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Postharvest GABA Application Effects on Some Biochemical Characteristics of *Anthurium* Cut Flowers under Cold Storage Conditions

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Abstract

Chilling damage to anthurium cut flowers exposed to the temperature of under 15°C impairs their vase life, quality and marketability. In the present experiment, the effect of GABA postharvest treatment (0, 1 and 5 mM) was studied on vase life, total soluble solid (TSS), H_2O_2 content, proline content, spathe browning, catalase, and superoxide dismutase activity of *Anthurium andraeanum* 'Sirion' during 10 days of storage at 10ºC and 5ºC. The results revealed that 1 mM GABA treatment at 10ºC was the treatment of choice with respect to vase life. GABA treatment had no special effect on reducing the browning incidence. GABA at 5 mM increased SOD activity. Furthermore, GABA reduced H_2O_2 levels compared to untreated control flowers. Moreover, under chilling stress, proline was the highest in GABA-treated flowers. Flowers treated with 1 mM GABA held higher catalase activity. The highest TSS belonged to 5 mM GABA under 5ºC.

Keywords: Chilling injury, Proline content, Spathe browning tropical flowers, γ–Amino butyric acid.

INTRODUCTION

Chilling stress causes major economic losses on tropical and semi-tropical crops (Luengwilai *et al*., 2012). Most of these crops are damaged by temperatures above freezing temperature and under 15ºC. The main damages that are caused by chilling during prolonged storage at 10 ºC are metabolic disorders (Lukatkin *et al*., 2012). The primary damages and disorders seem to be associated with the deterioration of membrane's structure while the metabolic changes occur as a result of unbalanced energy drainage during transpiration under cold storage conditions. However, the dominant change is the unavoidable conversion in cell membrane's dynamics and stability. During cold storage periods, the activity of membrane-anchored enzymes greatly declines; the major enzyme that is affected is ATP-ase, which is the proton transfer enzyme. The consequence of this membrane change is its phase transition from the flexible liquid crystalline into solid jelly state. This phase change becomes obvious by the appearance of those jelly parts on the bilayer phospholipids, which gets prominent during long lasting chilling periods. Meanwhile, the visual symptoms of the damage can be detected on the plant's tissues (Lukatkin *et al*., 2012).

GABA is an important amino-acid comprised of four carbons holding many functions in plants, e.g. the control of pH, N metabolism and response to several stresses like anaerobic conditions, light scarcity, drought, chilling stress, high temperature, mechanical damages, tolerance to pests and diseases, as well as the promotion of the overall plant's growth and development. The internal amount of GABA in plants is about 0.03 to 32.5 μM g^{-1} of its fresh weight (Kathiresan *et al*., 1997).

The evidence disclosed that GABA prevents the peroxidation of membrane lipids and the synthesis of saturated fatty acids induced by chilling stress. Lipid peroxidation by cold stress is due to the existence of ROS molecules and the over-activity of some other enzymes like lipoxygenase (Deng *et al*., 2010). Another research found evidence of chilling stress alleviation by the help of natural compounds such as GABA on peach fruits (Yang *et al*., 2011). There is another study reporting the effect of GABA treatment on spathe browning and up-surging vase life of anthurium (Soleimani Aghdam *et al*., 2016).

The aim of the present experiment was to study the effects of GABA treatment on some physiological traits of anthurium 'Sirion' cut flowers stored at two temperatures.

MATERIALS AND METHODS

Anthurium 'Sirion' cut flowers were supplied by the Kebriaee commercial greenhouse in Varamin, Iran. The flowers were harvested early morning and were homogeneous regarding flower openness and visual appearance (40-50 percent of the real flowers were fully opened on the spadix). The flowers were packed carefully and were transferred to a laboratory in vials containing water. The flower stalks were homogeneously cut to a height of 30 cm. GABA treatments (0, 1 and 5 mM) were sprayed on the flower spathes. GABA (γ–aminobutyric acid, $C_4H_9NO_2$) was obtained from Sigma Company. The treated cut flowers were placed in special vases and were stored at 5 and 10ºC with 12-h photoperiod regime for 10 days with a relative humidity of 85 to 90 percent. Thereafter, they were taken out of the cold storage and were kept individually in glass vials containing 20 ml of distilled water. To avoid water loss by transpiration, the vials were capped by parafilm. All traits were measured at four times after cold storage, starting on the first day after storage time and repeated in the 1st, 2nd and 3rd weeks after it. But, spathe browning as a chilling symptom was measured daily, and so was vase life which was delineated when the flowers lost their visual quality down to 50%.

