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Effects of Exogenous GA_3 on Wheat Cold Tolerance

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ABSTRACT

To clarify the underlying physiological mechanism of gibberellic acid (GA) in cold tolerance, the effects of exogenous GA_3 on malondialdehyde (MDA), osmoregulation substances and endogenous hormones levels in the tiller nodes of two wheat cultivars, namely, Dongnongdongmai 1 (a cold insensitive cultivar) and Jimai 22 (a cold sensitive cultivar), were investigated at three periods of cold winter (0, -10, -25°C). The results showed that low concentrations of GA_3 (0.1 and 1 µM) decreased the endogenous GA concentration in both cultivars, but only increased the abscisic acid (ABA)/GA ratio in Dongnongdongmai 1. High concentrations of GA_3 (10 and 100 µM) increased the MDA level, retarded the accumulation of soluble protein and sugar in both cultivars, but decreased the content of ABA and the ratio of ABA/GA only in Dongnongdongmai 1 and had no influence on those in Jimai 22. The re-greening rate of Dongnongdongmai 1 decreased as the concentration of exogenous GA_3 increased. Correlation analyses showed that MDA was negatively correlated with re-greening rate, while soluble protein, sugar ABA content, and ABA/GA ratio were positively correlated with re-greening rate. In conclusion, low exogenous GA_3 level could decrease endogenous GA content and elevate ABA/GA ratio and soluble protein content, which help to improve cold tolerance. However, high exogenous GA_3 level decreased the ABA content and ABA/GA ratio, resulting in lower soluble sugar and protein content and aggravated oxidative damage, and finally weakened cold tolerance. The endogenous GA metabolism and ABA/GA balance play central roles in exogenous GA_3 mediated cold tolerance.

Keywords: Cold stress, Physiological mechanism, Phytohormone, Tiller node, Winter wheat.

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INTRODUCTION

Suitable temperature is an important environmental condition for plant growth. Cold stress could limit plant growth by causing injury and death to plants, resulting in low crop yield (Qi *et al*., 2010; Kazemi Shahandashti *et al*., 2013). Under low-temperature stress, plants could initiate series of self-protection processes to adapt themselves to the cold environment, such as membrane permeability changes (Yu *et al*., 2005), osmolytes accumulation (JianMing *et al*., 2009), antioxidants increase (Xu and Sun, 2009), variation in metabolic enzymes (Minami *et al*., 2005), and changes of endogenous phytohormone level (Gusta *et al*., 2005).

Wheat is one of the most important crops in the world and 35% of the world population live on it (Paux *et al*., 2008). Hence, it is of great significance to study the mechanisms of cold tolerance in wheat. So far, progress has been achieved in cold tolerance mechanisms. The previous studies have always taken leaves as the main object for the cold tolerance study (Sharma *et al*., 2007; Sun *et al*., 2009). However, the tiller section is the main organ for wheat to achieve wintering in alpine regions, and it is also the organ reserving energy material which ensures the plant re-growth in the next spring (Yu *et al*., 2008a). Formation of tiller node can be divided into tiller bud formation and its subsequent outgrowth (Gerlach *et al*., 2003),

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which is regulated by various environmental and hormonal factors (Ding, 1997; Evers *et al*., 2006; Ferguson and Beveridge, 2009; Kim *et al*., 2010).

Exogenous application of some plant hormones was reported in plant species to enhance plant tolerance to many abiotic stresses, including cold (Rapacz, 2002), drought (Wei *et al*., 2006), and salt (Hamayun *et al*., 2010). Exogenous abscisic acid (ABA) (Rapacz *et al*., 2003) and 6-BA (Wang *et al*., 2009b) have been widely used to improve plant tolerance to cold stress. Studies also found that exogenous GA_3 enhanced the cold tolerance in rice (Xing, 2003) and Jincheng cucumber (Cheng and Du, 2008), but significantly reduced the tolerance in oilseed rape (Rood *et al*., 1989). Thus, the role of exogenous GA_3 on plant cold tolerance has not been demonstrated thoroughly and still needs further research.

Bred by Northeast Agricultural University, Dongnongdongmai 1 is the first wheat cultivar which could survive in the cold winter in Heilongjiang province (Zeng *et al*., 2011). This research aimed to understand the regulatory mechanisms of GA_3 in wheat cold tolerance by comparing Dongnongdongmai 1 and Jimai 22 cultivar (cold-sensitive cultivar). Our research could have an important significance in cultivating wheat with high yield and good quality in Heilongjiang province.

MATERIALS AND METHODS

Experimental Design

To study the mechanism of exogenous GA_3 in regulating wheat cold tolerance, two wheat cultivars with different cold sensitivity were used. The detailed wheat cultivation method, planting conditions, material treatments, and sample collection are given in the following segments. Each cultivar was divided into five groups for different concentrations of exogenous GA_3 treatments (0, 0.1, 1, 10, and 100 μM) under cold stress and each treatment was performed with at least three repetitions for a biological triplicate. After sampling, the contents of *MDA*, soluble sugar, and soluble protein were measured to study the effects of different concentrations of exogenous GA_3 on wheat tiller nodes physiological status under cold stress. Then, the endogenous GA and ABA contents and ABA/GA ratio were determined to probe the endogenous hormone metabolism under different treatments. The re-greening rates of two cultivars were calculated in the following spring and the correlation analyses between re-greening rate and aforementioned physiological and biochemical indicators under different cold stresses were processed to determine which indicators were related to increased wheat cold tolerance, which would help illuminating the mechanisms of exogenous GA_3 mediated wheat cold tolerance.

