



Full Length Article

Uniconazole Foliar Spray Treatment Alleviates Cold Stress in Adzuki Bean (*Vigna angularis*) Seedlings

Hongtao Xiang^{1†}, Wan Li^{1†}, Dianfeng Zheng², Yuting Liang³, Xueyang Wang¹, Xiaoyan Liang², Tongtong Wang¹, Manli Wang¹, Chunjie Yang¹, Jia Liu⁴ and Ning He^{1*}

¹Crop Tillage and Cultivation Institute, Heilongjiang Academy of Agricultural Sciences, Harbin 150086, Heilongjiang, China

²College of Agronomy, Heilongjiang Bayi Agricultural University, Daqing 163319, Heilongjiang, China

³East University of Heilongjiang, Harbin 150066, Heilongjiang, China

⁴Plant Protection Institute, Heilongjiang Academy of Agricultural Sciences, Harbin 150086, Heilongjiang, China

*For correspondence: Zpszl5@aliyun.com

†Contributed equally to this work and are co-first authors

Received 02 August 2019; Accepted 21 September 2019; Published 08 January 2020

Abstract

Uniconazole is a plant growth regulator that inhibits the cytochrome P450 family of enzymes, which are involved in the catabolism of abscisic acid (ABA), and promotes plant growth and stress resistance. To investigate the cold-stress resistance of adzuki bean (*Vigna angularis*) seedlings, the cold-tolerant accession Baoqinghong (BQH) and the cold-sensitive accession Tianjinhong (TJH) were foliar sprayed with 20 μ M uniconazole, followed by cold stress at 15°C for 5 d. Our results revealed that foliar spray of 20 μ M uniconazole considerably increased the crop yield and yield-related parameters, including the number of pods per plant, the number of seeds per pot, and the 100-grain weight, in both TJH and BQH under cold stress. The malondialdehyde (MDA) and osmolyte content as well as antioxidant enzymatic activity were higher in BQH than in TJH. Foliar treatment with uniconazole (20 μ M; 225 L/Ha) significantly increased the yields in both BQH and TJH under cold-stress exposure. Therefore, we concluded that foliar treatment with 20 μ M uniconazole effectively promoted the cold tolerance of the adzuki bean cultivars by interacting with endogenous ABA and physiological adaptation, including the activities of antioxidant enzymes and the levels of osmolytes. These results provide novel insights and are expected to aid in the development of more effective stress resistance breeding programs in adzuki bean production. © 2020 Friends Science Publishers

Keywords: Cold; Uniconazole; Adzuki bean; Seedling stage; Yield

Abbreviations: ABA, abscisic acid; CAT, catalase; IAA, indole acetic acid; JA, jasmonic acid; MDA, malondialdehyde; NBT, nitroblue tetrazolium; POD, peroxidase; SA, salicylic acid; SOD, superoxide dismutase

Introduction

Uniconazole is a triazole-based plant growth regulator that inhibits the activities of the cytochrome P450 family of enzymes, which catabolize abscisic acid (ABA) (Kojima *et al.* 1996). This plant-growth retardant is involved in the processes of somatic embryogenesis (Li and Wolyn 1995), seedling growth (Jiang *et al.* 1998), flowering (Wijayanti *et al.* 1996), and stress resistance (Leul and Zhou 1999). Uniconazole exerts powerful inhibitory effects on ABA 8'-hydroxylase, causing alterations in the hydrophilic functional groups and conformations of many enzymes (Todoroki *et al.* 2008), including CYP707A, a key enzyme for controlling the ABA concentration (Todoroki *et al.* 2009), CYP701B1 (Miyazaki *et al.* 2011), and CYP701A6 in rice (Todoroki *et al.* 2012). In addition, uniconazole suppresses trans-zeatin biosynthesis in *Arabidopsis* (Sasaki *et al.* 2013). ABA is a plant hormone involved in the response to abiotic and biotic stresses. Treatment with ABA has been found to increase the

cold resistance of many plant species, such as Bermuda grass (*Cynodon dactylon*) (Huang *et al.* 2017), *Elymus nutans* (Fu *et al.* 2017), and rice (Xiang *et al.* 2017).

Adzuki bean (*Vigna angularis*) is an important legume crop and is widely cultivated in the Heilongjiang province of China. The color of grain can be red, white, black (Yook *et al.* 2017), or gray (Yang *et al.* 2015). Previous studies have established the production of starch (Aslinah *et al.* 2018) and the contents of tubulin (Mizuno *et al.* 1981), glycosylsterol, and saponins (Kojima *et al.* 1989) in *V. angularis*. More importantly, aqueous extracts of *V. angularis* have been demonstrated to have potential beneficial medicinal effects as well as applications in disease therapy (Itoh and Furuichi 2005; Baracho *et al.* 2016). In view of its importance and usage, the growth and related regulatory mechanisms in *V. angularis* have been extensively studied. For example, the gibberellin and colchicine present in the plant tissues have a positive influence on the microfibril arrangement of epidermal cell walls (Takeda and Shibaoka 1981). Moreover,

gibberellin A3 has been shown to decrease the expression level and enzymatic activity of 1-aminocyclopropane-1-carboxylic acid oxidase (Kaneta *et al.* 1997). However, the growth of adzuki beans in Heilongjiang province (northeastern China) is often adversely affected by cold stress. Treatment with uniconazole is proposed to improve the yield and antioxidative defense capacity of *V. angularis* by enhancing cold tolerance during the seedling stage. The objective of the present study was to investigate the effects of uniconazole treatment on improvement of cold tolerance in *V. angularis*. The findings illustrate the physiological mechanism of uniconazole in enhancing cold tolerance of adzuki beans at the seedling stage to improve adaptation, growth, and yields.