Vase life

The time starting from the harvest to the flower shedding and spadix drying is considered

as the vase life. At the end of this period, spadix losses its shiny state and gets covered by black spots (Promyou *et al*., 2012). Thus, in the present test, vase life was supposed to be ended when the spathes of the samples lost half of their visual quality.

TSS

Total soluble solids were evaluated after standard curve calibration at 625 nm and were reported as mg/g FW according to Irigoyen *et al*. (1992).

H_2O_2

H_2O_2 was assayed based on a method suggested by Alexieva *et al.* (2001) as μM^{-1} FW.

Proline

Proline content was quantified by the method of Irigoyen *et al.* (1992) and the result was expressed as µg/ g FW.

Chilling injury (Spathe browning)

The disorder regarding low temperature and senescence is visualized by brown spots on flower spathes (Promyou *et al*., 2012). Chilling injury was assayed on a daily basis and was rated on a five-point scale according to the level of brown spots spread on the spathe in which 1 means 'no chilling damage', 2 means 'low damage declaring 10 to 20 percent of spathe affection', 3 refers to 'medium damage graded from 21 to 50 percent of spathe affection', 4 shows '51 to 80 percent browning', and 5 shows 'intensive damage from 81 to 100 percent of spathe browning' (Fig. 1). Chilling injury was calculated by the following equation.

Fig. 1. Spathe browning pattern (on a scale of 1-5) in anthurium cut flowers exposed to chilling injury. Numbers are showing the damage level as described within the text.

Antioxidants enzymes assay

About 0.2 g of spathe tissue was homogenized by buffer phosphate. The homogenate was centrifuged at 4°C for 18 min at 20,000 g and the supernatants were used to determine enzyme activity. The activity of CAT and SOD were determined by the methods developed by Aebi (1984) and Sen Gupta *et al*. (1993), respectively.

Experimental design and statistical analysis of the data

The study was conducted as a factorial experiment based on a Completely Randomized Design (CRD) with 18 flowers for morphological traits and 72 flowers for biochemical traits during four sampling times with three replications. The data were analyzed by SAS (9.4). Means were compared by Duncan test. The charts were drawn by MS-Excel 2014.

RESULTS AND DISCUSSION

Vase life

The results revealed that the main effects of temperature and GABA were significant on vase life ($P < 0.01$). But, GABA × temperature interactive effect was not significant on vase life (Table 1(. Means comparison showed that the flowers treated with 1 mM GABA demonstrated longer vase life than those treated with 5 mM GABA (Fig. 2). Regarding temperature, the longest vase life belonged to flowers stored at 10ºC and the lowest belonged to those stored at 5ºC (Fig. 3). Wang *et al*. (2014) reported that GABA treatment had a clear ameliorative effect on reducing the chilling stress and improving the storability of banana fruits. The reason was the enhanced proline accumulation plus the activated antioxidant enzymes. In another study, senescence in anthurium flowers was accelerated by chilling mainly due to the over-activity of phospholipase D (PLD) and lipoxygenase (LOX). GABA treatment improved vase life by reducing the pool of PLD and LOX under cold storage conditions so that senescence was delayed significantly (Soleimani Aghdam *et al*., 2016). Soleimani Aghdam (2015), in his study of chilling effects on anthurium during storage at 4ºC for 21 days, reported that pre-harvest GABA treatment at 1 mM and post-harvest treatment at 5 mM significantly prolonged vase life. The same results were obtained in the present study.

Table 1. ANOVA for the effects of different concentration of GABA and storage temperatures on vase life of anthurium cut flowers.