Materials

Two cultivars of wheat (*Triticum aestivum* L.) were used in this study. The Dongnongdongmai 1 (cold-tolerant cultivar) has a winter survival rate of 85%, while the Jimai 22 cultivar (cold-sensitive cultivar) has a winter survival rate of less than 2% (Liu *et al*., 2013). The two cultivars were kindly provided by School of Agriculture, Northeast Agricultural University. GA_3 was purchased from Beijing Chemical Reagent Company (CAS RN: 77-06-5).

Planting Condition and Sample Collection

The seeds of the two cultivars were sown in a field at Xiangfang Farm owned by Northeast Agriculture University, (45° 34′– 45° 46′ N, 126° 22′– 126° 50′ E) on September 12, 2008 (Row length: 4 m; Row spacing: 0.5m; Sowing depth: 5 cm, Plant spacing: 1 cm). The type of soil was chernozem soil (total nitrogen, 1.80 g/kg; available phosphorus, 49.3 mg/kg; available potassium, 158.5 mg/kg; PH, 7.11). The wheat plants were treated with normal fertilizer ($(NH_4)_2HPO_4$, 150 kg/hm^2; K_2SO_4, 75 kg/hm^2). At tiller initiation stage (September 26, 2008), different rows of plants were evenly irrigated with 2 L water solution containing 0, 0.1, 1, 10, and 100 μM GA$_3$. During the natural decreasing of field temperature, tiller nodes (the enlarged zone connecting the shoots and the roots) were randomly harvested when daily minimum temperature reached 0°C (October 1), -10°C (November 4), and -25°C (December 20) (Figure 1). All the tiller nodes were washed with de-ionized water and stored at -80°C after freezing in liquid nitrogen.

Malondialdehyde (MDA) Determination

The MDA content was measured according to Chen *et al*. (2013) with some modification. Half gram of frozen tiller node was homogenized in 10 mL trichloroacetic acid. After centrifugation, 2 mL supernatant and 2 mL (0.6%) 2-thiobarbituric acid were mixed and boiled for 15 minutes. The mixture was then centrifuged and cooled down quickly. And the 3 mL supernatant was used for colorimetric assay under three wavelengths: 600, 532, and 450 nm. The MDA concentration was calculated as shown below:

MDA [μmol g^{-1} fresh weight (FW)]= [$6.452\times(D_{532}-D_{600})-0.559\times D_{450}$]×10 mL 1.5 mL^{-1} 0.5 g^{-1}.

Soluble Sugar Content Determination

Soluble sugar content in the frozen tiller node was determined as previously described (Li *et al*., 2004). The absorbance was measured at 540 nm. Results were calculated by the standard regression equation as shown below:

y (Extinction)= 0.4893× (Sugar content) -0.4746.

Soluble Protein Content Determination

The soluble protein was extracted in the frozen tiller node according to previous studies (Ishimaru *et al*., 2001) and determined using BSA as a standard. The absorbance was measured at 595 nm. Results were calculated by the standard regression equation as shown below:

y (Extinction)= 0.995 × (Protein content)+0.003.

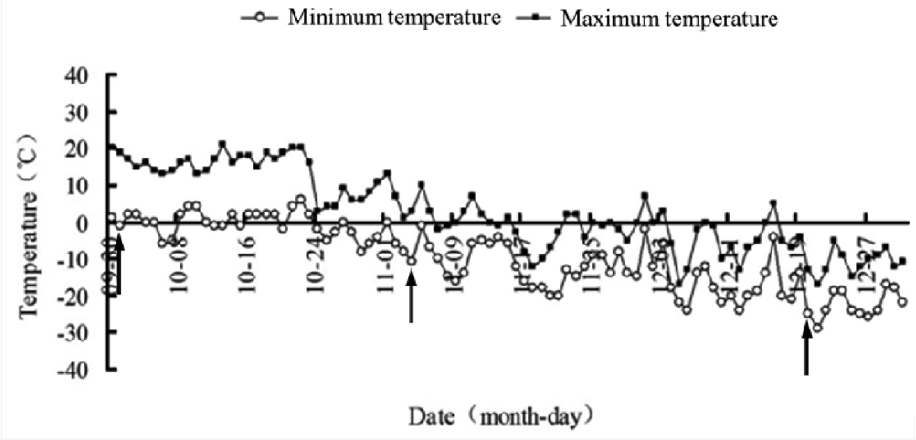

Figure 1. Temperature Trends during the sampling period. Daily maximum and minimum temperature from September 26 to October 31 were measured in the field. The sampling days are marked by arrows.

