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Effect of Exogenous Abscisic Acid (ABA), a Potential Growth Regulator on Physiological Response to Chilling Stress of Adzuki Bean (*Vigna angularis*) at Flowering Stage

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ABSTRACT

Background: Low temperatures during flowering severely affect crop growth and yield.

Methods: This experiment was conducted under potted conditions with LXD 4 and TJH as test materials and pre-sprayed with exogenous ABA at a concentration of 20 mg×L^{-1} at flowering stage, respectively and then with an average of 15°C condition for 5 days.

Result: The results showed that foliar spraying exogenous ABA effectively inhibited the increase of MDA content and relatively significantly improved the photosynthetic parameters such as *G*s and *Tr* and alleviated the loss of yield factors caused by chilling stress. Compared with spraying water, spraying exogenous ABA can significantly increase the yield per pot of LXD 4 by 6.72~17.77% and TJH by 6.41~37.04% under low temperature conditions. In summary, foliar spraying exogenous ABA ameliorated the effects of chilling stress on the physiological characteristics of adzuki bean leaves and improved yield as a result of improved antioxidant defense mechanisms which impeded lipid peroxidation. Thus, we conclude that foliar spraying exogenous ABA could decelerate the damages caused by chilling stress to some extent.

Key words: Adzuki bean, Anti-adversity defense system, Chilling, Exogenous ABA, Yield.

INTRODUCTION

Adzuki bean (*Vigna angularis*) is widely planted in China, it is planted in almost different regions of the country, especially in the Northeast region. Adzuki bean has the biological characteristics of light, temperature and infertile, but does not tolerate low temperature and is sensitive to chilling (Donnelly, 2021). In recent years, low temperatures have occurred frequently in spring and summer in Northeast China, which has a great impact on the production of adzuki bean. Sudden cold damage has become an important abiotic adversity stress that restricts adzuki bean production in Heilongjiang province (Xiang *et al*., 2019a).

Plant endogenous hormones regulate plant growth and response to stress (Klingler *et al*., 2010). ABA, an endogenous hormone discovered and identified in the 1960s, plays an important role in plant stress tolerance and resistance, under low temperature stress, a significant increase in the leaf abscisic acid content (at least 2.5-fold) was measured in plants (Pruvot *et al*., 1996). Exogenous treatment with abscisic acid (ABA) resulted in an improvement in growth and survival of nonacclimated, chilled seedlings (Anderson *et al*., 1994). Exogenous hormones regulate the physiological metabolism of plants by changing the levels of endogenous hormones. Exogenous ABA has multiple pathways in resisting low temperature stress. The impact of low temperature on crop yield is obvious and in severe cases it can even lead to production failure. This study proposes that exogenous ABA treatment can improve the yield and antioxidant defense capacity of adzuki bean by enhancing the cold tolerance at flowering stage. The main research purpose is to confirm that exogenous ABA treatment can improve the cold tolerance of adzuki bean.

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MATERIALS AND METHODS

Plant materials

The cold-tolerant and cold-sensitive adzuki bean varieties, LXD 4 and TJH were used as experimental materials. Exogenous ABA was provided from China National Practical Bean Technology System, purchased from Sigma Company. The experiment was carried out at the potted planting field of the Institute of Crop Cultivation and Tillage, Heilongjiang

Academy of Agricultural Sciences (45.57°N and 126.37°E) in Harbin, Heilongjiang province, northeastern China.

Experimental design

Adzuki bean seeds were planted in each resin pot (diameter of 30cm and height of 25 cm) filled with about 16 kg of soil. There are six treatments in this experiment (Three replication), as shown in Table 1 and the temperature change during the day is shown in Fig 1. When plants to grow to flowering stage (51 days after sowing), foliar spraying of exogenous ABA (concentration of 20 $mg\times L^{-1}$ with a spraying rate of 225 $L\times hm^{-2}$), was conducted and the pots were kept into the artificial climate room with different low temperature for chilling treatment. The duration is 1, 2, 3, 4 and 5 days, respectively. Each treatment was sampled separately and immediately placed in liquid nitrogen and then stored in a -80°C refrigerator for the determination of physiological indicators.

