Effect of exogenous Gama-aminobutyric acid on physiological tolerance of wheat seedlings exposed to chilling stress

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Abstract

Accumulation of γ -aminobutyric acid (GABA) is associated with stress factors in plant systems. The objective of the current study was to compare GABA concentration in wheat plants under chilling stress. After 48 h treatments of seedlings under chilling stress combined stresses with and without GABA, morphological and biochemical assays were conducted. It was observed that the inhibition of seedling roots elongation caused by chilling stress was significantly mitigated by GABA. The activities of antioxidant enzymes were changed; the content of malondialdehyde was increased in chilling stress but reduced in GABA treated seedlings. GABA can alleviate oxidative damage caused by chilling stress in wheat seedlings by activating antioxidant defense responses.

Keywords: γ -aminobutyric acid; wheat; chilling stress

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Introduction

γ -aminobutyric acid (GABA) is a ubiquitous non-protein amino acid that exists widely in prokaryotes, animals and plants, and is a neurotransmitter in the cerebrospinal fluid of mammals (Deewatthanawong, 2010). Metabolism of GABA takes place in two cellular compartments; GABA synthesis occurs in the cytosol whereas GABA is degraded in the mitochondrion. 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 (Renault et al., 2011). In plants, GABA has been mostly investigated as a metabolite and is thought to function in anaplerotic alimentation of the tricarboxylic acid (TCA) cycle and C/N balance control (Fait et al., 2008). In contrast, the role of GABA in plant development, particularly vegetative development, has received little attention, even though several studies have reported on its rapid accumulation in response to environmental cues (malekzadeh et al., 2012). As a result, the consequences of GABA accumulation in development remain unclarified (Renault et al., 2011).

GABA is regarded as an endogenous signal molecule that plays an important role in regulating the stress response, plant growth and development (Song et al., 2010). For example, exogenous GABA could alleviate oxidative damage caused by aluminum, and proton

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stresses in barley seedlings (Song, Xu et al., 2010). Shi et al. (2010) reported that GABA participated in regulating the expression of genes involved in H_2O_2 and ethylene production in *Caragana intermedia* roots under salt stress. Eexogenous GABA alleviated Chilling injury (CI) in cold-stored peach fruit (Shang et al., 2011). However, the mode of action of GABA in reducing CI has not been clearly elucidated.

Chilling injury (CI) is a physiological disorder that limits the storage of chilling-sensitive peach fruit at low, but non-freezing, temperatures (Deewatthanawong et al., 2010). CI symptoms in peach fruit Include flesh browning, flesh mealiness or woolliness, failure to ripen normally, increased susceptibility to decay, and accelerated senescence (Nilo et al., 2010; Zheng, 2011). Great efforts have been made to find treatments to control CI in postharvest peach fruit (Cao et al., 2010; Jin et al., 2009; Song et al., 2010).

Chilling can lead to increased concentrations of toxic oxygen compounds in susceptible tissues (Malekzadeh et al., 2012). A number of enzymes participate in protecting plants from oxidative damage (Deewatthanawong et al., 2010). Members of the enzymatic antioxidant defense system include superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.11), phenolic peroxidases such as guaiacol peroxidase (GPX; EC 1.11.1.7), and the ascorbate/ glutathione cycle that includes glutathione reductase (GR; EC 1.6.4.2). The superoxide radicle (O^{-2}) is dismutated to H_2O_2 by SOD, and CAT, APX and GPX metabolize H_2O_2 to H_2O. APX requires reduced ascorbate and GPX requires a phenolic compound like guaiacol to function. GR functions in the regeneration of reduced ascorbate after it is converted to monodehydroascorbate by APX.

One of the primary plant responses for adaptation to water deficit stress is the accumulation of solutes such as amino acids (e.g. proline), quaternary ammonium compounds (e.g. glycinebetaine), polyols, and sugars (e.g. mannitol, trehalose, sucrose, and fructans) that act as osmoprotectants (Deewatthanawong et al., 2010). Fructans, a class of water-soluble fructose polymers based on sucrose, accumulate in many bacterial and plant species, in which they serve as an important storage carbohydrate (Fait et al., 2008) and are implicated in protecting plants against water deficit caused by low matric potential, salinity, or low temperatures (Fait et al., 2008).