Endogenous Hormones Contents Determination

The measurement of endogenous hormones contents was conducted according to Yang *et al.* (2001) with some modification. Briefly, half gram of frozen tiller node was homogenized in 4 mL extraction buffer at 4°C. The homogenate was completely transferred into a 10 mL tube, and was suspended for 4 hours under 4°C. The supernatant was collected after centrifugation (6,000×g for 8 minutes). The pellet was re-extracted with 1 mL extraction buffer and the supernatant was collected. After mixing the two supernatants, the volume was measured. After flowing through the C-18 column, methanol in the supernatant was removed by rotary evaporating flask. The samples were dissolved by 1 mL dilution buffer. The ABA and GA contents were measured by ELISA kit, which was purchased from the China Agricultural University. The signal was detected with ANTHOS-2010.

Re-greening Rate Calculation

At the onset of tiller bud initiation, winter wheat was irrigated with different concentrations of GA_3. *The Re-greening rate= The re-greening number of seeding in the next spring/Total number of wheat seedling* (Yu *et al.*, 2008a)

Replication and Data Processing

All analyses were performed at least in triplicate. Excel 2003 was used for data processing and chart generation. The least significant difference (LSD) multiple comparison method (P= 0.05) from DPS 7.05 software was used for statistical comparison. Pearson correlation coefficient was used to assess the associations between the re-greening rate and physiological/biochemical indicators. The SPSS statistical software (version 12.0, IBM Company, Chicago, IL, USA) was used for this analysis. Statistical significance was assessed by calculating *p* value, where *p* values of < 0.05 were considered to be statistically significant and *p*-values of < 0.01 were considered to be statistically highly significant.

RESULTS

Effect of Exogenous GA_3 on MDA Content

Under all the tested conditions, the MDA contents in the tiller node of Dongnongdongmai 1 were significantly lower than that of Jimai 22, especially at -

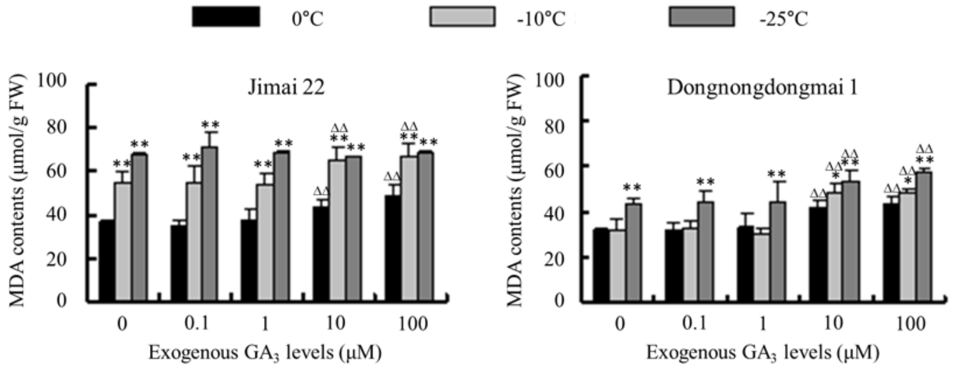

Figure 2. Influence of exogenous GA_3 on MDA content in Dongnongdongmai 1 and Jimai 22. (* P< 0.05 and ** P< 0.01 *vs.* 0°C; $^{\Delta}$ P< 0.05 and $^{\Delta\Delta}$ P< 0.01 *vs.* 0 µM GA_3 treatment).

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10°C and -25°C ($P < 0.01$) (Figure 2). As the temperature decreased, the MDA content gradually increased in the tiller node of Jimai 22 irrespective of the application of exogenous GA_3. However, significant increase was only observed at -25°C in Dongnongdongmai 1, irrespective of the application of exogenous GA_3. Under higher concentration (10 and 100 μM) GA_3 treatments, -10°C cold stress just induced a slight increase of MDA content compared with 0°C cold stress in Dongnongdongmai 1 (Figure 2).

Low concentrations of exogenous GA_3 (0.1 and 1 μM) had no significant effect on the MDA contents in the tiller node of the two cultivars, while higher concentrations (10 and 100 μM) markedly increased the *MDA* levels at 0 and -10°C (Figure 2). At -25°C, however, high GA_3 concentrations significantly increased the MDA content in the tiller node of only Dongnongdongmai 1 ($P < 0.01$).

Effect of Exogenous GA_3 on Soluble Sugar Content

Under all the tested conditions, Dongnongdongmai 1 had a higher soluble sugar level than Jimai 22 (Figure 3). Compared with that at 0°C, the soluble sugar in the tiller nodes of Jimai 22 gradually accumulated as the temperature decreased under no GA_3 treatment or low levels (0, 0.1 and 1 μM) of GA_3 treatment, while the accumulation was only observed at -25°C when irrigated with high levels of GA_3 (Figure 3). In Dongnongdongmai 1 without GA_3 treatment, the soluble sugar content increased as the temperature decreased (Figure 3). However, when treated with 0.1, 1 or 10 μM GA_3, increase of soluble sugar content was only observed when temperature fell to -25°C, but, when treated with 100 μM GA_3, no increase was tested.

