Jasmonic acid regulates ascorbate and glutathione metabolism in *Agropyron cristatum* leaves under water stress

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1. Introduction

Water stress is one of the main environmental factors that adversely affect plant growth, productivity and survival. Water stress usually induces the accumulation of reactive oxygen species (ROS), which cause oxidative damage to plants [1,2]. If not effectively and rapidly removed from plants, ROS can damage a wide range of cellular macromolecules such as lipids, enzymes and DNA [3]. Plants can protect themselves against oxidative damage by antioxidant system including antioxidative enzymes and nonenzymatic compounds [4].

Ascorbate and glutathione are two crucial nonenzymatic compounds involved in defence against oxidative stress. It is well known that plants can adjust ascorbate and glutathione contents by modulating the regeneration and biosynthesis of ascorbate and glutathione. L-Galactose pathway is the main biosynthetic pathway of ascorbate in plants, L-galactono-1,4-lactone dehydrogenase (GalLDH) is the key enzyme in this way [5]. Gamma-glutamylcysteine synthetase (γ-ECS) is the key enzyme for glutathione biosynthesis [6]. Ascorbate–glutathione cycle is the recycling pathway of ascorbate and glutathione regeneration. Thus, the ascorbate–glutathione cycle plays an important role in maintaining the contents of ascorbate and glutathione in plants. In this cycle, ascorbate peroxidase (APX) utilizes AsA as electron donor for reduction of H₂O₂, monodehydroascorbate is reduced to AsA by monodehydroascorbate reductase (MDHAR) and dehydroascorbate is reduced to AsA by dehydroascorbate reductase (DHAR). Oxidized glutathione (GSSG) produced in this cycle is reduced to GSH by glutathione reductase (GR) [7]. By this way, AsA and GSH are regenerated and H₂O₂ is scavenged.

Jasmonic acid and methyl jasmonate (MeJA), collectively termed jasmonates, are regarded as endogenous regulators that play important roles in regulating stress responses, plant growth and development [8]. Levels of endogenous jasmonates increase during stress conditions such as treatment with elicitors, wounding, ozone stress and water stress [9–18]. Evidence has showed that the expression of jasmonate-responsive genes (JRGs) is altered under stress conditions such as pathogen infection, wounding and ozone stress, and thus contributes to stress tolerance in plants [9–11,19–23]. It has been reported that JRGs can also be induced by drought [24,25]. Increasing evidence
showed that exogenously supplied JA or MeJA increased antioxidative ability of plants under water stress [26–28]. Ascorbate and glutathione metabolism are important parts of the antioxidant metabolism in plants. It has been documented that jasmonic acid is involved in the regulation of ascorbate and glutathione metabolism under ozone stress [23]. Previous studies have also showed that jasmonates play an important role in signaling drought-induced antioxidant responses, including ascorbate [29–31]. Gene expression in response to environmental stress is usually studied at the transcript level because this gives a more precise estimate of antioxidant gene activation than enzyme activity. Previous studies with respect to the regulation of ascorbate and glutathione metabolism by JA under water stress have been focused on the activities of enzymes, involved in ascorbate and glutathione metabolism. However, the transcriptional responses of these enzyme genes under water stress are still not clear.

*Agropyron cristatum* is a native grass with high nutritional value and a strong resistance to the drought stress, which naturally grows in the semiarid area of loess hilly-gully region on the Loess Plateau of northwestern China. In our previous observation, *A. cristatum* showed a strong antioxidant ability under water stress (data not shown). However, the regulation mechanism of antioxidant metabolism of *A. cristatum* is still unknown. Therefore, investigating the regulation mechanism of the ascorbate and glutathione metabolism is important for elucidating antioxidant mechanism of *A. cristatum* under water stress.

In this study, we investigated JA levels, the transcript levels and activities of enzymes involved in ascorbate and glutathione metabolism, and the contents of AsA, GSH, total ascorbate and total glutathione in the leaves of *A. cristatum* exposed to water stress induced by PEG-6000. The aim of the study was to elucidate how jasmonic acid regulates ascorbate and glutathione metabolism at both molecular and physiological levels under water stress, and provide new knowledge to antioxidant metabolism in plants under water stress.