Materials and Methods

Plant materials and treatments

The cold-tolerant Baoqinghong (BQH) and the cold-sensitive Tianjinhong (TJH) were treated with uniconazole to investigate its mechanism of action on the cold-stress resistance in terms of physiological and yield parameters. Fifteen-day-old uniformly grown seedlings were planted, with three seedlings per pot, in plastic pots filled with black soil in an experimental farm in Harbin City, Heilongjiang province (45°75'27"N, 126°63'19"E), China. The normal growth temperature was maintained at 25°C. The seedlings were randomly divided into four groups, in which Uniconazole15°C and Uniconazole25°C were foliar sprayed with 20 µM uniconazole (CAS number 83657-22-1. Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), and CK15°C and CK25°C pots sprayed with nothing. After being fully absorbed within 1 d, the Uniconazole15°C and CK15°C pots were moved to a growth chamber at a daily temperature of 15°C for 5 d and randomly arranged on growth chamber benches with three replicates, whereas the Uniconazole25°C and CK25°C pots were kept at 25°C with a complete random experimental design with three repeats. Leaves of all four groups were collected from the same positions at 1, 2, 3, 4, and 5 d after cold treatment. After cold-stress, all seedlings were transplanted in the field to determine yields, number of pods per plant, number of grains per pot, and 100-grain weight (Zhao *et al.* 2006). The experimental layout was a randomized block design with replicates.

Physiological measurements

The activities of catalase (CAT, EC1.11.1.6), peroxidase (POD, EC1.11.1.7), and superoxide dismutase (SOD, EC 1.15.1.1) as well as the contents of free proline, soluble sugar, and malondialdehyde (MDA) were determined with three replicates using the methods described in our previous study (Xiang *et al.* 2017).

Briefly, MDA content was assayed by the thiobarbituric acid method. Leaf samples (0.1 g) were homogenized in 5 mL of 0.05 M phosphate buffer (pH 7.8) at 4°C. The

homogenate was centrifuged at 12,000 × g for 20 min. Then, 2.5 mL of 20% (w/v) trichloroacetic acid (TCA) containing 0.5% (w/v) thiobarbituric acid (TBA) was added to 1.5 mL of the supernatant. The mixture was heated in boiling water for 10 min and then quickly cooled in an ice bath. After the tube was centrifuged at 1800 × g for 10 min, the absorbance of the supernatant was recorded at 532, 600, and 450 nm with a GE Ultrospec 2100 Pro UV/Visible spectrophotometer (GE Healthcare, USA). The MDA concentration was calculated by the formula:

$$\text{MDA (mM)} = 6.459 (\text{A}_{532} - \text{A}_{600}) - 0.569 \text{A}_{450}.$$

SOD (EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT). For the total SOD assay, 5 mL of the reaction mixture containing 50 mM HEPES (pH 7.6), 0.1 mM EDTA, 50 mM Na₂CO₃, 13 mM methionine, 0.025% (w/v) Triton X-100, 75 mM NBT, 2 mM riboflavin, and an appropriate aliquot of the enzyme extract. The reaction mixtures were illuminated for 15 min at a light intensity of 350 mmol (photon) m⁻² s⁻¹. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm. Peroxidase (POD, EC1.11.1.7) activity was assayed with guaiacol as the hydrogen donor with an extinction coefficient of 26.6 mM cm⁻¹ at 470 nm. The reaction mixture consisted of 0.25% (v/v) guaiacol and 0.1 M H₂O₂ in 10 mM sodium phosphate buffer, pH 6.0. A series of dilutions of the crude enzyme preparations (0.1 mL) were added to 3 mL of the reaction mixture. Changes in absorbance at 470 nm from 0, 1, 2, and 3 min were recorded, and the activity of peroxidase was expressed as mmol (product) min⁻¹ g⁻¹ (FM). Enzyme activities were measured with a GE Ultrospec 2100 Pro UV/Visible spectrophotometer (GE Healthcare, USA).

CAT (EC1.11.1.6) activity was determined by continuously monitoring the decomposition of H₂O₂ at an absorbance of 240 nm, according to the reported method with a minor modification (Aebi1983). The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0) and 0.14 mM H₂O₂. Reaction was started by adding mitochondria into 1 mL of the reaction mixture, and the absorbance at 240 nm was monitored by a spectrophotometer. The molar extinction coefficient of H₂O₂ at 240 nm is 36 M cm⁻¹.

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 GE Ultrospec 2100 Pro UV/Visible spectrophotometer (GE Healthcare, USA).

ABA, salicylic acid (SA), indole acetic acid (IAA), and jasmonic acid (JA) measurements

ABA measurements were performed with three replicates, as reported previously (Wang and Chen 2011; Xiang *et al.*

2017). SA was extracted from leaves excised from adzuki bean plants; then, its quantity was determined three times by reverse-phase high-performance liquid chromatography according to the procedures specified in the study by Mohr and Cahill (2007). Approximately 100 mg of fresh leaf tissue was used to establish the amount of free IAA, with three replicates. The sample preparation was performed according to the procedure reported previously by Ludwig-Muller *et al.* (2009). The concentrations of endogenous IAA were calculated based on the isotope dilution principle (Cohen *et al.* 1986). Then, monitoring of the quinolinium ions (ions derived from endogenous IAA and $^{13}\text{C}_6$ -IAA, respectively) was conducted at m/z 130/136 (Mittag *et al.* 2015). Sample preparation through vapor-phase extraction, followed by chemical ionization gas chromatography/mass spectrometry, was employed for the JA measurements (Schmelz *et al.* 2004; Acosta *et al.* 2009).