Low GA_3 levels had no significant effect on the soluble sugar content in these two cultivars, while high GA_3 levels decreased the soluble sugar content in the two cultivars ($P < 0.01$) (Figure 3).

Effect of GA_3 on Soluble Protein Content

Dongnongdongmai 1 had a slightly higher soluble protein content compared with Jimai 22. Generally, the soluble protein content in the tiller node of the two cultivars gradually increased as the temperature decreased (Figure 4). 0.1 μM GA_3 enhanced the accumulation of soluble protein in Jimai 22 at 0°C, while high concentrations of GA_3 showed the opposite effects at all tested temperatures (Figure 4). Low concentrations of GA_3 (0.1 and 1 μM) increased the soluble protein content in Dongnongdongmai 1 at -

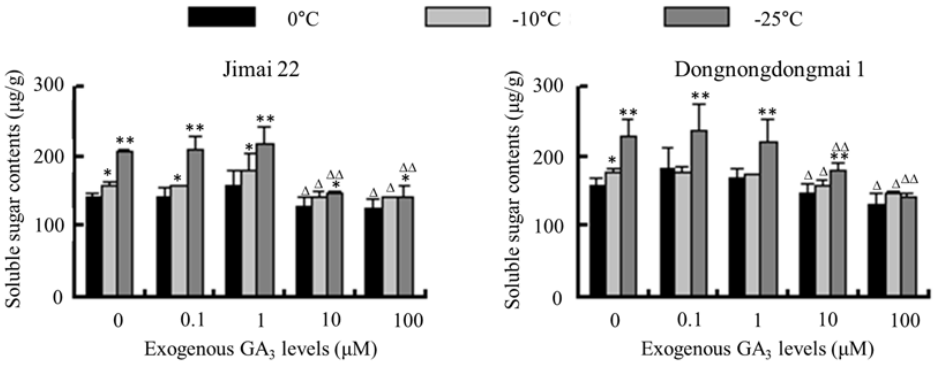

Figure 3. Influence of exogenous GA_3 on soluble sugar content in Dongnongdongmai 1 and Jimai 22. (* $P < 0.05$ and ** $P < 0.01$ *vs.* 0°C; $^{\Delta}P < 0.05$ and $^{\Delta\Delta}P < 0.01$ *vs.* 0 μM GA_3 treatment).

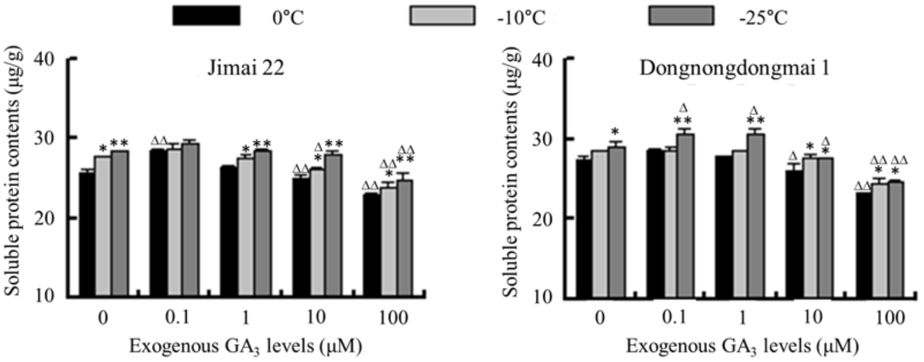

Figure 4. Influence of exogenous GA_3 on soluble protein content in Dongnongdongmai 1 and Jimai 22. (* $P< 0.05$ and ** $P< 0.01$ *vs.* 0°C; $^{\Delta}P< 0.05$ and $^{\Delta\Delta}P< 0.01$ *vs.* 0 μM GA_3 treatment).

25°C, whereas high concentrations showed significant inhibitory effects at all tested temperatures ($P< 0.01$).

Effect of GA_3 on ABA Content

The ABA content in Dongnongdongmai 1 was higher than that in Jimai 22, especially at -25°C. As the temperature dropped down, endogenous ABA content gradually increased in the tiller node of Dongnongdongmai 1, while that of Jimai 22 met an obvious increase at -10°C compared with that at 0°C, but a slight reduction at -25°C was observed compared with that at -10°C (Figure 5). Different GA_3 concentrations had no obvious effects on ABA content in the tiller node of Jimai 22, while high concentrations (10 and 100 μM) reduced the ABA content in the tiller node of Dongnongdongmai 1. It is noteworthy that 100 μM GA_3 completely prevented the cold-induced ABA accumulation in the tiller node of Dongnongdongmai 1 (Figure 5).