### 2. Materials and methods

#### 2.1. Plant material, growth conditions and treatments

The seeds of native grass *A. cristatum* were collected from Yan’an city in the semiarid area of loess hilly-gully region on the Loess Plateau of northwestern China.

Seeds of *A. cristatum* were sown in plastic trays filled with a sand/vermiculite matter mix (2:1, v/v) and grown in a greenhouse under the same conditions as described above. To test whether the effects of IBU could be reversed by exogenously supplied JA, another group of plants were pretreated with 5 mM IBU + 1 mM JA for 12 h, and then exposed to water stress treatment for 24 h. The control plants were treated with distilled water alone under the same conditions as above groups. After treatment of 0 h, 4 h, 8 h, 12 h, and 24 h, the fifth leaf of *A. cristatum* plants were collected and frozen in liquid nitrogen, and then kept at –80 °C until used for analyses.

#### 2.2. Analysis of APX, GR, DHAR, MDHAR

Enzymes were extracted according to Grace and Logan [32] with some modifications. Each frozen sample (0.5 g) was ground into a fine powder in liquid N₂ with a mortar and pestle. Fine powder was homogenized in 6 ml 50 mM KH₂PO₄ (pH 7.5) containing 0.1 mM ethylenediaminetetraacetic acid, 0.3% (v/v) Triton X-100, and 1% (w/v) insoluble polyvinylpolypyrrolidone. The extract was immediately centrifuged at 13,000 × g for 15 min at 2 °C. The supernatant was then used immediately for measuring the following enzymes.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured by monitoring the decrease in absorbance at 290 nm [33]. The assay mixture (2.5 ml) contained 50 mM phosphate buffer (pH 7.3), 0.1 mM ethylenediaminetetraacetic acid, 1 mM H₂O₂, 10 mM AsA and enzyme extract. The reaction was initiated by adding H₂O₂. One unit of enzyme was defined as the amount of APX catalyzing the oxidation of 1 μmol ascorbate per minute. A molar coefficient of 2.8 mM⁻¹ cm⁻¹ was used for the calculation of enzyme activity.

Glutathione reductase (GR, EC 1.6.4.2) activity was monitored at 340 nm in 3 ml reaction mixture containing 100 mM Tris–HCl (pH 8.0), 0.5 mM ethylenediaminetetraacetic acid, 0.5 mM MgCl₂, 10 mM oxidized glutathione (GSSG), 1 mM NADPH and enzyme extract. The reaction was initiated by adding NADPH [32]. One unit of GR activity was defined as the reduction of 1 μmol NADPH per minute. A molar coefficient of 6.2 mM⁻¹ cm⁻¹ was used for the calculation of enzyme activity.

Monodehydroascorbate reductase (MDHAR, EC 1.6.5.4) activity was assayed at 340 nm in 3 ml reaction mixture containing 50 mM Hepes–KOH (pH 7.6), 1 mM NADH, 2.5 mM AsA, 2.5 units AsA oxidase (EC 1.10.3.3) and enzyme extract. The reaction was initiated by adding AsA oxidase [34]. One unit of MDHAR activity was defined as the amount of enzyme that oxidizes 1 μmol NADH per minute. A molar coefficient of 14.6 mM⁻¹ cm⁻¹ was used for the calculation of enzyme activity.

Dehydroascorbate reductase (DHAR, EC 1.8.5.1) activity was measured at 265 nm in 3 ml assay solution containing 100 mM Hepes–KOH (pH 7.0), 20 mM GSH, and 2 mM DHA. The reaction was initiated by adding DHA [35]. One unit of DHAR activity was defined as the amount of enzyme that produces 1 μmol AsA per minute. A molar coefficient of 14.6 mM⁻¹ cm⁻¹ was used for the calculation of enzyme activity.

The specific enzyme activity for all the above enzymes was expressed as units mg⁻¹ protein.

