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Exogenous ABA Enhances the Antioxidant Defense System of Maize by Regulating the AsA-GSH Cycle under Drought Stress

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Abstract: When drought occurs during the maize-filling period, the probability of yield decline increases. Abscisic acid (ABA) plays a regulatory role in physiological and metabolic activities during plant development. However, its effect on the antioxidant system of maize leaves during the grain-filling stage is unclear. Maize plants (Zhengdan958) were used as an experimental material, and ABA was sprayed on the leaves during the grain-filling stage. The plants were placed under drought conditions to analyze the relationship between the ascorbate-glutathione (AsA-GSH) cycle and hydrogen peroxide (H_2O_2) removal. Exogenous ABA significantly reduced the malondialdehyde content, relative electrolyte leakage, and H_2O_2 under drought stress. This is similar to the exogenous ABA effect on the AsA-GSH cycle. Exogenous ABA upregulated the transcription of related genes and alleviated the inhibition of drought stress on the monodehydroascorbate reductase and dehydroascorbate reductase activities, thereby further increasing the ascorbate peroxidase and glutathione reductase activities. It contributed to an increase in the AsA and GSH levels and inhibited the decrease in the AsA/dehydroascorbic acid and GSH/oxidized glutathione ratios. Therefore, exogenous ABA plays an important role in improving the antioxidant capacity and drought resistance physiology of maize by enhancing antioxidant enzyme activity and stabilizing the AsA and GSH redox state.

Keywords: maize; drought stress; abscisic acid; AsA-GSH cycle; gene expression

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1. Introduction

Current estimates indicate that the world population will reach 9.6 billion by 2050 [1,2]. It is expected that grain production must increase by 50% in the next 40 years to meet the needs of the population [3]. Global warming leads to an increase in drought stress frequency. Water is a key factor in plant growth and water deficit leads to growth retardation, metabolism obstruction, and productivity decline. The sources of energy and protein in human nutrition makes maize (*Zea mays* L.) the third-largest crop globally [4]. Maize is sensitive to water deficits during the key growth and development period, such as the grain-filling stage. Therefore, it is imperative to improve the drought resistance of maize during the grain-filling period to ensure global food security and establish sustainable agriculture.

The imbalance between the production and clearance of reactive oxygen species (ROS) in plants inhibits its growth and photosynthetic capacity [5]. This includes superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and hydroxyl radicals (OH). Some studies have suggested that the damage to chloroplast development under water stress is caused by the metabolic imbalance of oxygen free radicals [6]. Although H_2O_2 is not a free radical, it is the source of many oxygen-containing free radicals and the conversion of other ROS. H_2O_2 is an important signaling molecule in plant cells, capable of mediating

the response of plants to stress [7]. H_2O_2 may mediate ABA-induced stomatal closure by controlling potassium channels on the cytoplasmic membrane and activating calcium channels [8]. H_2O_2 affects gene expression and metabolism and induces programmed death of plant cells [9–11]. The physiological regulation of H_2O_2 is crucial for metabolic activities, as abnormal accumulation causes damage to the membrane system of tissues and organs. Under water-deficit conditions, the massive ROS accumulation will destroy the stability of protein, sugar, and nucleic acid molecules [12]. This, in turn, results in the peroxidation of unsaturated fatty acids in the biofilm, that is, membrane peroxidation, resulting in a decrease in membrane fluidity, structural damage, and an increase in permeability [13,14]. The degree of membrane lipid peroxidation is reflected by the malondialdehyde (MDA) content. MDA poisons plant cells by causing intramolecular or intermolecular cross-linking of proteins [15].

Aerobic organisms continuously produce active oxygen, which is more serious under abiotic stress. The ROS level must be regulated. After long-term adaptation to their environment, plants have formed a relatively perfect active oxygen scavenging system. This system has evolved to regulate cellular redox status and coordinate gene expression to resist damage to the cell structure and function caused by ROS accumulation with strong oxidation ability and promote plant resistance [16–19]. The ascorbate-glutathione (AsA-GSH) cycle has proven to be the main metabolic pathway for scavenging H_2O_2 [20]. This cycle can remove reactive oxygen free radicals and protect the structure of the cell membrane and maintain its function. Therefore, it is reasonable to believe that the strength of intracellular antioxidant systems is the basis for determining the stress resistance of plants [21].

The AsA-GSH reduction system includes AsA, GSH, ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) [22]. They cooperate to complete the removal of H_2O_2 under drought stress [23]. The AsA-GSH cycle varied slightly in different tests, but the basic framework was similar, including some related metabolic reactions (Figure 1) [24]. APX is the first catalytic enzyme for H_2O_2 removal in the AsA-GSH cycle. It can catalyze AsA to reduce H_2O_2 to H_2O and form monodehydroascorbate (MDHA). However, the half-life of MDHA is not long and unstable. One part can be reduced to AsA by MDHAR, while the other can spontaneously and disproportionately produce dehydroascorbic acid (DHA). Under the catalysis of DHAR, DHA can use GSH as a reducing agent to form AsA and oxidized glutathione (GSSG) to complete the regeneration of AsA. GSSG can be catalytically reduced to GSH by GR, which is the key enzyme that maintains the stability of GSH levels in cells [25]. The AsA-GSH circulatory system exists in the chloroplasts, cytoplasm, mitochondria, and peroxisomes of plants. Moreover, several genes encoding AsA-GSH circulating enzymes have been identified, including two GR genes (GR1 and GR2) and three DHAR genes (DHAR1, DHAR2, and DHAR3) [26].