Effect of GA_3 on Endogenous GA Content

Dongnongdongmai 1 had a lower endogenous GA level in the tiller nodes than Jimai 22 under all tested conditions (Figure 6). Compared with the endogenous GA content at 0°C without exogenous GA_3 treatment, there was a significant increase in

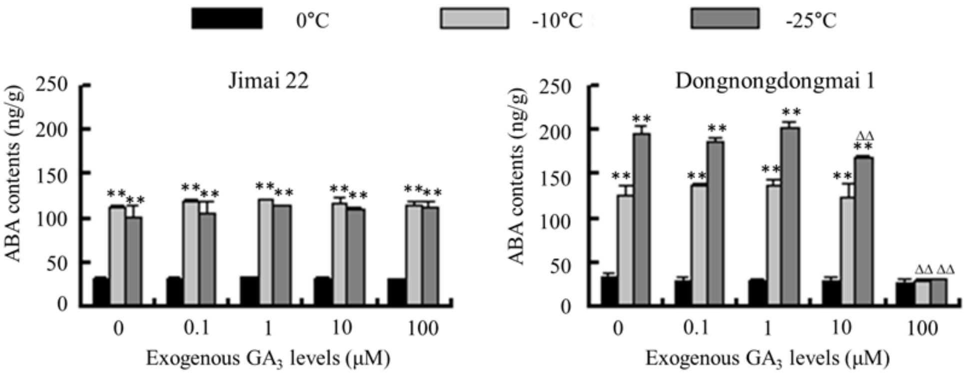

Figure 5. Influence of exogenous GA_3 on endogenous ABA in Dongnongdongmai 1 and Jimai 22. (* $P< 0.05$ and ** $P< 0.01$ *vs.* 0°C; $^{\Delta}P< 0.05$ and $^{\Delta\Delta}P< 0.01$ *vs.* 0 μM GA_3 treatment).

the endogenous GA content in Jimai 22 at -10°C and no significant difference was observed at -25°C. It seemed that the temperature had no effects on the endogenous GA content in Dongnongdongmai 1 as that almost kept the same level under three different temperatures without exogenous GA_3 treatment. Application of exogenous GA_3, especially at low concentrations, repressed the endogenous GA production in the tiller nodes of the two winter wheat cultivars (Figure 6) ($P< 0.01$). Moreover, it seemed that inhibiting effects of GA_3 treatments had little to do with the temperature as the GA contents were on the same level under a certain exogenous GA_3 content treatment (except for 100 μM GA_3 treatment at -25°C in both cultivars and 1 μM GA_3 treatment in Dongnongdongmai 1). Under 100 μM GA_3 treatments, the endogenous GA content at -25°C was significantly higher than that at 0°C and -10°C in both cultivars, which was an interesting phenomenon.

Effect of GA_3 on Endogenous ABA/GA Ratio

The ABA/GA ratio in Dongnongdongmai 1 was higher than that in Jimai 22. Endogenous ABA/GA ratio gradually increased in Dongnongdongmai 1 as the temperature decreased, while an increase at -10°C and a marginal reduction at -25°C were observed in Jimai 22 (Figure 7). No

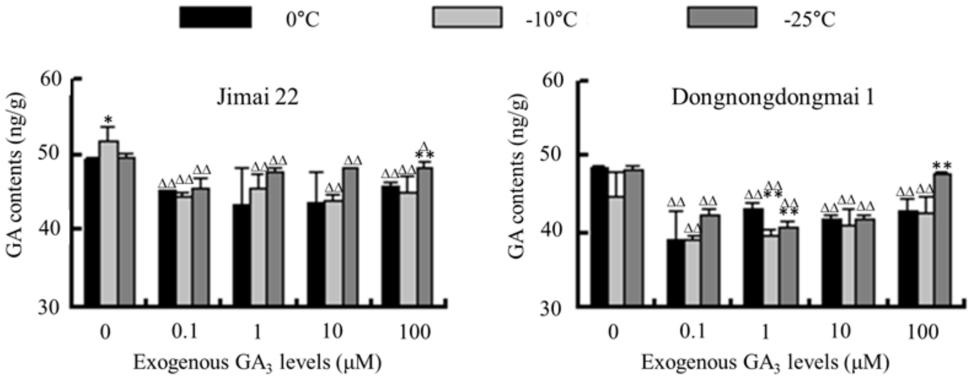

Figure 6. Influence of exogenous GA_3 on endogenous GA in Dongnongdongmai 1 and Jimai 22. (* $P< 0.05$ and ** $P< 0.01$ *vs.* 0°C; $^{\Delta}$ $P< 0.05$ and $^{\Delta\Delta}$ $P< 0.01$ *vs.* 0 μM GA_3 treatment).