SoV	df	MS
		Vase life (days)
GABA (G)	2	53.38**
Temperature (T)	1	6.05**
G × T	2	0.38ns
Error	12	0.27
CV (%)	-	3.57

** and ns: significant at $P < 0.01$and insignificant, respectively.

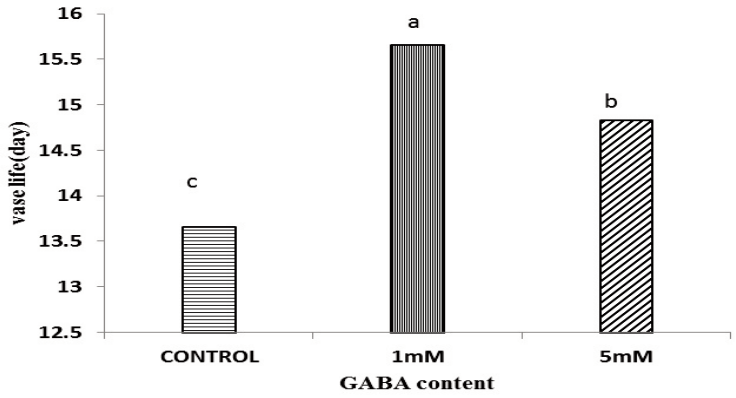

Fig. 2. Means comparison for the effect of GABA treatment on the vase life of anthurium 'Sirion'. Different letter on the column shows significant differences based on Duncan's multiple range test ($P < 0.01$).

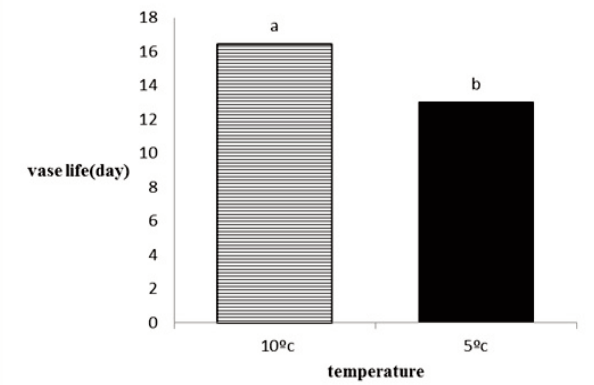

Fig. 3. Means comparison for the effect of temperature on the vase life of anthurium cut flowers 'Sirion.' Different letter on the column shows significant differences based on Duncan's multiple range test ($P < 0.01$).

TSS

Simple, double and triple effects of treatments were significant on TSS (Table 2). Means comparison revealed that the highest TSS belonged to 5 mM GABA during the 1st sampling time at 5ºC. The lowest was recorded in the 4th sampling time at 10ºC for 1 mM GABA treatment (Table 3).

Table 2. ANOVA for the effects of GABA treatment, sampling time, and storage temperatures on TSS, H_2O_2, proline content, and SOD and CAT activities of anthurium cut flowers.

SoV	df	MS				
		H_2O_2	Proline	TSS	SOD	CAT
Time	3	0.51**	0.047**	3416.28**	16.63**	50.21**
Temperature	1	0.43**	0.054**	604.08**	0.42**	27.57**
GABA	2	0.045**	0.047**	1125.10**	1.59**	5.07**
Time × Temperature	3	0.12**	0.017**	109.80**	1.78**	11.76**
Time × GABA	6	0.019**	0.011**	309.52**	1.88**	4.87**
Temperature× GABA	2	0.011**	0.00005^{ns}	27.90**	0.19 **	3.21**
Time × Temperature × GABA	6	0.007**	0.003**	12.84**	0.41**	4.08**
Error	48	0.0001	0.0002	1.05	0.022	0.039
CV (%)	-	5.47	11.87	4.36	9.08	7.75

** and ns: significant at $P < 0.01$and insignificant, respectively.

Table 3. Means comparison for the effect of temperature, GABA, and sampling time on TSS (mg/g FW) of anthurium 'Sirion' cut flowers.