Measurement items and methods

Determination of MDA content

The content of MDA (malondialdehyde) was determined using the thiobarbituric acid (TBA) method (Dhindsa *et al*., 1981).

Content of soluble sugar, soluble protein and free proline

Glucose equivalents were used to determine soluble sugar content to the anthrone-sulfuric acid method described by Khosrowshahi *et al*., 2020).

The soluble protein content was determined according to a method introduced by Chaturvedi *et al*. (2015).

For the free proline assay, leaf samples (0.5 g) were homogenized in 10 mL of 3% sulfosalicylic and the homogenate was filtered through filter paper. Extract (2 mL) was added to 2 mL of glacial acetic acid and 2 mL of acid ninhydrin and the mixture was bathed in water of 100°C for 30 min. After cooling down, 4 mL of methylbenzene was added with agitation. Absorbance of the red methylbenzene supernatant was taken at 520 nm with a spectrophotometer.

Table 1: Test design scheme.

Treatment code	Foliar spray treatment	Temperature treatment
T1	Spray ABA	Average 15°C
T2	Spray water	Average 15°C
T3	Spray ABA	Normal temperature
CK	Spray water	Normal temperature

Gas-exchange parameters

Net photosynthetic rate (*P*n), transpiration rate (*T*r), intercellular CO_2 concentration (*C*i) and stomatal conductance (*G*s) were measured on the third fully expanded trifoliate leaves from the main apex using a portable photosynthesis system (Li-Cor 6400, Li-Cor Inc., Nebraska, USA). Plants were measured under PPFD of 1,000 μmol(photon) $m^{-2}\times s^{-1}$, CO_2 concentration of 500 mmol mol^{-1}, 25 ± 3°C and 80% humidity.

Statistical analysis

Microsoft Excel 2013 and SPSS 25.0 was used to analyze the one-way ANOVA of all the collected data. Duncan test ($p<0.05$) was used to evaluate the difference within treatments and the significant differences among different materials were determined.

RESULTS AND DISCUSSION

Effect of exogenous ABA on adzuki bean yield under chilling stress

15°C during flowering stage can significantly affect the yield of adzuki beans. For LXD 4, the yield shows CK>T2 at each time and the result of significant analysis showed that CK was higher than T2 from 2d to 5d. In addition, with the extension of the treatment time, the greater the degree of yield loss, T2 of LXD 4, compare with 1d, the yield at 5d decreased by 10.30 g per pot, a decrease of 59.81%. For TJH, the yield shows that, CK was consistently higher than T2, significantly. Under low temperature conditions, spraying exogenous ABA can inhibit production reduction. For LXD 4, T1 was significantly higher than T2 by 6.72%, 7.69%, 10.85%, 9.92% and 17.77% during treatment from 1d to 5d. For TJH, T1 was significantly higher than T2 by 6.41%, 15.67%, 20.47% and 37.04% from 2d to 5d (Table 2).

As shown in Table 3, it is clear that chilling stress increased the hundred-grain weight. For LXD 4, T2 was significantly heavier than CK by 3.23%, 8.00%, 16.30%, 8.06% and 4.30% during treatment from 1d to 5d. For TJH,