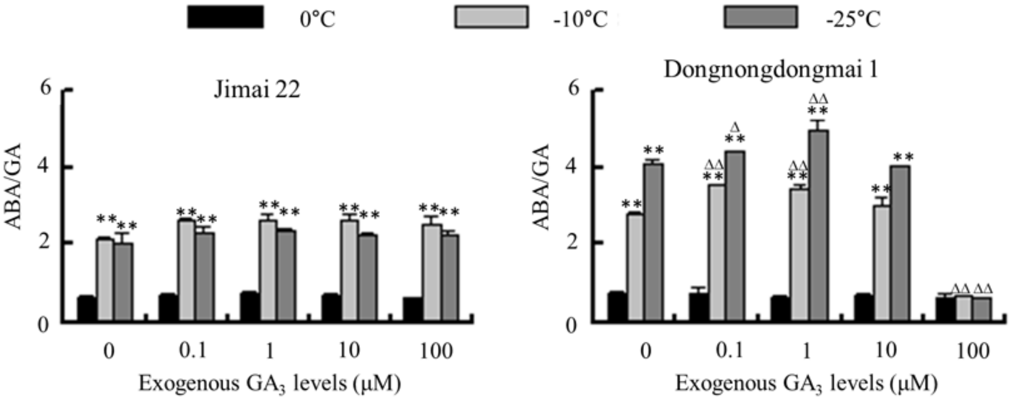

Figure 7. Influence of exogenous GA_3 on endogenous ABA/GA ratio in Dongnongdongmai 1 and Jimai 22. (* $P< 0.05$ and ** $P< 0.01$ *vs.* 0°C; $^{\Delta}$ $P< 0.05$ and $^{\Delta\Delta}$ $P< 0.01$ *vs.* 0 μM GA_3 treatment).

notable effects of exogenous GA_3 on the ABA/GA ratio were observed in the tiller node of Jimai 22. Low levels of GA_3 significantly increased the ABA/GA ratio in the tiller node of Dongnongdongmai 1 at -10 and -25°C ($P< 0.01$), while 100 µM GA_3 dramatically reduced the ABA/GA ratio ($P< 0.01$) (Figure 7).

Effect of GA_3 on Re-greening Rate of Winter Wheat

The re-greening rates of the two cultivars were also calculated and shown in Table 1.

The re-greening rates of Jimai 22 for all treatments were zero, while those of Dongnongdongmai 1 reduced gradually as the concentration of GA_3 increased, except for 0.1 µM GA_3 treatment.

Correlations between Re-greening Rate and Physiological/Biochemical Indicators

The correlation analyses results (Table 2) showed that as the temperature decreased, the MDA activity had a negative correlation with re-greening rate in the following spring. Correlation coefficients were -0.96, -0.89, and -0.97 for the 0, -10, and -25°C cold stress, respectively. ABA, soluble sugar, and soluble protein content were positively correlated with the following spring re-greening rate. Correlation coefficients between soluble sugar and re-greening rate were 0.89, 0.99, and 0.99 for the 0, -10 and -25°C cold stress, respectively. Correlation coefficients between soluble protein content and re-greening rate were 0.94, 0.91, and 0.91 for the 0, -10 and -25°C cold stress, respectively. The correlation coefficients between ABA and re-greening rates for the -10 and -25°C cold stress were, respectively, 0.84 and 0.87, while no correlation was observed at the 0°C cold stress.

Table 1. The re-greening rates of Dongnongdongmai 1 and Jimai 22 under different exogenous GA_3 concentrations treatments. LSD multiple comparison method ($P= 0.05$) was used for statistical comparison.[a]

	Cultivar	
GA_3 Level (µM)	Dongnongdongmai 1	Jimai 22
0	83.3±4.2 A	0±0 D
0.1	84.7±10.2A	0±0 D
1	62.3±8.4 B	0±0 D
10	31.6±3.8 C	0±0 D
100	0±0 D	0±0 D

[a] Values followed by the same letter are not significantly different at 0.05 probability level according to LSD test.

Table 2. Correlation analysis between re-greening rate and physiological indices of wheat under different cold stresses (-0, -10 and -25°C).

	Correlation index at 0°C	Correlation index at -10°C	Correlation index at -25°C
MDA	-0.96**	-0.89*	-0.97**
Soluble Sugar	0.89*	0.99**	0.99**
Soluble Protein	0.94**	0.91*	0.91*
ABA	0.63	0.84*	0.87*
GA	0.15	-0.16	-0.19
ABA/GA	0.8	0.81	0.81

* and **: Indicates the correlation is significant ($P< 0.05$) and highly significant ($P< 0.01$).

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DISCUSSION

The occurrence of cold injury in plants is caused by lipid peroxidation induced by the accumulation of free radicals in cell membrane during cold stress (Mayer and Harel, 1979; Wise and Naylor, 1987; Chen *et al*., 2000; Kazemi Shahandashti *et al*., 2013). Our results indicated that MDA content in Dongnongdongmai 1 was significantly lower than that in Jimai 22, which provides fundamental explanation for the fact that the cold damage of Dongnongdongmai 1 is less than Jimai 22. Relatively high exogenous GA_3 level could significantly increase the MDA content in the tiller node of Dongnongdongmai 1 (Figure 2), which signify excessive exogenous GA_3 aggravated cold stress induced oxidative stress. It is reported that MDA could reduce the stability of membrane, promote membrane leakage, and further increase the intramembranous peroxidation level (Imlay and Linn, 1988; Kazemi Shahandashti *et al*., 2013). In Jimai 22, high level GA_3-induced MDA accumulation was only observed at 0 and -10°C, not at -25°C. The possible explanation is that Jimai 22 is a cold sensitive cultivar and -25°C cold stress probably froze it to death, therefore, the *MDA* content didn't continue to increase. Contribution of osmotic adjustment substances to cold tolerance has been reported by many researches (Xin and Browse, 2000; Gustaa and Wisniewskib, 2012). Osmotic adjustment substances (soluble sugar and protein) were increased gradually in the two wheat varieties as the temperature decreased. Soluble sugar and protein contents in tiller node of Dongnongdongmai 1 were significantly higher than those of Jimai 22 (Figures 3 and 4), which is consistent with our previous work (Yu *et al*., 2008b). This study further demonstrated that Dongnongdongmai 1 has stronger cold resistance than Jimai 22. Relatively higher concentrations of GA_3 (10 and 100 μM GA_3) reduced osmotic adjustment matter contents in the tiller note of the two wheat cultivars, resulting in dehydration damage and great reduction of cold tolerance. But, relatively lower concentration of GA_3 (0.1 and 1 μM) enhanced the soluble protein content in Dongnongdongmai 1 under -25°C cold stress (Figure 4). Proteomics analysis demonstrated that Dongnongdongmai 1 expressed more proteins than Jimai 22 under cold stress (Yu *et al*., 2009). The correlation analyses results also showed that soluble protein content were positively correlated with the following spring re-greening rate (Table 2). We infer relatively lower concentration of GA_3 could promote Dongnongdongmai 1 cold tolerance by promoting protein synthesis.