Immunodetection of carbonylated proteins is a good indicator of protein damage due to oxidative stress and has been widely used in studies on human diseases such as Alzheimer's disease, chronic lung disease, chronic renal failure, diabetes and sepsis (Young et al., 2011). As an effective strategy for oxidative damage analysis, the identification of carbonylated proteins could act as a diagnostic biomarker and yield basic information to aid the establishment of efficacious antioxidant therapy (Deewatthanawong et al., 2010). It may also be a potential method for studying the effects of GABA on chilling-generated oxidative damage in crop plants.

Therefore this study was undertaken to determine the antioxidant defense response of wheat seedlings induced by GABA, to investigate whether the signal molecule is functional in alleviating the chilling-generated oxidative damage and to elucidate the underlying mechanism by which GABA inhibits the damage caused by chilling in wheat.

Materials and Methods

Plant material and growth conditions

The chilling-sensitive wheat (*Triticum aestivum* L.) cultivar Chamran developed in Iran was used in this study. Seeds were disinfested in 1% (active ingredient) sodium hypochlorite solution for 10 min to eliminate possible seed-borne microorganisms, rinsed for 1 min under running water then were dried for 30 min at room temperature.

For germination, seeds were soaked in distilled water for 2 h and then placed in a Petri dish with moist filter paper and kept in the dark for 24 h at 22–24 °C. Germinated seeds were transferred onto a mesh tray floating on a continuously aerated solution (pH 5.0, 2 L). The seedlings were kept in the dark at 22–24 °C for 24 h and then moved to a growth chamber at 24 ± 2 °C with a 12/12 h light/dark photoperiod. The

Table 1
Treatments applied to 3-day-old wheat seedlings

No	Chilling Stress	GABA (µM L^{-1})
control	0	0 (control)
0	1	0
1	1	100
2	1	250
3	1	500
4	1	750

solution applied to the seedlings was replaced daily. Three-day old seedlings were exposed to six treatments (Table 1) for different concentrations of GABA. The roots were sampled for subsequent determinations. Each treatment contained three replicates of 15 seedlings and the entire experiment was repeated twice.

Three days after spray application of GABA, all plants were subjected to chilling stress at 2±0.5 °C for 48 h under the same light regime as mentioned above. All plants were watered 2 h prior to and after the chilling stress to determine the extent of chilling injury.

Estimation of root and shoot elongation

Root and shoot elongation was estimated with 15 seedlings by measuring the length of the longest root and shoot with a ruler.

Assay of antioxidant enzyme activities and malondialdehyde content

Following the treatments listed in Table (1), 1 g samples of wheat seedling roots were collected and homogenized in 5 mL of ice cold extraction buffer and 0.1 g of polyvinyl poly pyrrolidone. For the analysis of catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) activities and malondialdehyde (MDA) content, 50 mM L^{-1} sodium phosphate (pH 7.0) was used as extraction buffer. The homogenates were centrifuged at 10000 rpm for 30 min and the supernatants were used directly for the assays. For enzyme assays, three repetitions of each treatment were used and the experiment was conducted twice.

CAT activity was determined by adding 0.2 mL of enzyme extract to 2.8 mL of 40 mM L^{-1} H_2O_2 dissolved in 50 mM L^{-1} sodium phosphate buffer (pH 7.0) as substrate. The decomposition of H_2O_2 was measured by recording the decline in absorbance at 240 nm. One unit (1 U) of CAT activity was defined as the amount of enzyme that converted 1 µM L^{-1} H_2O_2 min^{-1}. Specific activity was expressed as U mg^{-1} protein.

APX activity was assayed following the oxidation of ascorbate to dehydroascorbate at 290 nm by the modified method of Asada (1999). The assay mixture consisted of 50 mM sodium phosphate buffer pH 7.0 containing 1 mM EDTA, 1 mM sodium ascorbate, 10 mM H_2O_2 and enzyme extract. Addition of H_2O_2 started the reaction. Rates were corrected for the non-enzymatic oxidation of ascorbate by the inclusion of reaction mixture without enzyme extract. The activity was expressed in U/mg protein.

To determine the activity of SOD at 560 nm, the reaction mixture was made up of 130 mM methionine, 50 mM phosphate buffer (pH 7.8), 20 µM riboflavin, 75 µM nitro blue tetrazolium chloride and 15 µL of enzyme extract (Hwang et al., 1999). One unit of SOD activity was defined as the amount of enzyme that would inhibit 50% photoreduction of nitro blue tetrazolium chloride.