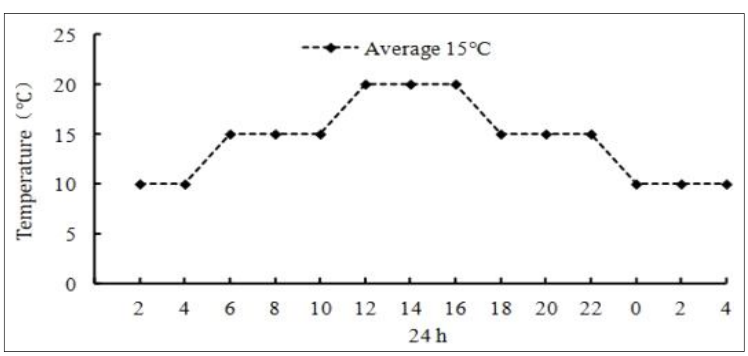
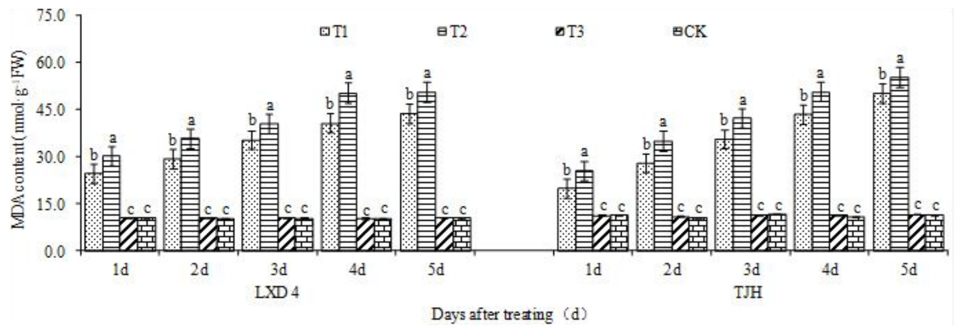


Fig 1: Changes in temperature during one day.

T2 was significantly heavier than CK by 19.24%, 10.82%, 12.04%, 16.71% and 10.88% during treatment from 1d to 5d. Spraying exogenous ABA reduced the hundred-grain weight of adzuki bean. For LXD 4, T1 was lighter than T2 by 0.73%, 0.83%, 1.38%, 1.97% and 1.24% during treatment from 1 to 5 days. For TJH, T1 was higher than T2 by 3.44%, 1.64%, 1.35%, 5.48% and 2.15% during treatment from 1 to 5 days.

Effect of exogenous ABA on MDA content in adzuki beans leaves under chilling stress

As shown in Fig 2, the MDA content showed an increasing trend after low temperature treatment during the flowering stage. Compared with CK, T2 of LXD 4 significantly increased by 19.68 nmol·g^{-1}, 25.45 nmol·g^{-1}, 30.00 nmol·g^{-1}, 39.89 nmol·g^{-1} and 40.03 nmol·g^{-1}. T2 treatment of TJH significantly increased by 14.17 nmol·g^{-1}, 24.42 nmol·g^{-1}, 30.48 nmol·g^{-1},



CK: Plant grown in natural environment + spraying H_2O; T1: Plant grown in Average 15°C+ spraying exogenous ABA; T2: Plant grown in Average 15°C + spraying H_2O; T3: Plant grown in natural environment + spraying ABA.

Fig 2: Effect of exogenous ABA on MDA content of adzuki beans leaves under chilling stress at flowering stage.

Table 2: Effect of exogenous ABA on the yield of adzuki beans under chilling stress at flowering stage (g·pot^{-1}).

Varieties	Treatment code	1d	2d	3d	4d	5d
LXD 4	T1	29.37±0.10b	28.01±0.11b	25.95±0.14c	22.82±0.28c	20.28±0.16c
	T2	27.52±0.19c	26.01±0.04c	23.41±0.18d	20.76±0.14d	17.22±0.05d
	T3	33.16±0.59a	33.16±0.53a	33.16±0.58a	33.16±0.42a	33.16±0.51a
	CK	28.97±0.62bc	28.97±0.65b	28.97±0.63b	28.97±0.78b	28.97±0.68b
TJH	T1	21.58±0.37bc	20.57±0.32c	19.27±0.24c	18.01±0.32c	14.91±0.06c
	T2	20.54±0.16c	19.33±0.21d	16.66±0.22d	14.95±0.14d	10.88±0.49d
	T3	27.28±0.39a	27.28±0.41a	27.28±0.38a	27.28±0.34a	27.28±0.38a
	CK	22.74±0.19b	22.74±0.20b	22.74±0.16b	22.74±0.07b	22.74±0.20b

Note: The data is the average value of ten repetitions; CK: Plant grown in natural environment + spraying H_2O; T1: Plant grown in Average 15°C + spraying exogenous ABA; T2: Plant grown in Average 15°C + spraying H_2O; T3: Plant grown in natural environment + spraying ABA. Different letters in the general column indicate the significant level of difference ($p<0.05$).