Statistical analysis

All measurements were expressed as the mean \pm standard error of three replicates by SPSS (V24.0, IBM Corporation, Armonk, NY, USA). The results were analyzed by one-way analysis of variance and Duncan's test. Statistical significance was considered at $P < 0.05$. OriginPro2016 software (OriginLab Corporation, Northampton, MA, USA) was utilized to draw the figures.

Results

Effects of uniconazole treatment on adzuki bean yield

After cold-stress exposure for 5 d, the number of pods per plant (Table 1), the number of grains per pot (Table 2), the 100-grain weight (Table 3), and the yield (Table 4) decreased from 37.45 to 22.56 g, from 4.49 to 4.44 g, from 9.69 to 8.76 g, and from 44.98 to 26.23 g pot⁻¹, respectively, with statistically significant differences ($p < 0.01$). These results indicated that the cold treatment mainly resulted in a diminished number of pods per plant and also minimally affected the 100-grain weight and the number of seeds per pot. Uniconazole treatment effectively increased the cold-tolerance potential of TJH. After treatment with uniconazole, the number of pods per plant, the number of grains per pot, the 100-grain weight, and the yield of cold-stressed TJH were increased (per pot) from 22.56 to 32.33, from 4.44 to 4.58 g, from 8.76 to 9.32 g, and from 26.23 to 37.74 g, respectively. Although treatment with uniconazole increased the yield and cold resistance of TJH, it only partially alleviated the adverse effects caused by cold stress. In contrast, cold stress exerted minimal effects on the number of pods per plant of BQH, but it decreased the number of grains per pot, the 100-grain weight, and the yield of BQH. Treatment with uniconazole increased the yield and cold resistance in BQH, enabling recovery to achieve its normal growth. These results reveal that the cold-resistance mechanism in cold-tolerant BQH is different from that of TJH.

Effects of cold stress and uniconazole treatment on antioxidant enzymatic activities

The MDA contents as well as CAT, POD, and SOD enzymatic activities were analyzed to determine the mechanism of the cold tolerance in BQH (Fig. 1). At the normal growth temperature (25°C), treatment with uniconazole exerted minimal effects on the MDA contents as well as CAT, SOD, and POD activities of both BQH and TJH. The main difference was that the cold-stress treatment increased the MDA content as well as CAT, SOD, and POD activities more significantly in BQH than in TJH ($P < 0.01$).

Effects of cold stress and uniconazole treatment on osmotic adjustment and hormone accumulation

We also analyzed the contents of osmolytes. The contents of free proline, soluble sugar, and soluble protein in BQH were higher than in TJH, especially after cold treatment for 3 d (Fig. 2). Importantly, treatment with uniconazole elevated the ABA contents significantly more at the normal and low temperatures in BQH than in TJH ($P < 0.01$). Conversely, the ABA content was significantly increased by uniconazole treatment only at 15°C. The main difference between BQH and TJH was the ABA content of their leaves. Another significant difference was found in the SA content, that is, treatment with uniconazole followed by cold-stress exposure increased the SA content in BQH but exerted a minimal effect on the same parameter in TJH. Likewise, cold stress induced a higher IAA content in TJH than in BQH.

Discussion

Cold tolerance is important in affecting the quantity and quality of bean production. Previous studies have shown that cold tolerance is related to photosynthetic reactions (Hussain *et al.*, 2018; Xu *et al.* 2019), the content and activities of enzymes in the glyoxysomal membrane and the mitochondria (Breidenbach *et al.* 1974), as well as those of fatty acid desaturase (Zhang *et al.* 2011) and ABA (Shinkawa *et al.* 2013). The main functions of ABA are associated with the metabolism of protein phosphatase 2C (Xue *et al.* 2008). Reportedly, interactions of ABA with other proteins and transcription factors regulate the stress response (Park *et al.* 2009). In the present study, cold-stress treatment for 5 d exerted significant effects on the evaluated indicators in the cold-sensitive accession TJH. Specifically, cold stress decreased the number of pods per plant, the number of seeds per pot, and the 100-grain weight in TJH as well as the yield of BQH (Tables 1–4). Furthermore, uniconazole foliar treatment followed by cold stress recovered the yield of BQH to normal levels, while it only partially recovered the yield of TJH.

Considerable changes were also observed in the antioxidative enzymatic activities and the contents of

Table 1: Effect of uniconazole on the number of pods per plant in adzuki bean seedlings grown under low-temperature stress