It is well known that plant hormones are involved in almost every aspect of plant growth and development as well as biotic and abiotic stress responses. In general, they often interact with each other and with the plant's environment to regulate plant vital movement (Kurepin *et al*., 2013b). Studying the wheat cold tolerance mechanism on plant hormone level may confer some new opinions. Thus, we detected the contents of endogenous ABA and GA as well as ABA/GA ratio values in the tiller node of the two wheat cultivars with and without application of exogenous GA_3. Consistent with the previous report (Wang *et al*., 2009a), ABA content in the tiller node of untreated Dongnongdongmai 1 reached the maximum at -25°C , which supports the assumption that the ABA accumulation is closely related with the strong cold tolerance of tiller node. The correlation analysis between re-greening rate and ABA content of wheat at relative severe cold stress (-10 and -25°C) also indicated a higher positive correlation (Table 2). GA_3 slightly decreased the ABA content in Dongnongdongmai 1 at 0°C. The possible explanation is that the strong cold-tolerant cultivar Dongnongdongmai 1 was insensitive to 0°C. High concentration of GA_3 treatments, especially 100 μM treatment, significantly decreased the ABA content in Dongnongdongmai 1, suggesting high exogenous GA_3 treatments levels could interfere the synthesis of ABA and result in weakened cold tolerance. Besides, Zentella *et al*. (2007) reported that GAs and ABA can down regulate each other's synthesis genes in *Arabidopsis*. Endogenous GA content in the tiller node of Dongnongdongmai 1 was lower than that of Jimai 22. Study on alfalfa also revealed a similar phenomenon, which supported that the weak cold-tolerant cultivars have much higher endogenous GA content than the strong cold-tolerant cultivars (Waldman *et al*., 1975). The treatment of low exogenous GA_3 level significantly reduced the endogenous GA level in the tiller node of both cultivars. But, the decline of endogenous GA level in Dongnongdongmai 1 was sharper than that in Jimai 22. Low endogenous GA level could inhibit the plant growth, promote stomatal closure, reduce transpiration, and increase the soluble protein content, resulting in enhanced cold tolerance (Luo, 1989; Dan Yue and Wang, 2008). We speculate low exogenous GA_3 may alter the metabolism of endogenous GA (may promote its catabolism) as application of exogenous growth-active GAs (such as GA_1, GA_3 or GA_4) could modify endogenous GA levels (Kurepin *et al*., 2013a).

Because plant hormones often respond to abiotic stress via positive and negative interactions (Peleg and Blumwald, 2011), the ABA/GA ratio may further illuminate the mechanism of exogenous GA_3-mediated cold tolerance. Treatments 0.1 and 1 μM GA_3 increased the ABA/GA ratio in the tiller node of Dongnongdongmai 1 at -10°C and -25°C (Figure 7), which could protect plant from cold injury. A previous study also demonstrated that increased cold tolerance was strongly associated with higher endogenous ABA levels and lower endogenous GA levels (Zhang *et al*., 2012). Our correlation analysis results (Table. 2) also showed that ABA content and ABA/GA ratio were positively related with wheat re-greening rate and endogenous GA content was not related with re-greening rate under cold stress, which signifies high ABA content and ABA/GA ratio facilitated wheat survival under cold stress. Furthermore, the balance between ABA and Gibberellin-like(GAs) has been proposed to be a common mediator for plant stress responses, not just cold stress (Zhang *et al*., 2012). Also, 100 μM GA_3 significantly reduced the ABA/GA ratio in the tiller node of Dongnongdongmai 1, which implied that high concentration of GA_3 treatments probably disturbed the balance between endogenous ABA and Gibberellin-like (GAs), and weakened the cold tolerance of Dongnongdongmai 1. This viewpoint was further confirmed by the re-greening rate calculation in the following spring as high concentration of GA_3 treatments had lowered the re-greening rate of Dongnongdongmai 1 (Table 1). Exogenous GA_3 treatments had no influence on ABA/GA ratio in Jimai 22, irrespective of the concentrations used (Figure 7). The possible explanation may be that different cultivars have different sensitivities to the same hormone levels. (Hu *et al*., 2010). Besides, Jimai 22 is a cold sensitive cultivar and cold stress probably results in growth-cessation and even death of the plant, thus, its ability to respond to external signals may be retarded and even lost. Our deduction are confirmed by the re-greening rate measurement, as cold sensitive cultivar Jimai 22 did not revive after the severe cold stress irrespective of the application of exogenous GA_3 or not (Table 1). It is really a pity that, because of the limitation of ELISA assay, we are not clear which GAs was measured, but just the sum of all GAs. But, it still can reflect the metabolism condition of endogenous GA to some extent under exogenous GA_3 treatment. The mass spectrometry analysis may further benefit the understanding of the regulating mechanisms of exogenous GA_3 to endogenous GAs metabolism and ABA/GA ratio. We will study this in the future.