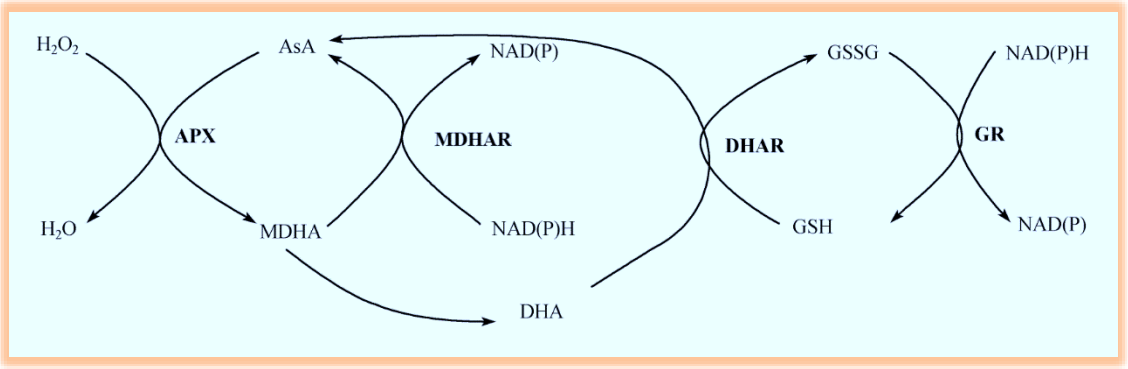


Figure 1. Illustration of the ascorbate-glutathione (AsA-GSH) cycle.

In different plant growth stages, the regulatory effect of plant hormones on stress responses has proven to be valuable. Abscisic acid (ABA) is a stress hormone that affects the growth and development of plants under abiotic stress (drought, heavy metals, heat, high salinity, low temperature, and radiation), especially water-related stress [27]. This allows the effective transport of ABA between cells because of its metabolism, perception, transport, and systemic effects [28]. Studies have shown that ABA, as a signal molecule, actively regulates fast and slow ABA communication pathways, endowing cells with the ability to resist dehydration, improve osmotic stress tolerance, and maintain the balance between plant internal growth and the environment. Under drought stress, the overexpression of 9-cis-epoxy carotenoid dioxygenase in tomatoes increased ABA accumulation and drought resistance and reduced transpiration levels [29]. It has been reported that ABA may be related to oxidative stress in maize cells [30]. Exogenous ABA is known to have a positive effect on plant stress tolerance. Some studies have found that ABA improves the activity of antioxidant enzymes in drought-resistant crops [31] and up regulates the expression of a variety of water stress-related genes. Environmental and human regulatory factors can affect the metabolic pattern and intensity of the AsA-GSH cycle [32]. It has been proven that it can induce antioxidant defenses by increasing the activities of APX, MDHAR, DHAR, and GR to provide plants with oxidative stress tolerance. Therefore, external regulation is an important method to improve plant antioxidant capacity.

Many photosynthetic products must be transported to the grain during the filling stage of maize. To improve the stress resistance of leaves, it is necessary to understand the regulatory mechanisms of exogenous hormones. In this study, the maize cultivar, Zhengdan958 (ZD958), was pretreated with exogenous ABA and subjected to water stress during grain filling. The effects of ABA on AsA-GSH in maize under water stress conditions were studied. This study provides a theoretical basis and technical support for exogenous ABA to improve antioxidant mechanisms and reduce the damage caused by drought stress.

2. Materials and Methods

2.1. Plant Material, Growth Conditions, Experimental Design, and Sampling

The inbred maize line, ZD958, was used as the test material (Henan Agricultural Materials Company, Zhengzhou, China). The experimental site was an artificial dry shed of the Northeast Agricultural University in Harbin. The Meidilianhua chemical company (Beijing, China) provided the chemical reagent ABA.

The treatment during the test was performed in the dry shed. The experimental plastic buckets measured 27.5 cm (top diameter) × 20 cm (bottom diameter) × 25 cm (height) with drainage holes at the bottom. Put 12.5 kg (pH 6.85) of black soil into each bucket. The main nutrients of black soil are analyzed as follows: total nitrogen (1.43 g·kg^{-1}), available potassium (254.55 mg·kg^{-1}), available phosphorus (33.93 mg·kg^{-1}), organic matter (4.86 g·kg^{-1}), and alkali hydrolysable nitrogen (186.17 mg·kg^{-1}). All the potted plants were placed on the same plot, and soil indicators were measured according to unified standards, applying fertilizer on time to ensure that they are not disturbed by climate and nutrient factors, and standardizing the system of pest control and weed removal.

After pollination, maize plants were treated with one of the following three treatments: (1) CK, untreated; (2) drought stress (for 4, 7, or 10 days); (3) drought stress + ABA, i.e., foliar sprayed with ABA (0.5 mmol L^{-1}) under drought stress. This ABA dose was selected based on previous experiments (data not shown). This experiment was repeated five times.

An SWP-100 portable soil water potential meter (Nanjing Institute of soil research, Chinese Academy of Sciences) was used to test soil water potential for 24 h. Three fixed measuring points were set for each treatment (the buried depth of the probe is about 15 cm). The amount of irrigation water was determined by a weighing method. The irrigation water for normal water supply and rewatering treatment was 80 mm (soil relative water content = 75–80%). When the soil water potential is lower than the set value, supplementary irrigation should be used to restore the preset water potential, and the irrigation water volume should be recorded. After

treatment, samples (leaves) were collected every three days. To determine the antioxidant index, the middle of the maize ear leaf was sampled, either fresh or frozen in liquid nitrogen (−80 °C).

2.2. Measurement of H_2O_2 Content

Fresh corn leaves (2 g) were added into a 4 mL buffer solution (PBS, 50 mM, pH 6.8), ground in an ice bath, and transferred to a centrifugal tube for centrifugation (10,000× *g*, 4 °C, 15 min). The supernatant was then added to a mixture of titanium disulfate and 20% sulfuric acid (*v*/*v*). Measure the absorbance value at 415 nm and calculate the H_2O_2 content according to the standard curve [33].

2.3. Measurement of MDA Content

Weigh 1g of corn leaves, grind with 5 mL buffer (TCA, 10%, *w*/*v*) and centrifuge (10,000× *g*, 20 min 4 °C); take the supernatant as the test sample. Take 2 mL of centrifuged sample, mix 2 mL of thiobarbituric acid (TBA, 0.6%, *w*/*v*), cool quickly after reaction (100 °C, 15 min), and centrifuge (10,000× *g*, 10 min, 4 °C) [34]. The absorbance values of the supernatant were measured at 450 nm, 532 nm, and 600 nm.