Accession	Treatment	1 d	2 d	3 d	4 d	5 d
BQH	CK25°C	22.67 ± 1.34 ^{Aa}	22.67 ± 1.34 ^{Aa}	22.67 ± 1.34 ^{Aa}	22.67 ± 1.34 ^{Aa}	22.67 ± 1.34 ^{Aa}
	CK15°C	23.67 ± 1.20 ^{Aa}	23.11 ± 0.70 ^{Aa}	24.22 ± 0.69 ^{Aa}	23.00 ± 1.00 ^{Aa}	23.11 ± 1.69 ^{Aa}
	Uniconazole25°C	23.00 ± 1.33 ^{Aa}	23.00 ± 1.33 ^{Aa}	23.00 ± 1.33 ^{Aa}	23.00 ± 1.33 ^{Aa}	23.00 ± 1.33 ^{Aa}
	Uniconazole15°C	23.56 ± 0.84 ^{Aa}	22.67 ± 0.67 ^{Aa}	23.33 ± 1.53 ^{Aa}	22.67 ± 0.58 ^{Aa}	22.78 ± 1.65 ^{Aa}
TJH	CK25°C	37.45 ± 2.34 ^{Aa}	37.45 ± 2.34 ^{ABa}	37.45 ± 2.34 ^{Aa}	37.45 ± 2.34 ^{ABa}	37.45 ± 2.34 ^{ABa}
	CK15°C	26.11 ± 3.34 ^{Aa}	30.22 ± 1.35 ^{Cb}	30.56 ± 3.25 ^{ABa}	28.67 ± 3.34 ^{Cb}	22.56 ± 1.02 ^{Cc}
	Uniconazole25°C	29.78 ± 2.27 ^{Aa}	39.78 ± 2.27 ^{Aa}	39.78 ± 2.27 ^{Aa}	39.78 ± 2.27 ^{Aa}	39.78 ± 2.27 ^{Aa}
	Uniconazole15°C	25.00 ± 3.18 ^{Aa}	32.67 ± 3.28 ^{BCb}	30.00 ± 2.34 ^{Bb}	31.44 ± 1.90 ^{BCb}	32.33 ± 2.52 ^{Bb}

Data are expressed as the mean ± standard error of three replicates. The uppercase and lowercase letters within each column indicate the differences at 0.01 and 0.05 levels of probability, respectively. CK15°C, plants were not treated with uniconazole and were grown at 15°C; uniconazole15°C, plants were treated with uniconazole and were grown at 15°C; CK25°C, plants were not treated with uniconazole and were grown at 25°C; and uniconazole25°C, plants were treated with uniconazole and were grown at 25°C

Table 2: Effect of uniconazole on the number of grains per pot of adzuki bean seedlings grown under low-temperature stress

Accession	Treatment	1 d	2 d	3 d	4 d	5 d
BQH	CK25°C	4.43 ± 0.13 ^{Aab}	4.43 ± 0.13 ^{Aa}	4.43 ± 0.13 ^{Aa}	4.43 ± 0.13 ^{Aa}	4.43 ± 0.13 ^{Aa}
	CK15°C	4.29 ± 0.10 ^{Ab}	4.24 ± 0.24 ^{Aa}	4.06 ± 0.14 ^{Ab}	4.06 ± 0.11 ^{Bb}	3.86 ± 0.18 ^{Bb}
	Uniconazole25°C	4.55 ± 0.14 ^{Aa}	4.55 ± 0.14 ^{Aa}	4.55 ± 0.14 ^{Aa}	4.55 ± 0.14 ^{Aa}	4.55 ± 0.14 ^{Aa}
	Uniconazole15°C	4.33 ± 0.06 ^{Aab}	4.50 ± 0.01 ^{Aa}	4.27 ± 0.27 ^{Aab}	4.47 ± 0.07 ^{Aa}	4.45 ± 0.12 ^{Aa}
TJH	CK25°C	4.49 ± 0.33 ^{Aa}	4.49 ± 0.33 ^{Aa}	4.49 ± 0.33 ^{Aa}	4.49 ± 0.33 ^{Aa}	4.49 ± 0.33 ^{Aa}
	CK15°C	4.30 ± 0.22 ^{Aa}	4.48 ± 0.12 ^{Aa}	4.27 ± 0.19 ^{Aa}	4.29 ± 0.05 ^{Aa}	4.44 ± 0.11 ^{Aa}
	Uniconazole25°C	4.58 ± 0.13 ^{Aa}	4.58 ± 0.13 ^{Aa}	4.58 ± 0.13 ^{Aa}	4.58 ± 0.13 ^{Aa}	4.58 ± 0.13 ^{Aa}
	Uniconazole15°C	4.33 ± 0.43 ^{Aa}	4.47 ± 0.33 ^{Aa}	4.39 ± 0.10 ^{Aa}	4.44 ± 0.18 ^{Aa}	4.29 ± 0.09 ^{Aa}

Data are given as mean ± standard error of three replicates. The upper- and lowercase letters in each column indicate $P < 0.01$ and $P < 0.05$, respectively

Table 3: Effect of uniconazole on the 100-grain weight (g) of adzuki bean seedlings grown under low-temperature stress

Accession	Treatment	1 d	2 d	3 d	4 d	5 d
BQH	CK25°C	14.83 ± 0.69 ^{Aab}	14.83 ± 0.69 ^{Aab}	14.83 ± 0.69 ^{ABa}	14.83 ± 0.69 ^{ABab}	14.83 ± 0.69 ^{Aab}
	CK15°C	13.27 ± 0.80 ^{Ab}	13.10 ± 1.03 ^{Ab}	13.46 ± 0.59 ^{Bb}	13.20 ± 0.60 ^{Bb}	13.40 ± 1.06 ^{Ab}
	Uniconazole25°C	15.80 ± 0.97 ^{Aa}	15.80 ± 0.97 ^{Aa}	15.80 ± 0.97 ^{Aa}	15.80 ± 0.97 ^{Aa}	15.80 ± 0.97 ^{Aa}
	Uniconazole15°C	14.93 ± 1.25 ^{ABab}	14.21 ± 1.03 ^{ABab}	15.08 ± 0.50 ^{ABa}	14.23 ± 1.09 ^{ABab}	14.53 ± 1.19 ^{ABab}
TJH	CK25°C	9.69 ± 0.25 ^{Aa}	9.69 ± 0.25 ^{Aa}	9.69 ± 0.25 ^{Aa}	9.69 ± 0.25 ^{ABa}	9.69 ± 0.25 ^{Aab}
	CK15°C	9.54 ± 0.56 ^{Aa}	9.63 ± 0.16 ^{Aa}	9.21 ± 0.29 ^{Aa}	8.68 ± 0.37 ^{Bb}	8.76 ± 0.71 ^{Ab}
	Uniconazole25°C	10.06 ± 0.52 ^{Aa}	10.06 ± 0.52 ^{Aa}	10.06 ± 0.52 ^{Aa}	10.06 ± 0.52 ^{Aa}	10.06 ± 0.52 ^{Aa}
	Uniconazole15°C	9.85 ± 0.24 ^{Aa}	9.75 ± 0.16 ^{Aa}	9.73 ± 1.06 ^{Aa}	9.49 ± 0.26 ^{ABa}	9.32 ± 0.48 ^{ABab}