#### 2.3. Analysis of GalLDH and γ-ECS

i-Galactono-1,4-lactone dehydrogenase (GalLDH, EC 1.3.2.3) was extracted and measured by the method of Tabata et al. [36] with slight modifications. Each frozen sample (0.1 g) was ground into a fine powder in liquid N₂ with a mortar and pestle. Fine powder was homogenized in 0.1 M potassium phosphate buffer (pH 7.4) containing 0.4 M sucrose. The extract was passed through 2 layers of miracloth and then immediately centrifuged at 300 × g for 10 min at 2 °C. The supernatant was centrifuged at 10,000 × g for 20 min at 2 °C. The sediment was suspended in 0.5 ml of the above buffer containing 0.4 M sucrose for the measurement of GalLDH activity. The assay mixture, in a final volume of 2.4 ml, was composed of enzyme solution (200 μl), 1.05 mg ml⁻¹ Cyt c (2 ml) and 56 mM i-Gal (200 μl). Before assay, the mixture was incubated at 25 °C for 1 min. The increase in absorbance at 550 nm was followed immediately after the addition of i-Gal. One unit of activity is defined as the amount of extract required to oxidize...
1 nmol of l-Gal (equivalent to the formation of 2 nmol of reduced Cyt c) per minute. A molar coefficient of 17.3 mM−1 cm−1 was used for the calculation of enzyme activity. The specific enzyme activity was expressed as units g−1 FW.

Gamma-glutamylcysteine synthetase (γ-ECS, EC 6.3.2.2) was extracted and measured by the method of Ogawa et al. [37] with some modifications. Each frozen sample (0.1 g) was ground into a fine powder in liquid N2 with a mortar and pestle. Fine powder was homogenized in 0.1 M HCl. The extract was immediately centrifuged at 20,000 × g for 10 min at 2 °C. The supernatant was then used for the assay of enzyme activity. The mixture of 200 μl supernatant and 400 μl 50 mM Tris–HCl (pH 7.6) containing 0.25 mM glutamate, 10 mM ATP, 1 mM dithioerythritol and 2 mM cysteine reacted at 25 °C for 1 h. Then 600 μl phosphorus agent containing 3 mM H2SO4, distilled water, 2.5% ammonium molybdate and 10% vitamin c was added and mixed adequately. The mixture was incubated at 45 °C for 25 min. The mixture was cooled at room temperature after reaction finished. The absorbance at 660 nm was measured. One unit of γ-ECS activity is defined as 1 μmol cysteine-dependently generated PO43− per minute. A molar coefficient of 5.6 mM−1 cm−1 −1 was used for the calculation of enzyme activity. The specific enzyme activity was expressed as units mg−1 protein.

2.4. Analysis of AsA, GSH, total ascorbate and total glutathione

AsA and DHA were measured according to Hodges et al. [38]. For each sample, DHA was estimated from the difference between total ascorbate and AsA.

Total glutathione, GSSG and GSH were measured according to Griffith [39]. For each sample, GSH was estimated from the difference between total glutathione and GSSG.

2.5. Measurement of protein concentration

Protein concentration was measured using bovine serum albumin as standard according to the method of Bradford [40].

2.6. Isolation of total RNA and semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was isolated from leaves by using TRNzol Total RNA Reagent (TIANGEN) according to the instruction supplied by the manufacturer. Approximately 3 μg of total RNA were reverse transcribed using oligo(dT) primer and TIANScript RT Kit (TIANGEN). cDNA was amplified with the GeneAmp PCR System 9700 Thermal Cycler by using primers designed according to the gene fragment of following enzymes in A. cristatum. The gene specific primers, accession number, melting temperatures, PCR protocols and size of amplified fragments are shown in Table 1. To standardize the results, the relative abundance of β-actin was also determined and used as the internal standard.

2.7. Determination of JA content

The contents of jasmonic acid in leaves were measured by gas chromatograph according to the method described by Lan et al. [14] and Deng et al. [41] with some modifications. Briefly, fresh leaves were ground to a fine powder in liquid N2 with a mortar and pestle. Extraction was done by adding 10 ml 80% methanol solution with 300 ng internal standard dihydrojasmonic acid (dHA) at 4 °C for 24 h [42]. Samples were centrifuged for 10 min at 4200 × g for 5 min. The supernatant was evaporated in vacuo to remove methanol and dissolved in phosphate buffer (pH 8.2), and then a few polyvinylpyrrolidone (PVP) was added and mixed. After standing for 5 min, above solution was centrifuged at 4200 × g for 5 min. The supernatant was then extracted by ether three times. The water phase was collected and extracted by the same volume of hexane, and concentrated to 1 ml under vacuum. The sample solution was dried by nitrogen and redissolved in the solution containing 1 ml benzene–petroleum ether (1:1, v/v) and 1 ml 1.6% KOH–methanol. After reacting at room temperature for 20 min, the solution was extracted by 8 ml distilled water. The upper solution was dried by nitrogen and redissolved in 200 μl ethanol, and immediately injected into gas chromatograph.