GABA concentration	Temperature (ºC)	1st sampling (Day 1)	2nd sampling (Day 7)	3rd sampling (Day 14)	4th sampling (Day 21)
Control	5	20.32^{j}	28.08^{f}	16.59^{l}	5.82^{o}
	10	19.15^{jk}	17.91^{k}	15.60^{l}	3.80^{p}
1 mM	5	44.16^{c}	35.93^{e}	26.21^{g}	7.11^{n}
	10	40.81^{d}	22.21^{i}	22.47^{i}	4.32^{p}
5 mM	5	59.64^{a}	39.58^{d}	24.90^{gh}	8.47^{m}
	10	47.28^{b}	25.06^{gh}	24.15^{h}	4.53^{p}

The period between each sampling time was 5 days starting the day of taking the flowers out of storage. Different letter(s) in the column shows significant difference based on Duncan's multiple range test ($P < 0.01$).

The accumulation of carbohydrates is a tolerance mechanism to confront chilling stress in flowers. Indeed, the increased carbohydrate levels function as osmolytes which improve the chilling tolerance in plant tissues by reducing osmotic potential and taking down freezing point (Ershadi and Taheri, 2013). Data presented in Table 3 show that the highest sugar accumulation occurred at the early experiment periods with the flowers treated by GABA under chilling temperatures compared to control plants. The low levels of carbohydrates under ambient temperature compared to chilled ones are seemingly associated with carbohydrate-starch equilibrium. Under non-stressed conditions, the dominance is with starch although, under stressful conditions, sugar conversion to the starch is reduced. Thus, soluble monosaccharides are accumulated within tissues (Sami e*t al*., 2016). The current study reports the effects of GABA on the accumulation of carbohydrates and the simultaneous chilling tolerance as was reported by Ershadi and Taheri (2013) too.

H_2O_2

Variance analysis for H_2O_2 and some other forthcoming traits are outlined in Table 2. According to the data presented in Table 4, the highest H_2O_2 belonged to the untreated plants stored at 5ºC in the 4th sampling time. Moreover, the lowest amount of H_2O_2 was recorded for the plants treated with 1mM GABA and stored at 10ºC in the 2nd sampling time. The control flowers at 5 and 10ºC in the 1st sampling time and the flowers treated with 5 mM GABA at 5 and 10ºC in the 1st sampling time as well as the 5 mM GABA-treated flowers stored at 10ºC for the 2nd sampling time had the same prohibition level. H_2O_2 is a pivotal signaling molecule from ROS groups playing an important role in stressful conditions. H_2O_2 induces tolerance in plants by improved lignification of cell walls and the cross linking structural membrane-anchored proteins as well as increasing the synthesis of phytoalexins. H_2O_2 over-accumulation in plant cells triggers oxidative stress, causes strong cell damage, and threatens cell viability. Normally, the half-life of H_2O_2 is about 1 ms; however, the half-life of other ROS radicals, namely O_2^- and OH^-, is much shorter than 2-4 µs. H_2O_2 molecule being considered as a secondary messenger plays pivotal roles in signaling pathways. It is due to the prolonged half-life of the molecule and its high permeability across cell membranes. H_2O_2 holds two contrasting roles; at low concentration, it is an important signaling molecule while, at high concentration, the scavenging of this molecule is disrupted under stressful environment, and the programmed cell death occurs (Neill *et al*., 2002). In the present experiment, the H_2O_2 accumulation rate was higher at lower temperatures than at ambient temperature. Furthermore, H_2O_2 was more accumulated in the last sampling times (3rd and 4th). The trend for H_2O_2 variation in the present experiment was the same as the results of Cao *et al*. (2009) in their study on loquat treated with methyl jasmonate.

Table 4. Means comparison for the effect of temperature, GABA and sampling time on H_2O_2 (µmol/g FW **)** of anthurium 'Sirion' cut flowers.

GABA concentration	Temperature (ºC)	1st sampling (Day 1)	2nd sampling (Day 7)	3rd sampling (Day 14)	4th sampling (Day 21)
Control	5	0.09^{k}	0.13^{j}	0.53^{c}	0.75^{a}
	10	0.09^{k}	0.13^{j}	0.18^{h}	0.36^{e}
1 mM	5	0.12^{j}	0.12^{j}	0.39^{d}	0.54^{c}
	10	0.08^{k}	0.07^{k}	0.15^{hi}	0.17^{hi}
5 mM	5	0.09^{k}	0.12^{j}	0.22^{g}	0.61^{b}
	10	0.08^{k}	0.08^{k}	0.16^{hi}	0.29^{f}

The period between each sampling time is 5 days starting the day of taking the flowers out of storage. Different letter(s) in the column shows a significant difference based on Duncan's multiple range test ($P < 0.01$).