Data are given as mean ± standard error of three replicates. The upper- and lowercase letters in each column indicate $P < 0.01$ and $P < 0.05$, respectively

Table 4: Effect of uniconazole on the yield (g-pot⁻¹) of adzuki bean seedlings grown under low-temperature stress

Accession	Treatment	1 d	2 d	3 d	4 d	5 d
BQH	CK25°C	44.55 ± 1.29 ^{ABab}	44.55 ± 1.29 ^{ABab}	44.55 ± 1.29 ^{ABa}	44.55 ± 1.29 ^{Ab}	44.55 ± 1.29 ^{ABA}
	CK15°C	40.60 ± 5.40 ^{Bb}	38.60 ± 4.42 ^{ABbc}	39.67 ± 1.78 ^{Bb}	36.98 ± 0.52 ^{Bc}	35.91 ± 3.68 ^{Bb}
	Uniconazole25°C	49.50 ± 1.68 ^{Aa}	49.50 ± 1.68 ^{Aa}	49.50 ± 1.68 ^{Aa}	49.50 ± 1.68 ^{Aa}	49.50 ± 1.68 ^{Aa}
	Uniconazole15°C	45.63 ± 1.87 ^{ABab}	43.45 ± 2.53 ^{ABbc}	45.10 ± 4.30 ^{ABa}	43.29 ± 4.10 ^{ABa}	44.19 ± 5.47 ^{ABa}
TJH	CK25°C	44.98 ± 2.07 ^{Ab}	44.98 ± 2.07 ^{ABb}	44.98 ± 2.07 ^{ABab}	44.98 ± 2.07 ^{ABab}	44.98 ± 2.07 ^{ABa}
	CK15°C	44.18 ± 1.65 ^{Ab}	39.16 ± 2.42 ^{Bc}	36.09 ± 3.25 ^{Bc}	32.11 ± 4.76 ^{Bc}	26.23 ± 0.71 ^{Cc}
	Uniconazole25°C	50.40 ± 4.19 ^{Aa}	50.40 ± 4.19 ^{Aa}	50.40 ± 4.19 ^{Aa}	50.40 ± 4.19 ^{Aa}	50.40 ± 4.19 ^{Aa}
	Uniconazole15°C	44.54 ± 1.15 ^{Ab}	42.48 ± 1.45 ^{Bbc}	38.50 ± 6.09 ^{Bbc}	39.82 ± 4.15 ^{ABb}	37.74 ± 3.74 ^{Bb}

Data are given as mean ± standard error of three replicates. The upper- and lowercase letters in each column indicate $P < 0.01$ and $P < 0.05$, respectively

osmoregulatory substances (Fig. 1–2). Osmolytes, including free proline, soluble sugars (Creelman *et al.* 1990; Bertrand *et al.* 1994), and soluble proteins (del Rio *et al.* 1977), play crucial roles in the response of crops to abiotic stresses (Shabala 2013). An increase in the contents of osmoregulatory substances was established to protect the plasma membrane from the damage caused by oxidative stress effectively. The main function of most antioxidative enzymes, including CAT (Shikanai *et al.* 1998), POD (Ivanov and Edwards 2000; Stiborova *et al.* 2000), and SOD (Alscher *et al.* 2002), is the scavenging of reactive oxygen species. We showed that under normal conditions and low-temperature exposure, the CAT, POD, and SOD activities in BQH were higher than in TJH (Fig. 1–2).

The effects of uniconazole on plant hormonal activities have been comprehensively studied. For example, it has been reported that uniconazole-P treatments increase the ABA content but decrease those of IAA and GA₃ in Satsuma mandarin fruitlets (Kojima *et al.* 1996). Uniconazole also inhibited the biosynthesis of trans-zeatin in Arabidopsis (Sasaki *et al.* 2013). Although uniconazole promoted the activities of stress hormones while suppressed those of growth hormones, it increased the starch yield (Liu *et al.* 2015; 2019) and heavy metal accumulation (He *et al.* 2017). In this study, we found that foliar treatment with uniconazole increased the ABA content in TJH, resulting in a partial yield recovery. However, treatment with uniconazole did not elevate the levels of growth hormones such as IAA (Fig. 3).