Our previous study of the two cultivars (Jimai 22 and Dongnongdongmai 1) revealed that the tiller node of winter wheat played an important role in cold tolerance

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(Yu *et al*., 2008b). In the present study, we further studied the regulatory role of exogenous GA_3 in wheat tiller node cold tolerance. According to the results, we speculate that low exogenous GA_3 may enhance wheat cold tolerance by altering endogenous GA metabolism (mainly decreasing the endogenous GA content), consequently, enhancing ABA/GA ratio and protein synthesis. But the enhancing effects reflected on antioxidant physiology (MDA content) and population level (reviving rate) were not observed, which may be related to the treatment methods used in this study and the extremely severe cold stress that lasted for the whole winter. Indeed, the minimum temperature reached -30°C in December, which may beyond the wheat physiological tolerance limit. However, high exogenous GA_3 level had the opposite effect: it even aggravated wheat cold stress. The possible mechanism was that high exogenous GA_3 inhibited the synthesis of ABA, then lowered the ABA/GA ratio and disturbed the ABA/GA balance, subsequently, the synthesis of protective substances (soluble sugar and protein) was suppressed, oxidative damage was sharpened, and lastly, wheat survival rate declined.

Our study results helps in understanding the function of exogenous GA_3 on withstanding cold stress in wheat during cold winter, which also provides a theoretical basis for improving crop production. Nevertheless, GA_3 treatments in our study didn’t improve the reviving rate in the following spring significantly, which could be caused by the concentration of exogenous GA_3 and treatment methods used in this study. Further studies are needed to unveil the underlying regulatory mechanism of exogenous GA_3 in wheat cold tolerance.

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اثر های جیبرلیک اسید(GA_3) خارجی روی تحمل سرما در گندم

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چکیده

به منظور روشن کردن سازوکارهای فیزیولوژیکی اسید جیبرلیک (GA) در رابطه با تحمل سرما، اثر های GA_3 خارجی روی مالون دی الدهاید (MDA)، مواد تنظیم اسمزی و مقدار هورمون های درونی در گره پنجه (tiller nodes) در دو رقم گندم به نام Dongnongdongmai 1 (رقم غیر حساس به سرما) و Jimai 22 (رقم حساس به سرما) در سه دوره سرد زمستان (۰، ۵-، و ۲۰- درجه سلسیوس) بررسی شد. نتایج نشان داد که غلظت های کم GA_3(۰/۱ و۱ میکرومول) منجر به کاهش غلظت GA درونی در هر دو رقم شد ولی فقط در رقم Dongnongdongmai 1 نسبت آبسیسیک اسید به جیبرلیک اسید (ABA)/GA) را افزایش داد. غلظت های زیادGA_3 (۱۰ و۱۰۰ میکرومول) منجر به افزایش مقدار MDA و جلوگیری از انباشت پروتئین و قند محلول در هر دو رقم شد ولی مقدار ABAو نسبت آبسیسیک اسید به جیبرلیک اسید(ABA/GA) را فقط در Dongnongdongmai 1 کاهش داد و در این مورد اثری روی Jimai 22 نداشت. با افزایش غلظت GA3 خارجی، نرخ(سرعت) بازسبزشدن (regreening) رقم Dongnongdongmai 1 کم شد. تحلیل وابستگی نتایج نشان داد که MDA رابطه ای منفی با نرخ بازسبز شدن داشت در حالیکه پروتئین محلول، قندها ABAو نسبتABA/GA با این نرخ رابطه مثبت داشتند. نتیجه کلی این است که غلظت های کم GA_3 خارجی می تواند مقدار GA درونی را کاهش داده و نسبت ABA/GA و پروتئین محلول را که در بهبود تحمل به سرما موثراند افزایش دهد. از سوی دیگر، مقادیر زیاد GA_3 خارجی ، مقدار ABA و نسبت ABA/GA را کم کرده و منجر به کاهش قند محلول و پروتئین و تشدید صدمات اکسیداتیو شده و نهایتاً تحمل به سرما را کم میکند. متابولیزم GA درونی و تراز ABA/GA نقش اساسی در تحمل به سرما به میانجیGA_3 خارجی دارند.

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