A GC-2010A gas chromatograph (Shimadzu Corporation, Japan) was used for the analysis. Compounds were separated on an HP-1MS column (30 m × 0.25 mm × 0.25 μm). Injection volume was 1 μl. The initial temperature was 50 °C and then temperature programmed at 20 °C/min to 280 °C, with nitrogen as the carrier gas (flow rate 50 ml/min). The identity of the compounds was determined by the comparison of their retention time with pure commercially available standards. The content of JA was quantified by the method of Deng et al. [41].

2.8. Measurement of malondialdehyde content and electrolyte leakage

Malondialdehyde content was measured by thiobarbituric acid (TBA) reaction as described by Hodges et al. [43]. Electrolyte leakage was determined according to Zhao et al. [44].

<table>
<thead>
<tr>
<th>Gene and accession no.</th>
<th>Primer sequences</th>
<th>Melting temperatures</th>
<th>PCR protocols</th>
<th>Size of amplified fragments (bp)</th>
</tr>
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</table>
| GalLDH (GQ457296)      | Forward: GCCTGAGTGTGAAATA
Reverse: GATCGAGTTGTTAAAT | 52.7
50.4 | 94 °C/2 min; 94 °C/30 s, 53 °C/30 s, 72 °C/2 min, 30 cycles; 72 °C/10 min | 280 |
| γ-ECS (GQ457297)       | Forward: TTCGCACTTTGAGGACTT
Reverse: CTGGCTAATGCGAAAT | 52.7
55.7 | 94 °C/2 min; 94 °C/30 s, 55 °C/30 s, 72 °C/2 min, 30 cycles; 72 °C/10 min | 305 |
| DHAR (GQ457298)        | Forward: CACCTTGGACGCCCTCCTG
Reverse: TTGCGGCGCCGAGTAG | 53.4
52.1 | 94 °C/2 min; 94 °C/30 s, 53 °C/30 s, 72 °C/2 min, 25 cycles; 72 °C/10 min | 130 |
| MDHAR (GQ457299)       | Forward: GGAAGTGGGCGCCACACTAC
Reverse: TCTACGGAGTTGCTAC | 57.3
57.5 | 94 °C/2 min; 94 °C/30 s, 55 °C/30 s, 72 °C/2 min, 30 cycles; 72 °C/10 min | 276 |
| APX (GQ457300)         | Forward: AGATGTCTTCTTAGTGGTG
Reverse: GCTGTCGGTAGTAGTG | 55.4
57.3 | 94 °C/2 min; 94 °C/30 s, 55 °C/30 s, 72 °C/2 min, 25 cycles; 72 °C/10 min | 140 |
| GR (GQ457301)          | Forward: CAATGGTGAGCCGACGAA
Reverse: ACTTATCGACACCCACCTT | 57.3
57.3 | 94 °C/2 min; 94 °C/30 s, 55 °C/30 s, 72 °C/2 min, 27 cycles; 72 °C/10 min | 166 |
| β-Actin (GQ457302)     | Forward: GAGCGTGTTACTTCATCA
Reverse: TGCGCTACAGATCTCCTTC | 55.4
57.3 | 94 °C/2 min; 94 °C/30 s, 55 °C/30 s, 72 °C/2 min, 30 cycles; 72 °C/10 min | 304 |
electrolyte leakage was expressed as the relative ion leakage, a percentage of the total conductivity after boiling.

2.9. Statistical analysis

The results presented were the mean of five replicates. Means were compared by one-way analysis of variance and Duncan’s multiple range test at the 5% level of significance.

3. Results

3.1. Effect of water stress and pretreatment with JA biosynthesis inhibitor (IBU) on JA content

The effects of water stress and IBU on JA content in A. cristatum leaves are shown in Fig. 1. Water stress led to an increase in JA content within the 24 h of treatment. A significant increase in JA content occurred within 12 h of treatment. After 12 h and 24 h of treatment, the content of JA increased 4.7-fold and 5.0-fold, respectively, compared to the control. Pretreatment with IBU markedly inhibited the accumulation of JA in leaves under water stress. After 12 h and 24 h, pretreatment with IBU inhibited the increase in JA content induced by water stress by 68.9% and 76.8%, respectively. However, the pretreatment with IBU alone did not affect JA content in non-stressed leaves. These results indicated that pretreatment with IBU could significantly inhibited the accumulation of JA in leaves of A. cristatum under water stress.