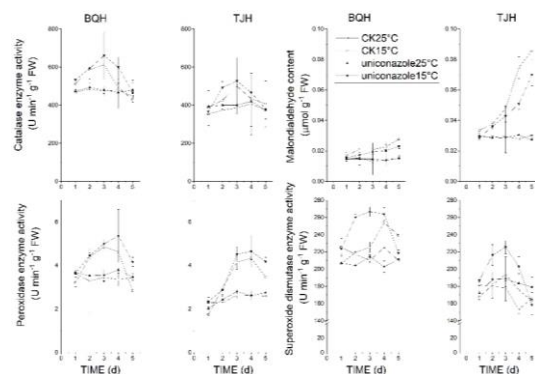


Fig. 1: CAT, POD, and SOD activities as well as MDA content in uniconazole-pretreated cold-tolerant BQH and cold-sensitive TJH after cold-stress exposure

FW, fresh weight; CK25°C and CK15°C, adzuki bean seedlings were grown at a normal temperature or under cold-stress exposure, respectively; uniconazole25°C and uniconazole15°C, adzuki bean seedlings were pretreated with 20 μM uniconazole for 1 d, followed by transferring to a temperature of 25°C or 15 °C for cold treatment for 1–5 days, respectively

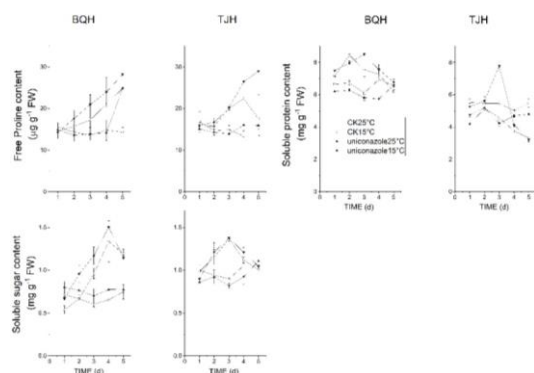


Fig. 2: Free proline, soluble protein, and soluble sugar contents in uniconazole-pretreated cold-tolerant BQH and cold-sensitive TJH after cold-stress exposure

Instead, under cold stress, this treatment induced a significant increase in the endogenous ABA and SA contents in both BQH and TJH due to the higher ABA content in the cold-tolerant BQH leaves than in the cold-sensitive TJH ones. However, cold-stress treatment reduced the BQH and TJH yields by different extents. Foliar treatment with uniconazole promoted an increase in the endogenous ABA content, resulting in full recovery of the BQH yield but only a partial recovery of the TJH yield after the cold treatment. These findings are beneficial for breeding programs that involve selection of adzuki bean accessions with high ABA contents.

Conclusion

Exogenous uniconazole treatment partially alleviated the adverse effects of cold stress on yield parameters of adzuki beans. The beneficial influence was caused by the increased ABA content induced by uniconazole, resulting in enhanced antioxidative enzymatic activities and osmolytes in the leaves of adzuki bean seedlings.

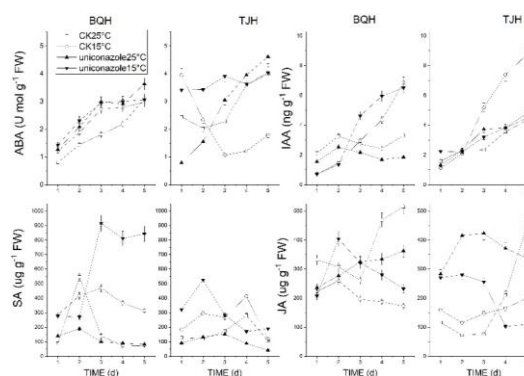


Fig. 3: ABA, IAA, SA, and JA contents in uniconazole-pretreated cold-tolerant BQH and cold-sensitive TJH after cold-stress exposure

Acknowledgments

This work was supported by open funds of the State Key Laboratory of Plant Physiology and Biochemistry (SKLPPBKF1803), the open funds of the National Center for Engineering and Technical Research of Coarse Grain (ZLKF201701, ZLKF201801), the earmarked fund for the China Agriculture Research System (CARS-08-02A), and Applied Research and Development Project of Heilongjiang Academy of Agricultural Sciences (2018YYF003).

References

Acosta IF, H Laparra, SP Romero, E Schmelz, M Hamberg, JP Mottinger, MA Moreno, SL Dellaporta (2009). *tasselseed1* is a lipoxxygenase affecting jasmonic acid signaling in sex determination of maize. *Science* 323:262–265

Aebi H (1983). Catalase *In:Methods of Enzymatic Analysis*, Vol 3, pp:273–277 Bergmeyer, H (ed) Verlag Chemie, Weinheim, Germany

Alscher RG, N Erturk, LS Heath (2002). Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J Exp Bot* 53:1331–1341

Aslinah LNF, M Mat Yusoff, MR Ismail-Fitry, (2018). Simultaneous use of adzuki beans (*Vigna angularis*) flour as meat extender and fat replacer in reduced-fat beef meatballs (bebola daging). *J Food Sci Technol* 55:3241–3248

Baracho NC, NF Monteiro, MG Borges, RR Arguelho (2016). Effect of aqueous extract of the *Vigna angularis* in rats subjected to an experimental model of moderate chronic kidney disease. *Acta Cir Bras* 31:527–532

Bertrand A, G Robitaille, P Nadeau, R Boutin (1994). Effects of soil freezing and drought stress on abscisic acid content of sugar maple sap and leaves. *Tree Physiol* 14:413–425

Breidenbach RW, NL Wade, JM Lyons (1974). Effect of chilling temperatures on the activities of glyoxysomal and mitochondrial enzymes from castor bean seedlings. *Plant Physiol* 54:324–327

Cohen JD, BG Baldi, JP Slovin (1986). C(6)-[benzene ring]-indole-3-acetic Acid: a new internal standard for quantitative mass spectral analysis of indole-3-acetic Acid in plants. *Plant Physiol* 80:14–19