Table 3: Effect of exogenous ABA on the hundred-grain weight of adzuki beans under chilling stress at flowering stage (g).

Varieties	Treatment code	1d	2d	3d	4d	5d
LXD 4	T1	16.39±0.09b	17.13±0.11a	18.35±0.14a	16.95±0.13a	16.43±0.22bc
	T2	16.51±0.06b	17.28±0.09a	18.60±0.22a	17.29±0.10a	16.64±0.06b
	T3	17.39±0.04a	17.39±0.07a	17.39±0.11b	17.39±0.09a	17.39±0.14a
	CK	16.00±0.08c	16.00±0.06c	16.00±0.05c	16.00±0.15b	16.00±0.06c
TJH	T1	12.91±0.15a	12.22±0.11b	12.39±0.13b	12.37±0.34b	12.16±0.12b
	T2	13.37±0.05a	12.42±0.16b	12.56±0.09b	13.08±0.04ab	12.43±0.20b
	T3	13.37±0.07a	13.37±0.08a	13.37±0.16a	13.37±0.20a	13.37±0.09a
	CK	11.21±0.15b	11.21±0.12c	11.21±0.03c	11.21±0.12c	11.21±0.11c

Note: The data is the average value of ten repetitions; CK: Plant grown in natural environment + spraying H_2O; T1: Plant grown in Average 15°C + spraying exogenous ABA; T2: Plant grown in Average 15°C + spraying H_2O; T3: Plant grown in natural environment + spraying ABA. Different letters in the general column indicate the significant level of difference ($p<0.05$).

39.79 nmol·g^{-1} and 43.91 nmol·g^{-1}. Exogenous ABA can significantly inhibit the increase of MDA content under low temperature conditions. The analysis of variance showed that after treatment for 1 to 5 days, both LXD 4 and TJH showed that T1 was significantly lower than T2.

Effect of exogenous ABA on photosynthesis in adzuki beans leaves under chilling stress

It can be seen from Fig 3 that, with the extension of the stress time, compared with CK, *P*n, *G*s, *C*i and *T*r in T2 treatment reduced significantly both of LXD 4 and TJH. At the same time, under natural environment, spraying exogenous ABA has the effect of increasing *P*n, *G*s, *C*i and *T*r, especially *P*n, *G*s and *T*r can be significantly increased (Fig 3A, 3B, 3C and 3D).

Under low temperature conditions, spraying with exogenous ABA can improve the photosynthesis. As shown in Fig 4A, compared with T2, T1 treatment of LXD 4 increased by 4.84%, 14.02%, 12.01%, 21.31% and 18.39% and the ANOVA results showed that there was a significant difference at the 4th and 5th day. TJH showed that, T1 was significantly higher than T2 by 14.43%, 9.19%, 21.18%, 10.93% and 16.39%.

With the *G*s, it can be seen from Fig 3B, compared with T2, T1 treatment of LXD 4 significantly increased by 45.84%, 19.46%, 57.66% and 20.56% from 2d to 5d. TJH showed that T1 was significantly higher than T2 by 17.39%, 4.11% and 42.23% from 3d to 5d.

As shown in Fig 3C, compared with T2, T1 of LXD 4 increased by 5.67%, 8.30%, 9.77%, 19.54% and 13.95%, the analysis of variance showed that T1 was significantly higher than T2 at each time. For TJH, T1 was significantly higher than T2 by 6.90%, 10.58% and 11.47% during treatment from 3 to 5 days.