3.2. The selection of suitable JA treatment concentration

In order to select a suitable JA treatment concentration, we investigated the effects of different JA concentrations on the contents of AsA, total ascorbate, GSH and total glutathione under water stress. The JA concentrations are 0.1 μM, 1 μM, 5 μM, and 10 μM, respectively. The results showed that water stress increased the contents of AsA, total ascorbate, GSH and total glutathione (Fig. 2A–D). Pretreatment with IBU significantly decreased the content of AsA, total ascorbate, and GSH in the water-stressed leaves but not the content of total glutathione, compared to water stress treatment. Meanwhile, exogenous JA was added at different concentrations together with IBU to determine...
whether the effects of IBU could be reversed by exogenously supplied JA under water stress. Among different JA concentrations, application of 1 μM JA significantly increased the content of AsA, total ascorbate, and GSH under water stress, but not the content of total glutathione, compared to water stress treatment. These results suggested that 1 μM JA was a suitable concentration to study the regulation of JA in ascorbate and glutathione metabolism in A. cristatum leaves under water stress. Above results also showed that JA could significantly affect the contents of AsA, total ascorbate and GSH but not the content of total glutathione under water stress.

3.3. Effects of water stress and pretreatment with IBU on the transcript levels and activities of key enzymes in ascorbate and glutathione metabolism

Figs. 3–8 show that the transcript levels and activities of key enzymes in ascorbate and glutathione metabolism, including APX, GR, DHAR, MDHAR, γ-ECS and GalLDH, increased under water stress. An obvious increase in the transcript levels of APX (Fig. 3A), GR (Fig. 4A), DHAR (Fig. 5A), MDHAR (Fig. 6A), γ-ECS (Fig. 7A), and GalLDH (Fig. 8A) occurred from 4 h to 24 h of treatment, compared with the control. The activities of APX (Fig. 3B), GR (Fig. 4B), DHAR (Fig. 5B), MDHAR (Fig. 6B), γ-ECS (Fig. 7B) and GalLDH (Fig. 8B) also increased significantly from 4 h to 24 h of treatment, compared with the control. After 12 h of treatment, the activities of APX, GR, DHAR, MDHAR, γ-ECS and GalLDH increased by 82.8%, 56.3%, 95.4%, 90.5%, 46.9%, and 89.4%, respectively. After 24 h of treatment, the activities of APX, GR, DHAR, MDHAR, γ-ECS and GalLDH increased by 68.1%, 54.1%, 77.3%, 90%, 46.3%, and 96.2%, respectively.

In order to determine whether the increases in the transcript levels and activities of key enzymes in ascorbate and glutathione metabolism were related to the accumulation of JA in stressed leaves, the effects of IBU on the transcript levels and activities of above enzymes were investigated. At the same time, 1 μM JA was added together with IBU in order to determine whether the effects of IBU could be reversed by exogenously supplied JA. The results showed that the pretreatment with IBU significantly reduced the transcript levels of GalLDH, APX, GR, DHAR and MDHAR induced by water stress. Pretreatment with IBU alone did not affect the transcript levels and activities of these enzymes in non-stressed leaves (Figs. 3–8). The exogenous application of 1 μM JA prevented the reduction in the transcript level and activity of γ-ECS in stressed leaves (Fig. 7A and B). Pretreatment with IBU alone did not affect the transcript level and activity of γ-ECS in non-stressed leaves.
compared with the control. These results suggested that the accumulation of JA was involved in the regulation of ascorbate and glutathione metabolism under water stress.