Chilling injury (Spathe browning)

ANOVA for chilling injury of the treated cut flowers is manifested in Table 5. Means comparison revealed that at GABA × temperature interaction, 5ºC treatment with all GABA levels showed the highest chilling injury. The lowest browning incidence at all GABA levels belonged to 10 ºC (the same significance) (Fig. 4).

Table 5. ANOVA for the effects of GABA, sampling time, and storage temperatures on chilling injury of anthurium cut flowers.

SoV	df	MS
		Chilling injury
Time	19	1439.42**
GABA	2	2873.17**
Temperature	1	14930.23**
Time × GABA	38	3.97ns
Time × Temperature	19	162.32**
GABA × Temperature	2	2651.23**
Time × GABA× Temperature	38	4.89 ns
Error	240	52.40
CV (%)	_	10.92

*, **, and ns: significant at P < 0.05, P < 0.01and insignificant, respectively.

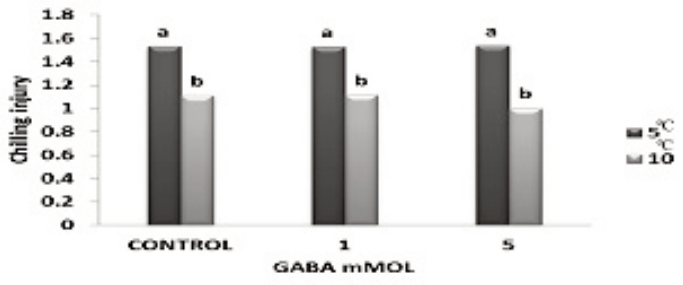

Fig. 4. Means comparison for the effect of temperature × GABA treatment on chilling injury of anthurium 'Sirion' cut flowers. Different letters on the bars show significant difference based on Duncan's multiple range test (P <0.01).

With respect to time × temperature interaction, the 5ºC treatment from Day 5 to Day 20 and the 10ºC treatment from Day 14 to Day 20 were related to the highest browning rate together. Furthermore, at 10ºC, from Day 1 to Day 5, the browning of spathe was the least (Table 6). It seems that temperature was more efficient in controlling the browning than GABA treatment. Adaxial anthurium spathe surface is covered with a thick cuticle with no stomata. However, the abaxial face contains 6 to 7 stomata per mm^2 (Criley and Paull, 1993). During the chilling times, stomata will remain open allowing greater water loss and the occurrence of brown spots (Criley and Paull, 1993).

Moreover, during chilling periods, free phenolic content is reduced mainly due to the fact that they are the initial substances for the biosynthesis of polyphenolics responsible for the browning behavior. The polyphenol oxidase (PPO) activity is the predominant factor related to the browning phenomenon. If chilling injury influences the membrane of an organelle like vacuole, its phenolic compounds will encounter PPO enzyme (Nguyen *et al*., 2003). In response to chilling,

Table 6. Means comparison for the effect of temperature × sampling time treatment on the chilling injury of anthurium 'Sirion' cut flowers.

Day	5 ºC	Means comparison	10 ºC	Means comparison
1	0.7	h	0.33	j
2	0.7	h	0.33	j
3	1.o7	e	0.33	j
4	1.4	cd	0.36	j
5	1.67	a	0.36	j
6	1.67	a	0.51	i
7	1.67	a	0.55	i
8	1.67	a	0.77	gh
9	1.67	a	0.88	fg
10	1.67	a	0.96	ef
11	1.67	a	1.29	d
12	1.67	a	1.48	bc
13	1.67	a	1.59	ab
14	1.67	a	1.63	a
15	1.67	a	1.67	a
16	1.67	a	1.67	a
17	1.67	a	1.67	a
18	1.67	a	1.67	a
19	1.67	a	1.67	a
20	1.67	a	1.67	a