Statistical analysis

All data were subjected to one-way analysis of variance. Mean separations were performed using Duncan's multiple range test. Differences at $P \leq 0.05$ were considered significant.

Results

Visual damage symptoms

GABA, applied within the range 100-750 µM L^{-1}, was effective in reducing visual injury symptoms of wheat seedlings subjected to chilling stress.

Under cold stress in concentrations of 100, 250, 500 and 750 µM L^{-1} GABA stem and root length decreased in comparison with the control. We also observed that chilling stress led to chlorosis and necrosis in leaves.

With applying different GABA concentrations, we observed that in 250 and 500

Table 2
The mean squares and levels of significance of an analysis of variance for the selected wheat seedling in response to chilling stress in different GABA Treatments.

Source of Variation	df	MDA	CAT	APX	SOD	Root L	Shoot L
Treatment	5	3.510*	2.680*	1.032	92.933*	12.659*	9.926*
Error	12	.820	.281	.372	18.778	.691	1.410

- Show that significantly at p = 0.05

Fig. I. Morphological differences in wheat seedlings after 72 h treatments under chilling stress, combined stresses with and without GABA.

µM concentrations of GABA, the symptom of chilling stress declined, but in 750 µM of GABA the effect of chilling in leaf and stem remarkably reduced.

Table 3
Effect of different concentration of GABA on root and shoot elongation in wheat seedling in response to chilling stress.

	Treatments	Mean ± SE
Root Length	Control	8.96 ± 0.87^{b}
	0	5.63 ± 0.32^{d}
	100	$6.96 \pm 0.45^{c,d}$
	250	9.23 ± 0.92^{b}
	500	11.60 ± 0.60^{a}
	750	$8.03 \pm 1.36^{b,c}$
Shoot length	Control	11.59 ± 0.81^{b}
	0	8.46 ± 1.3^{c}
	100	$9.73 \pm 0.25^{b,c}$
	250	11.36 ± 0.81^{b}
	500	13.83 ± 2.25^{a}
	750	10.90 ± 1.97^{b}

Means at the same time in a column followed by a different letter differ significantly at p = 0.05 by Duncan's multiple range test. Data are accompanied by standard error.

Estimates of root and stem elongation

The results presented in Fig. (I) reveal the growth responses of wheat plants grown under different GABA concentrations in the chilling stress. There was a significant reduction in length and fresh weight of shoot and root of wheat seedlings in the 0 GABA / chilling stress with control but the results show that in the presence of GABA with the concentration of 100, 250,500 and 750 µM the root and shoot length increased significantly. The results indicate that different concentrations of GABA had a significant influence on shoot and root dry weight of wheat plants.

Table 3 shows that, various concentrations of GABA affected root growth of wheat seedlings under chilling stress. Root and shoot length significantly increased and different concentrations of GABA significantly reduced chilling symptoms.

As Table 2 shows, there is a significant difference ($p < 0.05$) between the main characteristics of wheat seedlings in response to chilling stress.

Effect of exogenous GABA treatment on antioxidant and MDA content

MDA is a measure of lipid peroxidation. The MDA value increased significantly ($p<0.05$) after 72 h at 2 $^{\circ}$C treatment in 0 concentration of GABA (Table 5). The MDA content in wheat seedlings during chilling stress and GABA treatment decreased significantly ($p<0.05$) compared to those with chilling stress only but without the GABA treatment (Table 5).

Catalase content was increased in chilling stress compared with control. GABA applied to plants under cold stress significantly ($p<0.05$) increased catalase content. Catalase content decreased with increasing concentration of GABA in the seedlings (Table 4).

APX content was increased in chilling stress compared with control. But application of GABA made no significant difference in APX content (Table 4).

SOD content was increased in chilling stress compared with control (Table 5). SOD activity decreased with increasing concentration of GABA in the seedlings (Table 5).