3.4. Effects of water stress and pretreatment with IBU on the content of AsA, total ascorbate, GSH, total glutathione and the redox state of ASA and GSH

To further investigate whether the ascorbate and glutathione metabolism were related to the accumulation of JA in the leaves of A. cristatum under water stress, the effects of pretreatment with IBU on the contents of AsA, total ascorbate, GSH and total glutathione were studied. Exogenous JA was added together with IBU to determine whether the effects of IBU could be reversed. Pretreatment with IBU significantly reduced the contents of AsA, GSH and total ascorbate induced by water stress. Pretreatment with IBU alone did not affect the contents of these antioxidants in non-stressed leaves (Fig. 9A–C). Exogenous application of JA prevented the reduction in the contents of AsA, GSH and total ascorbate induced by water stress (Fig. 9C). However, the content of total glutathione was not obviously affected by supplying JA or pretreating with IBU under water stress (Fig. 9D). These results suggested that the accumulation of JA increased the contents of AsA, GSH and total ascorbate but not the content of total glutathione under water stress. Above results suggested once again that the accumulation of JA was involved in the regulation of ascorbate and glutathione metabolism under water stress.

The changes in the redox state of ASA and GSH are shown in Fig. 9E and F. Water stress caused a decrease in the ratios of AsA/DHA and GSH/GSSG, compared with the control. Pretreatment with IBU significantly decreased the ratios of AsA/DHA and GSH/GSSG of stressed leaves, compared with the control and water stress treatment. Exogenous application of JA could prevent the decrease in the ratios of AsA/DHA and GSH/GSSG induced by IBU in stressed leaves. These results suggested that JA had an important role in maintaining the redox state of ASA and GSH under water stress.

3.5. Effects of water stress and pretreatment with IBU on the malondialdehyde content and electrolyte leakage

To further investigate whether JA has an important role for water stress tolerance in A. cristatum, the effects of pretreatment with IBU on the malondialdehyde content and electrolyte leakage were studied under water stress. Water stress caused an increase in the malondialdehyde content and electrolyte leakage (Fig. 10A and B), suggesting that oxidative stress was induced in leaves. Pretreatment with IBU significantly increased the malondialdehyde content and electrolyte leakage of stressed leaves compared with the control and water stress treatment, suggesting that oxidative stress was enhanced in IBU-inhibited plants. Exogenous application of JA could reverse the effects of IBU. These results suggested that JA had an important role for acquisition of water stress tolerance in A. cristatum.
4. Discussion

AsA is an important compound of plant antioxidant system and a major redox compound in plants. A novel AsA biosynthetic pathway in plants has been proposed by Wheeler et al. [5]. GalLDH is the key enzyme in this AsA biosynthetic pathway. Thus, the content of AsA in plants is closely related to the GalLDH activity. Besides, the activation of the AsA recycling pathway is also important for increasing the cellular content of AsA [45]. In this study, we found that JA regulated the ascorbate metabolism by increasing the transcript levels and activities of GalLDH, APX, DHAR and MDHAR, and the contents of AsA and total ascorbate under water stress. Under ozone stress, it has been documented that JA induces the accumulation of AsA and increases the transcript level and activity of DHAR [23], which was consistent with our experimental results under water stress. Besides, our study also indicated that the accumulation of JA increased the transcript levels and activities of MDHAR, APX and GalLDH under water stress.

GSH is another important compound of plant antioxidant system. The cellular content of GSH can be determined by γ-ECS and GR, which are the key enzymes for glutathione biosynthetic and recycling pathway, respectively. The results of our study showed that JA could induce the GSH metabolism by increasing the transcript level and activity of GR, and the content of GSH under water stress. It has been reported that JA induces glutathione biosynthesis by increasing the transcript levels of γ-ECS (GSH1) and GSH2 encoding glutathione synthetase (GSHS) under ozone stress [23]. Xiang and Oliver [46] also reported that jasmonic acid treatment increased the transcript levels of γ-ECS and GSH2, as well as GR. Our results also showed that JA increased the transcript level and activity of GR under water stress. However, our results gave no evidence that JA affected the transcript level and activity of γ-ECS under water stress obviously. In this study, we did not investigate whether the transcript level and activity of GSHS was regulated by JA under water stress. Thus, further investigation of the transcript level and activity of GSHS can help us to elucidate the regulation of glutathione metabolism by JA.