Chilling injury was calculated based on the following equation using Fig. 1 photographical explanations. Equation: The intensity of chilling damage * the number of flowers affected / total number of flowers in a group. Different letter(s) in the column shows significant difference based on Duncan's multiple range test ($P < 0.01$).

cells will save phenolic compounds within their walls which will later have easier reactions with PPO in the apoplastic environment (Nguyen *et al*., 2003). PAL (phenylalanine ammonia lyase) is another marker enzyme exposing damage, especially under stressful conditions. It diverts phenylalanine into mono-phenol and di-phenols which are the substrates of PPO enzyme. The activity of PAL rises in the course of chilling stress (Nguyen *et al*., 2003). Overall, although the incidence of chilling damage is reversely related to free phenolic compound levels, it directly depends on the PPO and PAL availability and activity (Nguyen *et al*., 2003). In the study of Soleimani Aghdam *et al*. (2015), the anthuriums that were treated with 1 or 5 mM GABA before and after harvest had more PAL and less PPO during storage at 4ºC. Moreover, the flowers under 1 mM pre-harvest treatment had higher total phenolic content rather than control samples. The results from the present experiment were different from theirs. Seemingly, the discrepancy observed was due to the missing of pre-harvest GABA treatment and possibly its effect on the endogenous GABA production and other related antioxidant regents, which have a crucial role in combating the delaying of senescence phenomena in flowers.

Proline contents

Mean comparisons revealed that the highest proline content was recorded in the flowers treated with 1 and 5 mM GABA at 5ºC in the 4th sampling time. The least data for proline content belonged to the control plants at 5 and 10ºC, 5 mM GABA at 10ºC for the 1st sampling and controls at 10ºC in the 2nd sampling time (Table 7).

Proline is a proteinous amino acid which maintains osmotic pressure and stabilizes protein structure and membrane integrity, especially under stressful conditions. Furthermore, proline plays

Table 7. Means comparison for the effect of temperature, sampling time treatment, and GABA on proline content (µg/ g FW) of anthurium 'Sirion' cut flowers.

GABA concentration	Temperature (°C)	1st sampling (Day 1)	2nd sampling (Day 7)	3rd sampling (Day 14)	4th sampling (Day 21)
Control	5	0.03^{k}	0.06$^{i\,j}$	0.14^{ef}	0.24^{b}
	10	0.04^{jk}	0.05^{ijk}	0.10^{gh}	0.07^{hi}
1 mM	5	0.12^{fg}	0.23^{b}	0.2^{c}	0.28^{a}
	10	0.11^{fg}	0.24^{b}	0.1^{gh}	0.16^{de}
5 mM	5	0.15^{de}	0.07^{hi}	0.14^{ef}	0.3^{a}
	10	0.05^{ijk}	0.08^{hi}	0.13^{ef}	0.17^{cd}

The period between each sampling time is 5 days starting the day of taking the flowers out of storage. Different letters in the column are showing significant difference based on Duncan's multiple range test ($P < 0.01$).

a pivotal role in acclimation for the chilling injury conditions (Ghorbani *et al*., 2009). Proline prevents the denaturation of proteins and integrates with phospholipids' bilayer to protect soluble proteins. Moreover, proline has a role in scavenging hydroxyl radicals to help maintain the energy levels and nitrogenous source. Proline mediates tolerance against unstable environments by controlling the osmotic pressure (Claussen, 2005). The compound carries out the action by preventing the cells from dehydration while simultaneously helping the constancy of osmotic potential. Previous studies have indicated that since GABA and proline have the same initial substrate, so proline receptors are able to identify GABA as a functional substrate.

