the 10 μM methyl jasmonate in combination with 2 mM salicylic acid treatment ameliorated CI of lemon fruit, which were associated with higher PAL enzyme activity and total phenols accumulation along with lower POD and PPO enzymes activity. The present study suggested that GABA treatment got involved in promoting the total phenols accumulation in anthurium cut flowers, which results from higher PAL/PPO enzymes activities. The accumulation of phenols can lead to an enhanced antioxidant capacity, which may contribute to the chilling tolerance in anthurium cut flowers.

Anthurium cut flowers spathe browning is associated with chilling injury. CI as an oxidative stress is associated with accumulation of ROS, which led to membrane lipid peroxidation, disrupt cellular membrane structure and cause the loss of cellular compartmentalization, resulting in PPO contacting phenolic substrates and oxidizing phenolics to form brown polymers (Siboza et al., 2014), which, in turn, might reduce the storage quality and marketability of anthurium cut flowers. Alleviation of anthurium cut flowers spathe browning treated with GABA at pre and postharvest stage can be due to enhancing of DPPH scavenging

activity of flowers, which led to diminishing of membrane lipid peroxidation. Increased DPPH scavenging activity was positively correlated with chilling tolerance in cucumber (Kang and Saltveit, 2002). The present study suggested that GABA treatment stimulate PAL activity, as well as the total phenols accumulation. The accumulation of phenols can lead to an enhanced antioxidant capacity, which may contribute to the chilling tolerance in anthurium cut flowers. Wang et al. (2014) reported that the GABA treated banana fruits showed significantly higher antioxidant capacity assayed by DPPH and FRAP, which may be due to higher total phenols accumulation. Also, except of indirect effect on enhancing the antioxidant capacity, GABA itself have ROS scavenging activity and enhanced antioxidant capacity of banana fruit under GABA treatment can be due to endogenous GABA accumulation (Wang et al., 2014).

5. Conclusion

Electrolyte leakage and MDA increase were retarded by exogenous GABA treatment. GABA treatment increased the accumulation of proline. Higher content of total phenols was also observed in the GABA-treated anthurium cut flowers which accompanied by an increase in PAL activity associated with decreasing PPO enzyme activity. DPPH radical scavenging activity was significantly promoted in GABA treated anthurium cut flowers during the storage. CI mitigation of anthurium cut flowers under exogenous GABA treatment could be attributed to enhancing total antioxidant capacity, enhancing accumulation of proline as powerful osmolyte and enhancing PAL enzyme activity associated with decreasing PPO enzyme activity which led to accumulation total phenols, all of them resulting in an increase of membrane integrity by reducing membrane lipid peroxidation, and, ultimately diminishing ROS accumulation, it has a positive impact on CI tolerance.

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