2.4. Determination of Electrolyte Leakage

The leaves were washed with deionized water, and the surface water was absorbed using filter paper. Deionized water (10 mL) was added to the weighing bottle containing the leaves and placed in a vacuum drying oven, and the air was pumped out with a vacuum pump for 10 min. The weighing bottles were placed on the oscillator and oscillated for 1 h. Measure the initial conductivity (S1) with a conductivity meter. After measurement, each weighing bottle was placed in a boiling water bath for 10 min to kill the plant tissue. After removing the test tube, it was cooled with tap water, shaken evenly. The final conductivity value (S2) was measured according to the formula for electrolyte leakage (EL) = S1/S2 × 100.

2.5. Measurement of Antioxidant Enzyme Activities

To determine APX enzyme activity, we followed the methods described by Zhu [35]. The reaction system consisted of 850 µL phosphate-buffered saline (PBS, pH 7.0, containing 0.1 mM EDTA), 50 µL AsA (5 mM), and 50 µL H_2O_2 (20 mM). The AsA oxidation was initiated by monitoring H_2O_2 levels (extinction coefficient ε = 2.8 mM cm^{-1}). The enzyme activity was calculated by the decrease in absorbance at 290 nm.

The decrease in absorbance value per minute at a wavelength of 349 nm (by the oxidation of NADH, ε = 6.2 mM cm^{-1}) was used to measure the activity of MDHAR. The reaction system included Tris-HCl buffer (50 mM, pH 7.5), NADH (0.2 mM), AsA (2.5 mM), AsA oxidase (0.15 U), and 50 µL enzyme solution [36].

DHAR activity was determined according to the method described by Doulis [37]. The reaction system included 50 µL enzyme activity, 850 µL PBS (pH 7.0, containing 0.1 mM EDTA), 50 µL GSH (2.5 mM), and 50 µL DHA (0.1 mM). Record the change in absorbance per minute at 265 nm (by forming ASA, ε = 14 mm cm^{-1}) to calculate DHAR activity.

GR activity was calculated by recording the change in absorbance per minute at 340 nm (through the oxidation of NADPH, ε = 6.2 mM cm^{-1}). The reaction system consisted of 1.2 mL Tris-HCl buffer (100 mM, pH 8.0), 0.4 mL GSSH (1 mM), 0.3 mL NADPH (0.2 mM), and 0.1 mL enzyme solution [38].

The standard curve of bovine serum protein was used to calculate the protein concentration [39].

2.6. Measurements of AsA/DHA and GSH/GSSG in Leaves

Weigh 0.5 g of corn leaves, grind with 5 mL phosphoric acid (5%, *v*/*v*) buffer, and centrifuge (20 min, 10,000× *g*, 4 °C). Take the supernatant after centrifugation as the determination sample. The contents of AsA, DHA, and total AsA were determined according to Hodges' method [40]: DHA = total AsA-AsA. The contents of GSH, GSSG, and total GSH were determined according to Griffith's method [41]: GSH = total GSH-GSSG.

2.7. RNA Isolation and Real-Time Quantitative Polymerase Chain Reaction

First, total RNA was isolated from ear position leaves of maize. Trizol reagent (Invitrogen, Carlsbad, CA, USA) was used. Specific primers for each gene were designed from the 3′ ends of the gene sequences using Primer Premier 5.0 (Table 1). The primer melting temperature (T_m) value was between 55–60 °C. The cDNA sample was diluted five times for use as a template for computer detection. The reagent used for real-time quantitative PCR was 2× SG Fast qPCR Master Mix (B639271, BBI), and PCR was performed on a LightCycler 480 II (Roche Molecular Systems Inc., Pleasanton, CA, USA) [26]. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative transcript levels.

Table 1. Primer sequences for real-time RT-PCR.

Gene	Forward Sequence	Reverse Sequence
GR1	5′-CGGTGCAATAGTGGTTGATG-3′	5′-CCTATTGGTGGTTGGGAGAA-3′
GR2	5′-CGATATTGCGGTTAAATGTG-3′	5′-AAGTTCGTCTTTGGCTTGGA-3′
DHAR1	5′-CATCAAGACTAAGCCCACCAA-3′	5′-TAGAAACATGGCCACCACAA-3′
DHAR2	5′-CAATGTCCATGCCTACACCA-3′	5′-CAGGTAGCACCAAAGCACAA-3′
DHAR3	5′-CGAGGAAAAATGGATTGGTG-3′	5′-TGTTCCATCGCTTGGATCTT-3′

2.8. Statistical Analysis

Experimental data are represented as the mean ± standard deviation (SD). Microsoft Excel 2007 and SPSS 17 software were used for statistical analysis. Fisher's least significant difference test was used to compare the treatment methods, and the significance level was $p < 0.05$.

3. Results

3.1. Effects of Drought Stress and/or ABA Application on the Relative Water Content, Relative Electrolyte Leakage, MDA Content, and H_2O_2 Content of Leaves

Drought stress significantly reduced the relative water content of maize leaves, and exogenous ABA alleviated the downward trend (Figure 2a). Drought stress significantly increased the relative EL of maize plants (Figure 2b). With the extension of drought stress time, the relative EL of the plants increased gradually. The application of exogenous ABA inhibited the increase in the relative EL under drought stress conditions. Compared to drought treatment alone, ABA treatment decreased the relative EL by 20.86%, 19.3%, and 27.36% on the 4th, 7th, and 10th day of drought treatment, respectively.