Previous study showed that expression of GSH1 gene in Arabidopsis was increased and no increase of APXs and GRs genes was observed by JA treatment [23]. These results are different from our data by using A. cristatum. Since JA was not completely inhibited by IBU in our study, perhaps the low levels of JA present were able to upregulate the expression of GSH1 gene and thus trigger total glutathione metabolism responses but not ascorbic acid responses. The discrepancy in the expression of GSH1 gene between Arabidopsis and A. cristatum may be due to above reason. While the discrepancy in the expression of APXs and GRs genes between Arabidopsis and A. cristatum may be due to the difference of plant species.

The plant hormone abscisic acid (ABA), as a stress signal, also increases as a result of water stress. Increasing evidence indicated that one mode of ABA action may be related to its role in the oxidative stress in plants [47]. It has been documented that ABA can induce the expression of antioxidant genes encoding APX and GR, and increase the activities of APX and GR in maize seedlings [23]. Xiang and Oliver [46] also reported that jasmonic acid treatment increased the transcript levels of γ-ECS and GSH2, as well as GR. Our results also showed that JA increased the transcript level and activity of GR under water stress. However, our results gave no evidence that JA affected the transcript level and activity of γ-ECS under water stress obviously. In this study, we did not investigate whether the transcript level and activity of GSHS was regulated by JA under water stress. Thus, further investigation of the transcript level and activity of GSHS can help us to elucidate the regulation of glutathione metabolism by JA.
ABA can also increase the contents of AsA and GSH [49,50]. These results suggested that ABA was involved in the regulation of ascorbate and glutathione metabolism under water stress. In the present study, our results also showed that JA increased the transcript levels and activities of APX and GR, and the contents of AsA and GSH in leaves under water stress. Besides, evidence has showed that JA has many similar characteristics to ABA, such as inducing stomatal closure, inhibiting plant growth, promoting senescence and being involved in stress response, and they can cooperate dependently or independently with each other [51]. Peña-Cortés et al. discovered that ABA and MeJA operated through a common signaling pathway for the activation of the wound-inducible expression of Pin2 in potato and tomato [52]. Wounding led to enhanced ABA levels which stimulated jasmonate biosynthesis and jasmonate then caused accumulation of Pin2 transcripts. However, Lan et al. [27] presumed that water stress led to enhanced JA levels which stimulated ABA biosynthesis and ABA then increased drought-resistant ability of apple plants. Bandurska et al. reported that application of exogenous JA increased the content of ABA under water deficit in two barley genotypes [26]. There is also evidence showing that jasmonate signaling can be uncoupled from abscisic acid signaling in barley [53]. Up to now, the relationship between ABA and JA in regulating ascorbate and glutathione metabolism in plants under water stress is still unknown. Therefore, it is very interesting to investigate the relationship between ABA and JA in regulating ascorbate and glutathione metabolism in leaves under water stress.

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expression of specific genes, have been reported [55]. Therefore, it is also very interesting to investigate the relationship between NO and JA in regulating glutathione metabolism in plants under water stress. Although some signaling pathways that activate transcription of each enzyme gene have been reported, whether there are other signaling pathways are still not clear. Thus, the signaling pathways that regulate the transcript levels of key enzymes in ascorbate and glutathione metabolism are still needed to be further studied. JA, as a stress signal, increases as a result of water stress and plays important roles in the regulation of plant responses. However, the signal transduction of JA in regulating the ascorbate and glutathione metabolism is still unknown. It has been reported that NO, H₂O₂, and MAPK all participate in the process of JA signal transduction [56–58]. However, whether NO, H₂O₂, and MAPK participate in the signal transduction of jasmonic acid in regulating the transcript levels and activities of key enzymes in ascorbate and glutathione metabolism remains unknown. Therefore, it is necessary to study the roles of NO, H₂O₂, and MAPK in the ascorbate and glutathione metabolism regulated by JA, in order to provide more new knowledge to the antioxidant metabolism in plants.

In conclusion, our results clearly suggested that water-stress-induced JA accumulation participated in the regulation of ascorbate and glutathione metabolism by increasing the transcript levels and activities of APX, GR, MDHAR, DHAR, GAlDLH, and the contents of AsA, GSH, total ascorbate, which, in turn, enhanced the antioxidant ability and protects A. cristatum against oxidative stress induced by water stress. Our results suggested that the transcriptional control of antioxidant metabolism enzymes was a main way in the regulation of ascorbate and glutathione metabolism by JA. These results provide new knowledge to the antioxidant metabolism in plants under water stress conditions.

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