Creelman RA, HS Mason, RJ Bensen, JS Boyer, JE Mullet (1990). Water deficit and abscisic acid cause differential inhibition of shoot versus root growth in soybean seedlings: analysis of growth, sugar accumulation, and gene expression. *Plant Physiol* 92:205–214

Del Rio LA, M Gomez, J Lopez-Gorge (1977). Catalase and peroxidase activities, chlorophyll and proteins during storage of pea plants of chilling temperatures. *Rev Esp Fisiol* 33:143–148

- Fu J, Y Wu, Y Miao, Y Xu, E Zhao, J Wang, H Sun, Q Liu, Y Xue, Y Xu, T Hu (2017). Improved cold tolerance in *Elymus nutans* by exogenous application of melatonin may involve ABA-dependent and ABA-independent pathways. *Sci Rep* 7:39865
- He J, L Lin, Q Ma, M Liao, X Wang, Y Lai, D Liang, H Xia, Y Tang, J Wang, L Wang (2017). Uniconazole (S-3307) strengthens the growth and cadmium accumulation of accumulator plant *Malachium aquaticum*. *Intl J Phytoremed* 19:348–352
- Huang X, H Shi, Z Hu, A Liu, E Amombo, L Chen and J Fu (2017). ABA is involved in regulation of cold stress response in bermudagrass. *Front Plant Sci* 8:1613
- Hussain S, AA Khan, A Shakoob, A Goheer, T Qadir, MM Khan, Z Hussain (2018). Effect of cold and heat stress on different stages of wheat: a review. *J Glob Innov Agric Soc Sci* 6:123–128
- Itoh T, Y Furuichi (2005). Hot-water extracts from adzuki beans (*Vigna angularis*) stimulate not only melanogenesis in cultured mouse B16 melanoma cells but also pigmentation of hair color in C3H mice. *Biosci Biotechnol Biochem* 69:873–882
- Ivanov B, G Edwards (2000). Influence of ascorbate and the Mehler peroxidase reaction on non-photochemical quenching of chlorophyll fluorescence in maize mesophyll chloroplasts. *Planta* 210:765–774
- Jiang S, T Honma, T Nakamura, I Furukawa, F Yamamoto (1998). Regulation by uniconazole-P and gibberellins of morphological and anatomical responses of *Fraxinus mandshurica* seedlings to gravity. *IWA J* 19:311–320
- Kaneta T, T Kakimoto, H Shibaoka (1997). Gibberellin A3 causes a decrease in the accumulation of mRNA for ACC oxidase and in the activity of the enzyme in azuki bean (*Vigna angularis*) epicotyls. *Plant Cell Physiol* 38:1135–1141
- Kojima K, A Goto, S Nakashima (1996). Effects of Uniconazole-P on Abscission and endogenous ABA, IAA, and GA-like substances levels of Satsuma Mandarin fruitlet. *Biosci Biotechnol Biochem* 60:901–902
- Kojima M, M Ohnishi, S Ito, Y Fujino (1989). Characterization of acylmono-, mono-, di-, tri- and tetraglycosylsterol and saponin in Adzuki bean (*Vigna angularis*) seeds. *Lipids* 24:849–853
- Leul M, WJ Zhou (1999). Alleviation of waterlogging damage in winter rape by uniconazole application: effects on enzyme activity, lipid peroxidation, and membrane integrity. *J Plant Growth Regul* 18:9–14
- Li B, DJ Wolyn (1995). The effects of ancymidol, abscisic acid, uniconazole and paclobutrazol on somatic embryogenesis of asparagus. *Plant Cell Rep* 14:529–533
- Liu C, N Feng, D Zheng, H Cui, F Sun, X Gong (2019). Uniconazole and diethyl aminoethyl hexanoate increase soybean pod setting and yield by regulating sucrose and starch content. *J Sci Food Agric* 99:748–758
- Liu Y, Y Fang, M Huang, Y Jin, J Sun, X Tao, G Zhang, K He, Y Zhao, H Zhao (2015). Uniconazole-induced starch accumulation in the bioenergy crop duckweed (*Landoltia punctata*) II: transcriptome alterations of pathways involved in carbohydrate metabolism and endogenous hormone crosstalk. *Biotechnol Biofuels* 8:64
- Ludwig-Muller J, S Julke, NM Bierfreund, EL Decker, R Reski (2009). Moss (*Physcomitrella patens*) GH3 proteins act in auxin homeostasis. *New Phytol* 181:323–338
- Mittag J, A Gabrielyan, J Ludwig-Muller (2015). Knockout of GH3 genes in the moss *Physcomitrella patens* leads to increased IAA levels at elevated temperature and in darkness. *Plant Physiol Biochem* 97:339–349
- Miyazaki S, T Katsumata, M Natsume, H Kawaide (2011). The CYP701B1 of *Physcomitrella patens* is an ent-kaurene oxidase that resists inhibition by uniconazole-P. *FEBS Lett* 585:1879–1883
- Mizuno K, M Koyama, H Shibaoka (1981). Isolation of plant tubulin from azuki bean epicotyls by ethyl N-phenylcarbamate-Sepharose affinity chromatography. *J Biochem* 89:329–332
- Mohr PG, DM Cahill (2007). Suppression by ABA of salicylic acid and lignin accumulation and the expression of multiple genes, in Arabidopsis infected with *Pseudomonas syringae* pv tomato. *Funct Integr Genom* 7:181–191