As shown in Figure 2, the MDA content in functional maize leaves continued to increase with the extension of drought stress time. The MDA content reached a maximum on the 10th day of drought stress treatment, which increased by 145.31% compared to the control treatment. On the 4th, 7th, and 10th days of drought stress, after exogenous ABA treatment, the content of MDA in the functional leaves of ZD958 decreased by 17.35%, 34.50%, and 22.24%, respectively, compared with drought treatment alone. The most obvious effect of ABA was on the seventh day. This shows that ABA can effectively inhibit membrane lipid peroxidation damage to the cell membrane under drought stress and protect the integrity of the cell membrane.

Under drought stress, the H_2O_2 content in maize plants increased gradually with increasing stress time. On the 4th, 7th, and 10th days of drought stress, the H_2O_2 content increased by 97.40%, 105.90%, and 135.56%, respectively, compared to the control; the application of ABA decreased by 21.46%, 21.79%, and 30.91%, respectively. The effect of exogenous ABA was almost the same on the 4th and 7th days of drought stress and reached a maximum on the 10th day.

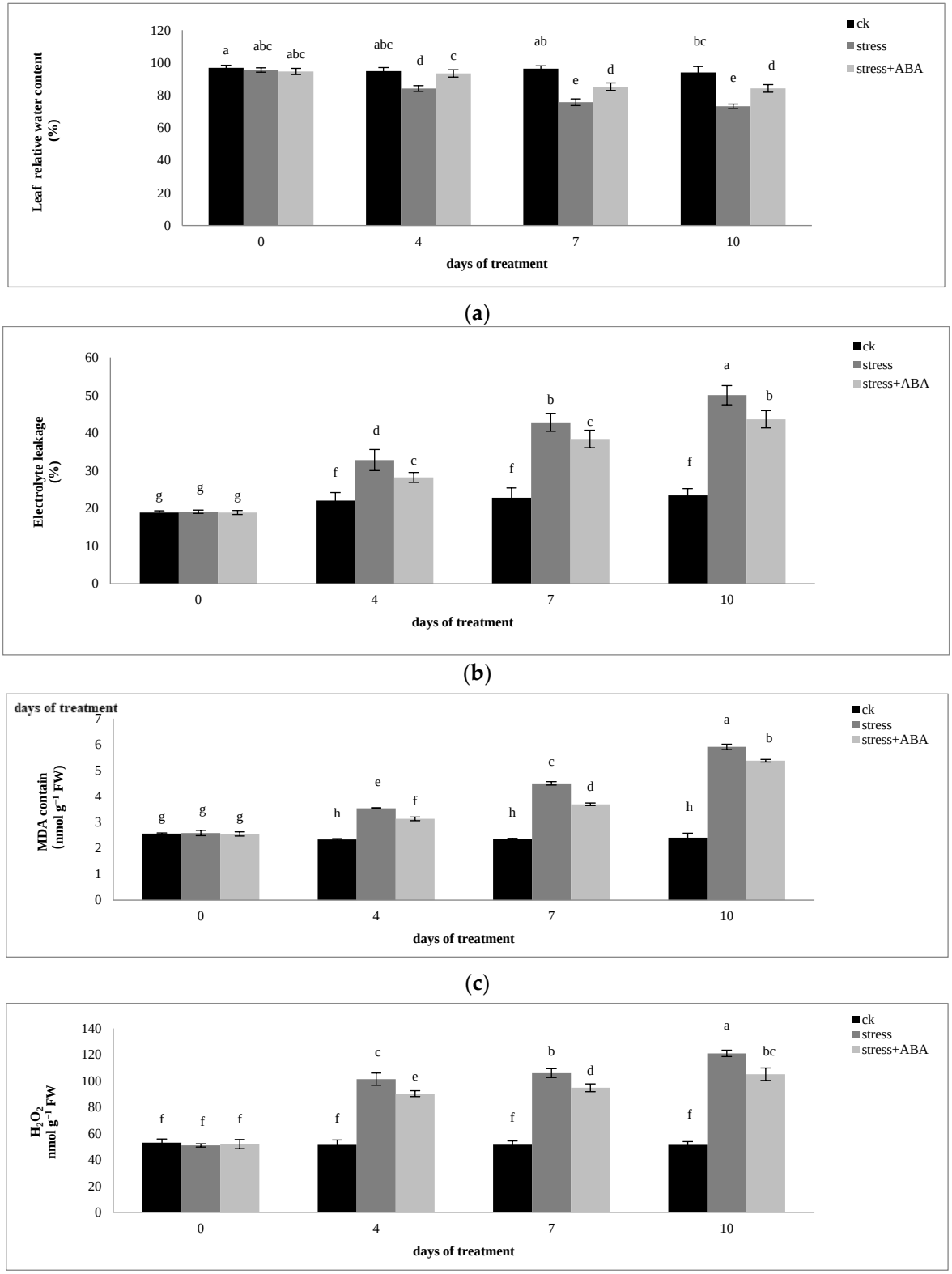
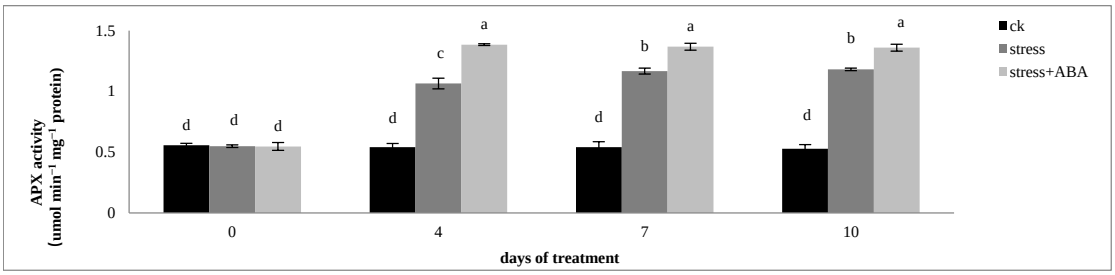


Figure 2. Effects of exogenous ABA application on (**a**) Relative water content, (**b**) EL, (**c**) MDA content, and (**d**) H_2O_2 content in leaves of maize exposed to drought stress for 10 days. The data represents the means of independent measurements for five replicates. Standard deviation (SD) is indicated by vertical error bars. Values with the same lowercase letters on the bars are not significantly different ($p < 0.05$).