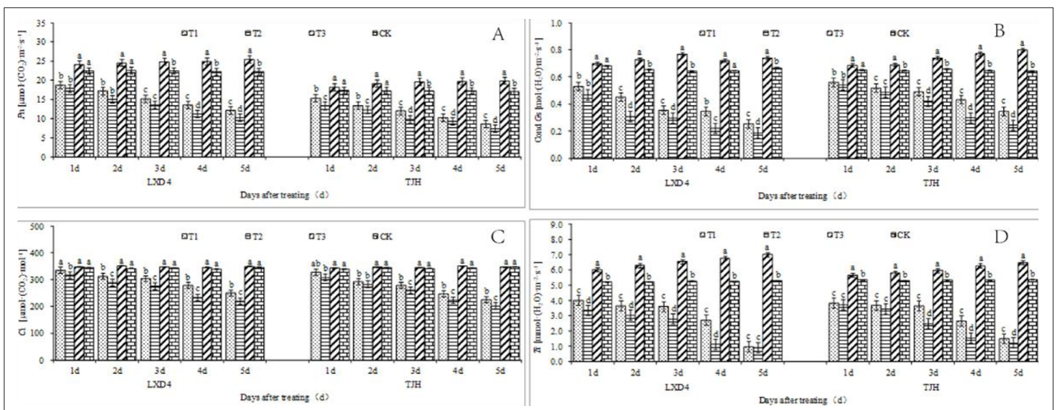
It can be seen from Fig 3D that, spraying exogenous ABA can inhibit the reduce of *T*r, compared with T2, T1 of LXD 4 was significantly higher than T2 by 19.54%, 20.33%, 28.17% and 140.35% from 1 to 4 days. For TJH, T1 was significantly higher than T2 by 47.05%, 72.28% and 20.91% from 3 to 5 days.

Effect of exogenous ABA on the content of osmotic regulation substances in adzuki beans leaves under chilling stress

Under the normal temperature condition, soluble sugar, soluble protein and proline content in adzuki bean leaves showed a stable change trend, but the content of these substances in leaves had an upward trend with the increase of chilling time intensity. As shown in Fig 4, the soluble sugar, soluble protein and proline contents in T2 were always significantly higher than CK.

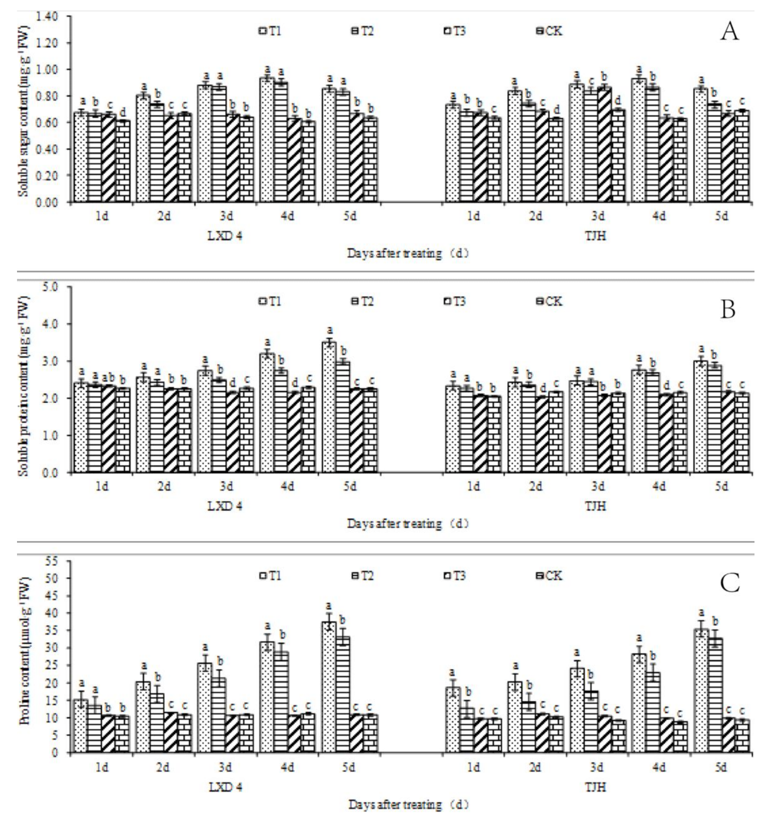
Under low temperature condition, spraying with exogenous ABA can further increase the content of osmotic regulation substances. As shown in Fig 4A, compared with T2, T1 treatment of LXD 4 significantly increased than T2 by 1.07% and 8.93% at 1d and 2d. TJH showed that, T1 was significantly higher than T2 at each time. With the soluble protein, it can be seen from Fig 4B that, compared with T2, T1 treatment of LXD 4 increased by 1.74%, 6.00%, 10.12%, 16.71% and 17.40%, the analysis of variance showed that after treatment 3 to 5 days, it showed that T1 was significantly higher than T2. TJH showed that, when treated from 4 to 5 days, T1 was significantly higher than T2 by 2.53% and 4.05%. The increasing effect of exogenous ABA on proline is very obvious. As shown in Fig 4C, compared with T2, T1 was always significantly higher than T2 both of LXD4 and TJH.