GABA levels increase under glutamine-deficient environments or protein biosynthesis reduction or protein compounds' breakdown rate acceleration. Altogether, GABA may act as an intermittent nitrogen source, a biological sensor for nitrogen amounts and a C/N balancer or as a signaling molecule between organelles. Since the activity of glutamate decarboxylase is pH-dependent, GABA could be considered as an index of cell pH evaluation. There is evidence that GABA biosynthesis like proline may be up to 16 times higher than normal conditions (Mazzucotelli *et al*., 2006). In addition, at lower temperatures, Ca^{2+} availability increases inside the cells due to membrane breakage and the disturbances in ionic pumps anchored in cell membranes, so the described phenomena have enough signals to stimulate the biosynthesis of proline and GABA. Proline amounts add up during environmental stressful conditions like chilling. However, proline accumulation in chilling-sensitive plants is not ever enough to devote them to chilling tolerability. Meanwhile, proline accumulation prevents the great pH descending in cell medium (Afshari and Parvane, 2013). Proline biosynthesis is in equilibrium with the activity of proline 5-carboxylate synthetase (P5CS) and the proline dehydrogenase (PDH), which breaks down the molecule. Chilling temperatures are able to decline or stop the activity of PDH, thereby promoting proline biosynthesis and accumulation. Wang *et al*. (2014) reported that the high activity of P5CS in banana peel treated with GABA under chilling temperature was higher than control. Furthermore, the activity of PDH was an indication of proline accumulation in GABA-treated fruits. Means comparison in the present experiment showed that in the GABA-treated flowers under chilling conditions, proline accumulation was higher during the last sampling times. Seemingly, this is related to the onset of senescence in spathes since during normal conditions, proline content is autonomously regulated within cells preventing its over-accumulation. But, under senescing conditions, the cell machinery losses its control on the proline content and the compound over-accumulates inside the cell. In another study, conducted on the anthurium cut flowers treated with 1 and 5 mM of GABA during pre- and post-harvest times under chilling stress, proline accumulation was higher than control treatments (Soleimani Aghdam, 2015). Our results are in agreement with the findings of those researchers.

SOD activity

Means comparison revealed that the highest SOD activity belonged to the flowers treated with 5 mM GABA at 5ºC in the 1st sampling time. It is noteworthy that the lowest activity of this enzyme was recorded in the control flowers treated at 10ºC in the 4th sampling time (Table 8).

The idea is that ROS scavengers like SOD, CAT, APX, and GR show higher scavenging efficiency by the help of GABA. In addition, they increase antioxidant system capacity and act as the first barriers against ROS molecules converting O_2^- into H_2O_2. Then, H_2O_2 is digested by the activity of CAT and APX. The high amount of catalase, APX, and GR activities in GABA-treated flowers reduce H_2O_2 levels and increase antioxidant enzymes activity (Soleimani Aghdam, 2015). Cao *et al*. (2009) reported that SOD activity was increased in control and MeJA-treated loquat fruits with regard to the time lapse. Higher SOD activity enables tissues to scavenge O_2^- more efficiently. Overall, the high unsaturated to saturated fatty acids ratio, the high energy level, and the low LOX and PLD enzymes' activity, as well as the systematic antioxidant enzymes such as SOD, CAT, GPX, GST, APX, DHAR and MDHAR activities, help to minimize cell membrane damage and to prevent ROS accumulation which will eventually upgrade chilling tolerance (Cao *et al*., 2009). Means comparison showed that SOD activity in the early times of experiment was higher than that in the last times. Owing to the results obtained, the SOD activity patterns are in line with the findings of Cao *et al*. (2009) for loquat fruits treated with MeJA, Yang *et al*. (2011) for chilling tolerance improvement of peach fruits treated with GABA, and Soleimani Aghdam and Bodbodak (2014) for heat treatment effects on reducing the chilling damage on loquat, cucumber, pomegranate, and mandarin.

Table 8. Means comparison for the effect of temperature, sampling time treatment, and GABA on SOD activity (U/mg protein) of anthurium 'Sirion' cut flowers.

GABA concentration	Temperature (ºC)	1st sampling (Day 1)	2nd sampling (Day 7)	3rd sampling (Day 14)	4th sampling (Day 21)
Control	5	2.23^{e}	1.26^{f}	1.21^{f}	0.77^{g}
	10	2.74^{bc}	0.76^{g}	0.56^{ghi}	0.41^{i}
1 mM	5	2.60^{cd}	2.23^{e}	0.54^{ghi}	0.46^{hi}
	10	2.84^{bc}	2.14^{e}	1.38^{f}	0.77^{g}
5 mM	5	3.14^{a}	2.89^{b}	0.67^{gh}	0.63^{ghi}
	10	2.39^{de}	2.24^{e}	1.17^{f}	0.57^{ghi}

The period between each sampling time is 5 days starting from the day of taking the flowers out of storage. Different letter(s) in the column shows significant difference based on Duncan's multiple range test ($P < 0.01$).