3.2. Effects of Drought Stress and/or ABA Application on APX, MDHAR, DHAR, and GR Activity

As shown in Figure 3a,b, under drought stress, the activities of APX and GR in maize leaves were significantly higher than those in the control. Exogenous application of ABA further increased the activities of the two enzymes. On the 4th, 7th, and 10th days of drought stress, the activities of APX and GR in maize leaves increased by 96.89%, 115.48%, and 124.05%, and 48.71%, 30.32%, and 27.65%, respectively. After exogenous ABA treatment, the activities of APX and GR increased by 156.01%, 152.53%, and 157.81%, and 143.94%, 154.14%, and 166.25%, respectively. The degree of stress influenced the changes in APX and GR activities. There was no significant difference between the activities of APX and GR in maize plants on the 10th day of drought stress and those on the seventh day of drought stress. However, they were significantly higher than those on the fourth day of drought stress. The effect of exogenous ABA on APX activity did not change with the stress duration. However, the effect of ABA on GR activity under drought stress escalated with increasing stress days.



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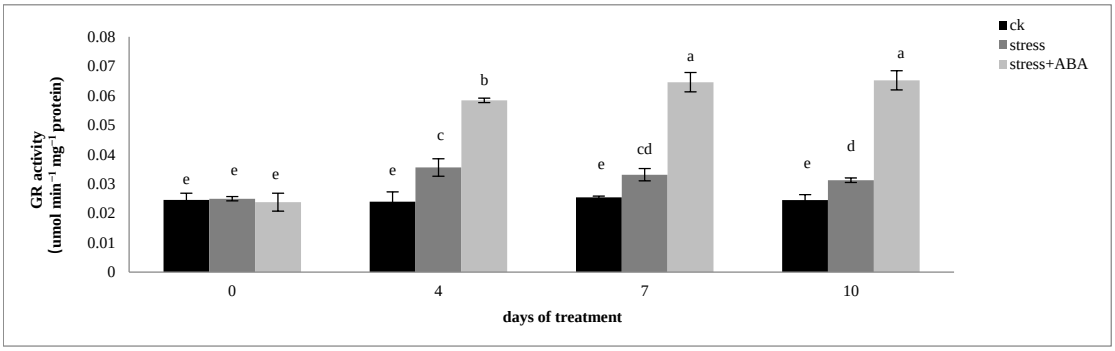

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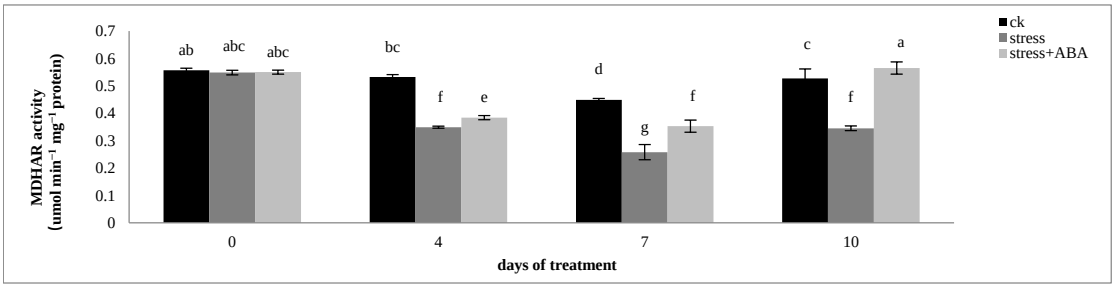

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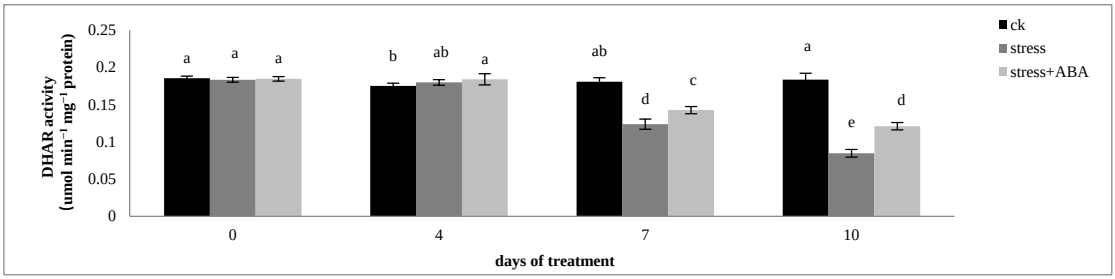


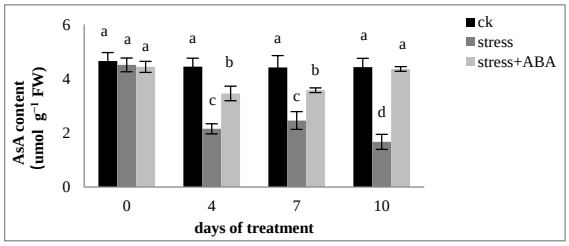
(**d**)

Figure 3. Effects of exogenous ABA application on the activities of (**a**) APX, (**b**) GR, (**c**) MDHAR, and (**d**) DHAR in leaves of maize exposed to drought stress for 10 days. The data represent the means of independent measurements for five replicates. SD is indicated by vertical error bars. Values with the same lowercase letters on the bars are not significantly different ($p < 0.05$).

Drought stress reduced the activities of MDHAR and DHAR in maize leaves, and the application of ABA inhibited the decline in enzyme activity. On the 4th, 7th, and 10th days of drought stress, the activity of MDHAR in maize leaves decreased by 34.43%, 42.54%, and 34.56%, respectively. After exogenous ABA treatment, MDHAR activity increased by 6.53%, 21.2%, and 41.77%, respectively. On the 7th day of drought stress, the DHAR activity of maize leaves decreased by 31.48% compared to that of the control and then continued to decline. On the 7th and 10th days of drought stress, there was no difference in the effect of exogenous ABA on DHAR activity in maize leaves under drought stress conditions.