Photosynthesis is a determinant of crop productivity and an important indicator of crop response to environmental stress. Photosynthesis is most sensitive to chilling stress (Allen *et al*., 2001) and chilling stress significantly inhibits *P*n, resulting in a decrease in yield (Strauss *et al*., 2007). This study also found that low temperature led to a significant decrease in *P*n and at the same time, *G*s, *C*i and *T*r were



CK: Plant grown in natural environment + spraying H_2O; T1: Plant grown in Average 15°C + spraying exogenous ABA; T2: Plant grown in Average 15°C + spraying H_2O; T3: Plant grown in natural environment + spraying ABA.

Fig 3: Effect of exogenous ABA on net photosynthetic rate (*P*n) (A), stomatal conductance (*G*s) (B), intercellular CO_2 concentration (*C*i) (C) and transpiration (*T*r) (D) of adzuki beans leaves under chilling stress at flowering stage.



CK: Plant grown in natural environment + spraying H_2O; T1: Plant grown in Average 15°C + spraying exogenous ABA; T2: Plant grown in Average 15°C + spraying H_2O; T3: Plant grown in natural environment + spraying ABA.

Fig 4: Effect of exogenous ABA on osmotic regulation substances content of adzuki beans leaves under chilling stress at flowering stage.

also significantly reduced. We believe that this is because low temperature causes ROS metabolism disorder, which affects the structure and activity of photosynthetic organs, which in turn affects the capture, transformation and distribution of light energy in leaves. This view is also consistent with (Ayub *et al*., 2011).

When encountering adversity stress, plants will automatically turn on the protection system to resist the threat of adversity factors and then try to maintain normal physiological and metabolic activities and avoid damage (Xiang *et al*., 2019b). Plants subjected to low temperature stress will turn on a series of stress self-protection mechanisms and these protection systems coordinate organically with each other to minimize damage (Xiang *et al*., 2019a). Exogenous ABA has the function of regulating the stress resistance physiology of plants and has multiple pathways in resisting low temperature stress. The main stress response is to increase the content of osmotic regulators to regulate the content of MDA decreased. This study found that, exogenous ABA can effectively change the physiological indicators of stress resistance in adzuki bean under low temperature conditions. The application of exogenous ABA can significantly increase the content of osmotic adjustment substances, further reduce the content of MDA (Fig 2).

We can also see from the results of this study that spraying of exogenous ABA can significantly increase the yield per pot of adzuki bean (Table 2) after low temperature stress and effectively alleviate the impact of low temperature on yield.

CONCLUSION

The flowering stage is the crucial stage for yield formation and we found that low temperature during this stage causes a significant reduction of yield of adzuki bean. At the same time, the MDA content in adzuki beans leaves was increased. The content of soluble sugar, soluble protein and proline are increased. The photosynthetic parameters such as *P*n, *G*s, *C*i and *T*r were reduced in chilling condition. Exogenous ABA has the function of resisting low temperature and reducing crop damage. The results of this study indicated that, it can effectively increase the content of soluble sugar, soluble protein and proline and promote photosynthesis such as *G*s and *T*r and then improve the resistance of adzuki beans to low temperature stress and maintain normal physiological activities. Chilling during the flowering period caused changes in yield factors and low temperature caused a significant decrease in the grain weight per pot. Spraying exogenous ABA can significantly increase the yield per pot of LXD 4 by

6.72~17.77% and TJH by 6.41~37.04% under low temperature conditions. Thus, we conclude that exogenous ABA solution application at 20 mg×L^{-1} at flowering stage can enhance the adzuki bean production under chilling stress.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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