Table 4
Effect of different concentrations of GABA on CAT and APX activity in wheat seedlings in response to chilling stress

	Treatments	Mean ± SE
CAT activity	Control	2.93 ± 0.28[c]
	0	5.89 ± 1.00[a]
	100	4.44 ± 0.23[b]
	250	4.54 ± 0.27[b]
	500	4.24 ± 0.43[b]
	750	4.24 ± 0.51[b]
APX activity	Control	2.56 ± 0.47[b]
	0	3.40 ± 0.20[a,b]
	100	3.93 ± 0.90[a]
	250	4.09 ± 0.62[a]
	500	4.01 ± 0.44[a]
	750	3.25 ± 0.75[a,b]

Means at the same time in a column followed by a different letter differ significantly at p = 0.05 by Duncan's multiple range test. Data are accompanied by standard error.

Table 5
Effect of different concentrations of GABA on CAT and APX activity in wheat seedlings in response to chilling stress

	Treatments	Mean ± SE
SOD activity	Control	26.02 ± 2.81[b]
	0	38.23 ± 3.76[a]
	100	30.90 ± 3.26[a,b]
	250	35.90 ± 5.62[a]
	500	27.70 ± 4.15[b]
	750	39.00 ± 5.25[a]
MDA content	Control	5.32 ± 1.24[b]
	0	8.35 ± 0.83[a]
	100	7.01 ± 0.77[a,b]
	250	6.23 ± 0.90[b]
	500	5.72 ± 0.73[b]
	750	6.60 ± 0.91[b]

Means at the same time in a column followed by a different letter differ significantly at p = 0.05 by Duncan's multiple range test. Data are accompanied by standard error.

Discussion

GABA is a major amino acid during wheat development (Akihiro et al., 2008; Saito et al., 2008). In this study, we compared the effects of exogenous GABA on wheat seedling development under chilling stress (Table 1).

Chlorosis of leaves is the first visual symptom of stress leading to senescence (Zhang et al., 2007) and is associated with a concomitant decline in concentration of photosynthetic pigments (Zhang et al., 2007). The leaves of control plants after low temperature stress were chlorotic and the photosynthetic pigments chlorophylls markedly decreased.

We showed that when seedlings were exposed to chilling stress, root and shoot length reduced significantly. The study also suggested that GABA significantly increased root and shoot growth and the best growth of plant was observed in 250 and 500 µM L^{-1} concentrations of GABA (Table 3).

As Table 2 shows, various concentrations of GABA significantly affected biochemical and physiological parameters of wheat seedlings.The study revealed that there was not difference between APX activities compared with control.

Chilling conditions may cause an increase in reactive oxygen species (ROS), starting oxidative damage to the membrane system of plants (Zhang et al., 2007, Malekzadeh et al., 2012). The protective mechanisms adapted by plants to scavenge free radicals and peroxides include several antioxidative enzymes such as SOD, CAT and POD. The antioxidative enzymes are important components in preventing the oxidative stress in plants as this is based on the fact that the activity of one or more of these enzymes is generally increased in plants when

exposed to stressful conditions (Malekzadeh et al., 2012).

Tables 4 and 5 indicate that antioxidant enzymes (CAT, APX and SOD) activity in wheat plants significantly increased under chilling stress. Based on these results, the mechanism related to physiological interactions between the GABA concentration and wheat seedlings include increased protein synthesis as well as induction of antioxidant enzymes, to avoid chilling stress.

In this study the higher levels of CAT and SOD observed in GABA-treated wheat seedlings compared with the seedlings under stresses without GABA treatment suggested that GABA treatment induced the activities of antioxidant enzymes in wheat seedling. Therefore, additional studies are needed to explore the behavior of GABA in various plant species and families for plant protection under various abiotic stresses.

Cell membrane stability was affected by lipid peroxidation caused by active oxygen species under various stress conditions (Su et al., 2010), and the concentration of MDA was an indicator of lipid peroxidation in plant cells (Young et al., 2011). The decline of MDA concentrations in different concentration of GABA under low temperature stress in wheat seedlings suggested that seedlings reduced lipid peroxidation. MDA was produced when polyunsaturated fatty acids in the membrane underwent peroxidation (Malekzadeh et al., 2012). Our observations are consistent with these earlier reports.

In conclusion, application of exogenous GABA reduced the protein and lipid damage caused by chilling stress in wheat seedlings, suggesting that GABA is critical for cellular stress response to chilling stress in plants. Considering its important role in coordinating cellular redox homeostasis, further research should be carried out to provide more insights into the mechanism of function of GABA in plant defense response against stress.

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