3.3. Effects of Drought Stress and/or ABA Application on Non-Enzymatic Antioxidants

As shown in Figure 4, under drought conditions, the AsA content and AsA/DHA ratio in maize leaves were significantly lower than those in the control treatment, but the DHA content increased significantly. Exogenous application of ABA significantly increased the AsA content and the AsA/DHA ratio in maize leaves and inhibited the increase in DHA content. The decrease in AsA content in the leaves of ZD958 escalated with increasing stress days. On the 10th day of stress, the DHA content in leaves of ZD958 was the highest, increasing 103.26% higher than the control.



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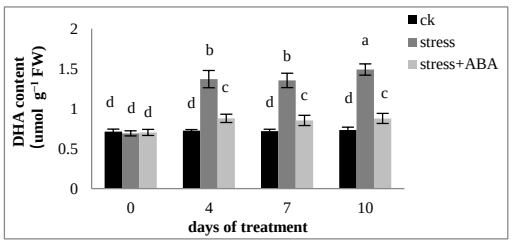

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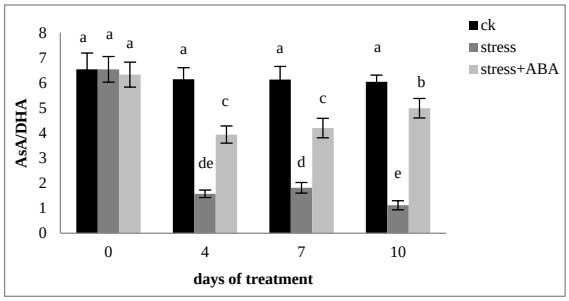

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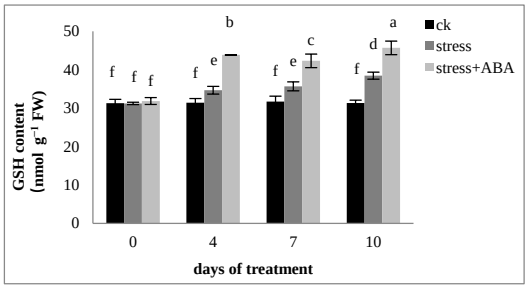

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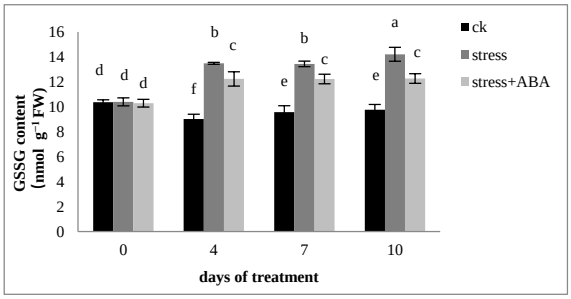

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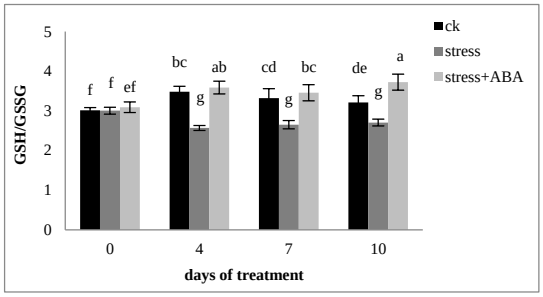


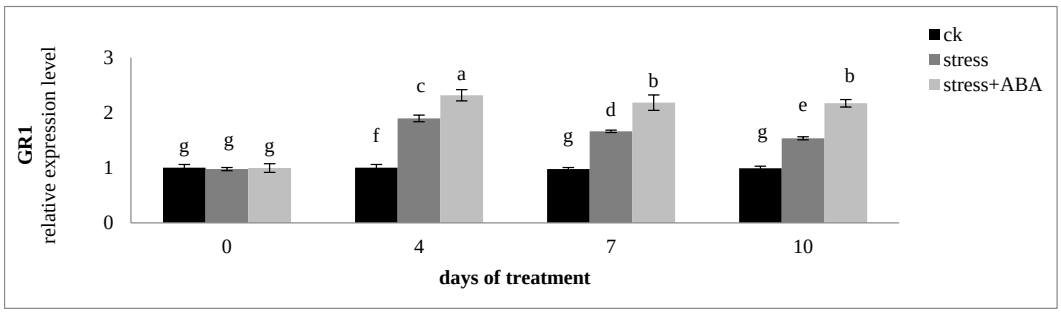
(**f**)

Figure 4. Effects of exogenous ABA application on the contents of (**a**) AsA and (**b**) DHA, (**c**) the AsA/DHA ratio, (**d**) reduced GSH, (**e**) oxidized GSSG, and (**f**) the GSH/GSSG ratio in leaves of maize exposed to drought stress for 10 days. The data represents the means of independent measurements for five replicates. SD is indicated by vertical error bars. Values with the same lowercase letters on the bars are not significantly different ($p < 0.05$).

The contents of GSH and GSSG in the functional leaves of maize increased significantly under drought stress. The application of exogenous ABA further increased the GSH content and inhibited the increase in GSSG under drought stress conditions. Exogenous ABA increased the GSH content by 21.01–29.26% and reduced the GSSG content by 12.68–19.87% compared to the drought stress alone. The GSH/GSSG ratio in maize leaves reduced significantly under drought stress, and the ABA application could increase it by 24.28–31.76%.

3.4. Effects of Drought Stress and/or ABA Application on the Expression of Genes Encoding AsA-GSH Cycle Enzymes

Drought-induced changes in GR activity (Figure 3b) were also evident at the mRNA levels (Figure 5a,b). The relative levels of GR1 and GR2 mRNAs increased under drought stress conditions (Figure 5a,b), and the highest levels were both observed on the fourth day. GR1 mRNA levels under drought stress conditions + ABA were higher than only drought stress conditions, but GR2 mRNA levels showed the opposite result.