- Park SY, PFung, NNishimura, DR Jensen, HFujii, Y Zhao, S Lumba, J Santiago, ARodrigues, TFChow, SE Alfred, DBonetta, RFinkelstein, NJPrivart, D Desveaux, PLRodriguez, PMcCourt, JK Zhu, JISchroeder, BF Volkman, SR Cutler (2009). Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* 324:1068–1071
- Sasaki E, T Ogura, K Takei, M Kojima, N Kitahata, H Sakakibara, T Asami, Y Shimada (2013). Uniconazole, a cytochrome P450 inhibitor, inhibits trans-zeatin biosynthesis in Arabidopsis. *Phytochemistry* 87:30–38
- Schmelz EA, J Engelberth, JH Tumlinson, A Block, HT Alborn (2004). The use of vapor phase extraction in metabolic profiling of phytohormones and other metabolites. *Plant J* 39:790–808
- Shabala S (2013). Learning from halophytes: Physiological basis and strategies to improve abiotic stress tolerance in crops *Ann Bot*, 112:1209–1221
- Shikanai T, T Takeda, H Yamauchi, S Sano, KI Tomizawa, A Yokota, S Shigeoka, 1998 Inhibition of ascorbate peroxidase under oxidative stress in tobacco having bacterial catalase in chloroplasts *FEBS Lett* 428:47–51
- Shinkawa R, A Morishita, K Amikura, R Machida, H Murakawa, K Kuchitsu, M Ishikawa (2013). Abscisic acid induced freezing tolerance in chilling-sensitive suspension cultures and seedlings of rice. *BMC Res Notes* 6:351
- Stiborova M, HH Schmeiser, E Frei (2000). Oxidation of xenobiotics by plant microsomes, a reconstituted cytochrome P450 system and peroxidase: a comparative study. *Phytochemistry* 54:353–362
- Takeda K, H Shibaoka (1981). Effects of gibberellin and colchicine on microfibril arrangement in epidermal cell walls of *Vigna angularis* Ohwi et Ohashi epicotyls. *Planta* 151:393–398
- Todoroki Y, K Kobayashi, H Yoneyama, S Hiramatsu, MH Jin, B Watanabe, M Mizutani, N Hirai (2009). Enlarged analogues of uniconazole, new azole containing inhibitors of ABA 8'-hydroxylase CYP707A. *Bioorg Med Chem Lett* 19:5782–5786
- Todoroki Y, K Kobayashi, H Yoneyama, S Hiramatsu, MH Jin, B Watanabe, M Mizutani, N Hirai (2008). Structure-activity relationship of uniconazole, a potent inhibitor of ABA 8'-hydroxylase, with a focus on hydrophilic functional groups and conformation. *Bioorg Med Chem* 16:3141–3152
- Todoroki Y, K Naiki, T Muramatsu, T Ohnishi, K Ueno, M Mizutani, N Hirai (2012). A conformationally restricted uniconazole analogue as a specific inhibitor of rice ent-kaurene oxidase, CYP701A6. *Bioorg Med Chem Lett* 22:3240–3243
- Wang LF, YY Chen (2011). Photosynthetic characterization at different senescence stages in an early senescence mutant of rice *Oryza sativa* L. *Photosynthetica* 49:140–144
- Wijayanti L, S Fujioka, M Kobayashi, A Sakurai (1996). Effect of uniconazole and gibberellin on the flowering of *Pharbitis nil*. *Biosci Biotechnol Biochem* 60:852–855
- Xiang H, T Wang, D Zheng, L Wang, Y Feng, Y Luo, R Li, Z Li, Y Meng, W Li (2017). ABA pretreatment enhances the chilling tolerance of a chilling-sensitive rice cultivar. *Braz J Bot* 40:853–860
- Xu C, Z Yang, M Wang, XZQ Zheng, J Li, HHL Wang, Y Zou (2019). Effects of low temperature on photosynthesis and antioxidant enzyme activities of *Panax notoginseng* during seeding stage. *Intl J Agric Biol* 21:1279–1286
- Xue T, D Wang, S Zhang, J Ehrling, F Ni, S Jakab, C Zheng, Y Zhong (2008). Genome-wide and expression analysis of protein phosphatase 2C in rice and Arabidopsis. *BMC Genomics* 9:550
- Yang K, Z Tian, C Chen, L Luo, B Zhao, Z Wang, L Yu, Y Li, Y Sun, W Li, Y Chen, Y Li, Y Zhang, D Ai, J Zhao, C Shang, Y Ma, B Wu, M Wang, L Gao, D Sun, P Zhang, F Guo, W Wang, Y Li, J Wang, RK Varshney, J Wang, HQ Ling, P Wan (2015). Genome sequencing of adzuki bean (*Vigna angularis*) provides insight into high starch and low fat accumulation and domestication. *Proc Natl Acad Sci USA* 112:13213–13218
- Yook JS, KA Kim, M Kim, YS Cha (2017). Black Adzuki bean (*Vigna angularis*) attenuates high-fat diet-induced colon inflammation in mice. *J Med Food* 20:367–375
- Zhang YM, CC Wang, HH Hu, L Yang (2011). Cloning and expression of three fatty acid desaturase genes from cold-sensitive lima bean (*Phaseolus lunatus* L). *Biotechnol Lett* 33:395–401
- Zhao, BH, P Wang, HX Zhang, QS Zhu, JC Yang (2006). Source-sink and grain-filling characteristics of two-line hybrid rice Yangliangyou 6. *Rice Sci* 13:34–42