Catalase activity

Means comparison indicated that the flowers treated with 1 mM GABA at 10ºC in the 2nd sampling time had the highest catalase activity. Furthermore, the lowest CAT activity belonged to the control flowers at 5ºC in the 3rd and 4th sampling times and to the flowers treated with 1 and 5 mM at 5ºC in the 4th sampling time (at the same probability level of $P < 0.01$) (Table 9).

The period between each sampling time is 5 days starting from the day of taking the flowers out of storage. Different letter(s) in the column shows significant difference based on Duncan's multiple range test ($P < 0.01$).

Oxidative stress induced by H_2O_2, O_2^-, and hydroxyl radicals causes the development of chilling stress. Antioxidant enzymes, such as SOD, CAT, and APX, play a dominant role in scavenging free toxic radicals and alleviating chilling stress. Protection against oxidative stress guaranties the cell viability under stressful environments and seemingly is the most important mechanism in combating chilling damages. There exist several reports that chilling tolerance im-

Table 9. Means comparison for the effect of temperature, sampling time treatment, and GABA on CAT activity (µmol H_2O_2/ min/g FW) of anthurium 'Sirion' cut flowers.

GABA concentration	Temperature (ºC)	1st sampling (Day 1)	2nd sampling (Day 7)	3rd sampling (Day 14)	4th sampling (Day 21)
Control	5	3.59^{d}	2.16^{g}	0.89^{lmno}	0.61^{o}
	10	4.31^{c}	2.27^{fg}	1.37^{hijk}	1.11^{jklm}
1 mM	5	3.17^{e}	2.31^{fg}	1.04^{klmn}	0.80^{mno}
	10	3.31^{de}	8.59^{a}	1.63^{h}	1.39^{hij}
5 mM	5	4.26^{c}	2.5^{f}	1.30^{hijk}	0.74^{no}
	10	4.49^{c}	7.09^{b}	1.48^{hi}	1.18^{ijkl}

The period between each sampling time is 5 days starting from the day of taking the flowers out of storage. Different letter(s) in the column shows significant difference based on Duncan's multiple range test ($P < 0.01$).

provement in horticultural products during post-harvest storage depends on the elevation of antioxidant enzyme activity (Cao *et al*., 2009). The increased SOD and CAT activity along with reduced LOX activity ends with a great reduction in H_2O_2 and O_2^- levels that will reduce the oxidative damage. According to the results, the flowers treated with GABA at chilling temperatures had higher CAT activity compared to the control plants. This is in accordance with the findings of Yang *et al*. (2011) for peach fruits treated with GABA. Furthermore, CAT activity was more intense at the early sampling times than at the last stages. Moreover, CAT activity was higher in the GABA-treated flowers whereas the activity of this enzyme was greatly higher at 10ºC than at 5ºC. The pattern of CAT activity in the present experiment is in accordance with the studies of Promyou *et al*. (2012) on anthurium cut flowers treated with salicylic acid and with the study of Cui *et al*. (2013) on tobacco plants under stressful conditions.

CONCLUSIONS

All in all, GABA treatment ameliorated chilling damages in low temperature treatments while its efficiency was not justified at 5ºC with 1 mM or 5 mM treatments. For the vase life, 1 mM GABA at 10ºC had a more promising effect than 5 mM treatment. TSS values were also influenced by the GABA treatment and the plants receiving GABA had more chilling tolerance as well. Seemingly, soluble solids accumulation was the criterion for improving chilling damage escape. The high amounts of H_2O_2 traced with the untreated plants showed the positive effects of GABA treatment on preventing ROS agglomeration and chilling tolerance induction. Using 5 mM GABA at 10°C hugely reduced the browning appearance in anthurium cut flowers. However, the temperature was more effective in browning incidence than the GABA treatment. Proline content and the activity of CAT and SOD enzymes in the GABA-treated flowers were higher than in control under chilling conditions.

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