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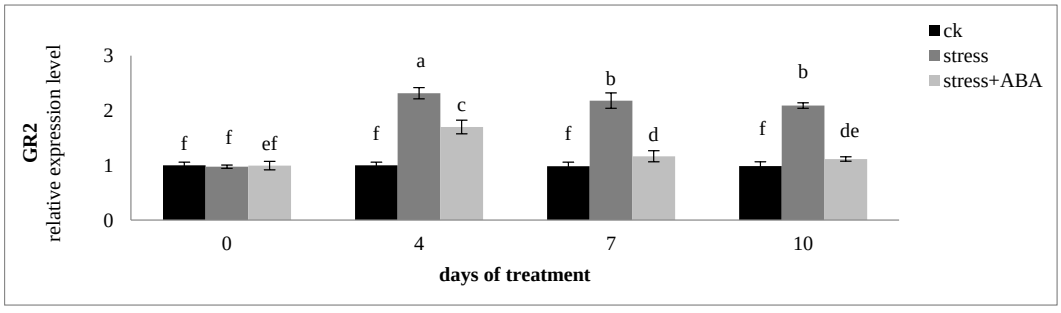

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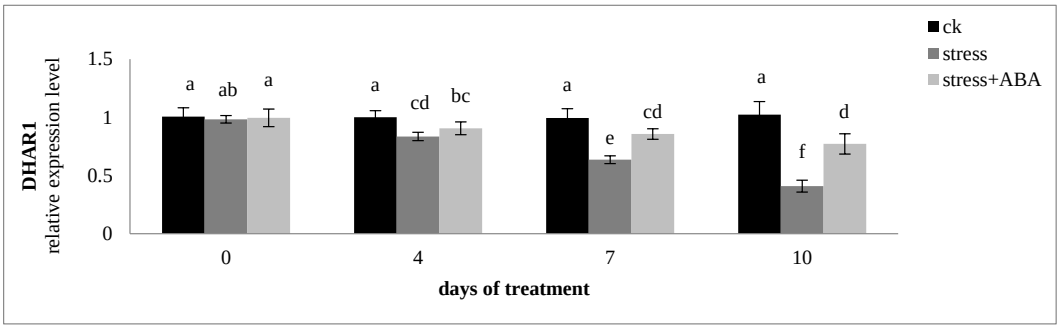

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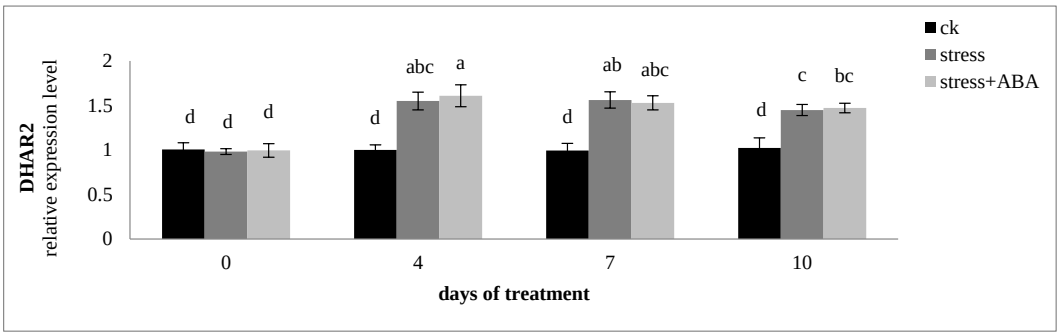

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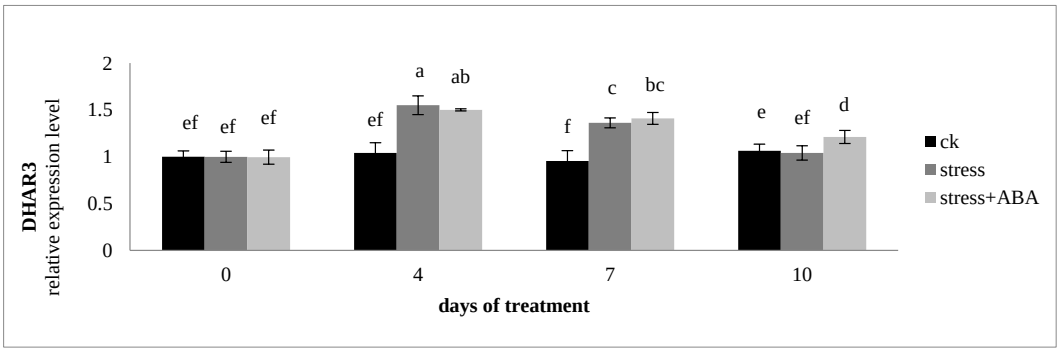

(**e**)

Figure 5. Effects of exogenous ABA application on the relative expression level of (**a**) GR1, (**b**) GR2, (**c**) DHAR1, (**d**) DHAR2, and (**e**) DHAR3 in leaves of maize exposed to drought stress for 10 days. The data represent the means of independent measurements for five replicates. SD is indicated by vertical error bars. Values with the same lowercase letters on the bars are not significantly different ($p < 0.05$).

On the fourth and seventh days, the relative levels of DHAR2 and DHAR3 under drought stress were higher than those in well-watered controls. Under drought stress, the transcription level of DHAR1 decreased, and the decline in the transcription level was inhibited by exogenous ABA, having a significant effect on the 7th and 10th days. Furthermore, on the fourth and seventh days, the transcription level of DHAR3 was upregulated to a greater extent than that of DHAR2 under water-deficit conditions. Lastly, the effect of ABA treatment on the levels of DHAR2 and DHAR3 mRNAs did not differ significantly from that of drought stress.

4. Discussion

The antioxidant defense system of leaves has attracted much attention. One of its principles is that leaves are the main sensor of drought stress. Moreover, some research results show that the drought tolerance of maize varieties is closely related to the ability of antioxidant systems [42]. In this experiment, the H_2O_2 content in maize plants increased in water deficit conditions, which explains the role of H_2O_2 as a stress signal molecule [43]. With

the aggravation of water stress, H_2O_2 attacks the cell membrane as a free radical [44]. When treated with exogenous ABA, the ability to scavenge H_2O_2 was enhanced in maize plants. Between the fourth and seventh days, the increase in H_2O_2 content in ZD958 was not different, which proved the effectiveness of oxidative stress control. On the 10th day of drought stress, the H_2O_2 content of maize plants treated with exogenous ABA did not decrease significantly. One possible explanation is that exogenous ABA treatment increases the internal ABA content, resulting in H_2O_2 accumulation (a signal molecule that activates antioxidant enzymes) [45]. This theory has been confirmed in rice [46] and maize [30]. The accumulated H_2O_2 maximally activated the enzyme activity participating in the clearance reaction.

Malondialdehyde (MDA), the end-product of membrane damage peroxidation caused by the accumulation of reactive oxygen species, can react with a variety of components in cells and damage a variety of enzymes and membrane systems. This is a sign of plant leaf senescence. Drought conditions exhibited greater accumulation in MDA contents, a product of lipid peroxidation in maize plants, fulvic acid-treated plants considerably lowered MDA contents which reported that lower MDA contents could be correlated with stress tolerance [47]. Under water-deficit conditions, the average content of MDA in maize leaves was higher, and the application of ABA significantly reduced membrane damage. Other studies observed that ABA treatment of maize significantly alleviated membrane damage (decreased MDA levels) [31]. Some studies found that, in the later stage of stress, the MDA and H_2O_2 contents in resistant crop varieties decreased, which may be because the antioxidant enzyme activity was activated to a maximum extent, thus reducing oxidative damage [48]. However, in this study, the MDA and H_2O_2 contents in maize leaves did not decrease under water stress conditions with an increase in stress days. This may be because the imbalance between the accumulation and clearance of reactive oxygen species (ROS) leads to the accumulation of MDA, which, in turn, partially inhibits the activity of antioxidant enzymes.

AsA and GSH are non-enzymatic substances with low moleculars, which widely exist in cells and participate in antioxidant metabolic reactions. They participate in the scavenging of reactive oxygen species through the AsA-GSH cycle. The AsA oxidation and reduction system consist of AsA, MDHA, and DHA. AsA is oxidized to MDHA by APX and further converted to DHA during H_2O_2 removal. MDHAR can reduce MDHA to AsA, and DHA can regenerate AsA through DHAR. Therefore, AsA/DHA can represent oxidation and reduction states in cells. The concentrations of AsA and GSH vary with the external environment, especially abiotic stress [49]. Under drought stress, the AsA content in ZD958 decreased continuously. This is consistent with the relevant test conclusions for maize [50,51] and *Brassica* [20]. This may be because the water deficit improved the activity of APX and promoted the H_2O_2 scavenging reaction of AsA in this experiment. At the same time, it reduces the activities of MDHAR and DHAR and hinders the regeneration of AsA.

APX is the core enzyme in the AsA-GSH cycle and plays a crucial role in H_2O_2 metabolism [52]. In a certain range, high temperatures [53], water shortages [54], and high salt contents [55] can lead to an increase in APX activity. This is consistent with the results of the present study. The APX activity of ZD958 significantly increased under drought stress, and the application of exogenous ABA further improved its activity. The high activity of APX and DHAR can improve the salt and alkali tolerance of cabbage [56]. Conversely, decreased APX, GR, and MDHAR activities at high temperatures resulted in more severe membrane lipid peroxidation [57]. It can be inferred that exogenous ABA enhanced the activity of APX, which can increase the drought resistance of maize and reduce the content of MDA.

The AsA/DHA ratio measures the level of available AsA [22]. Exogenous application of ABA inhibited the decrease in the AsA/DHA ratio in maize plants under water stress conditions. This can be explained by the significant increase in MDHAR and DHAR activities in ZD958 treated with exogenous ABA. Therefore, exogenous ABA can stabilize the redox state of maize cells by improving the activity of antioxidant enzymes. However, according to the conclusion of Li [51], it is predicted that if the stress time is prolonged

in this experiment, ABA may not continue to improve the antioxidant capacity of maize. When the mediated ability of AsA-GSH reaches its maximum, its mediated ability decreases with the extension of stress time, resulting in plant damage.

As an important antioxidant related to AsA regeneration in the AsA-GSH cycle, GSH also regulates the metabolism of H_2O_2 [14]. As a regulator of enzyme activity, GSH activates various defense mechanisms by participating in redox signals, such as cold injury [58], heat injury [59], salt injury [5], and water stress [19], thus protecting cells from adverse stress. In this signaling pathway, GSH interacts with the plant hormone abscisic acid (ABA) [60]. In this study, drought stress increased the GSH content in maize plants. This is consistent with the results for tomatoes [53] under mildly high-temperature stress. However, a water deficit reduced the ratio of GSH/GSSG in ZD958. Because GSH is oxidized in the process of scavenging accumulated H_2O_2, this is consistent with the results of previous studies. However, Selote [12] believed that the changes in GSH content and the GSH/GSSG ratio are not synchronous under different drought intensities. The redox state of GSH affects the adaptation of crops to the stress environment, and the amount of GSH involved in the response largely determines the efficacy of AsA. Exogenous ABA restored the high GSH/GSSG ratio in maize and could effectively protect plants from reactive oxygen species induced by abiotic stress. A high ratio of GSH/GSSG is a sign of plant tolerance. The transformation of GSSG to GSH can enhance the tolerance of plants to environmental stress [61]. The mechanism may operate through increasing GR activity and GR1 expression, increasing GSH synthesis, and reducing GSH degradation [60].

Under abiotic stress, GR, an antioxidant enzyme, plays an important regulatory role in the glutathione cycle. The increase in GR activity under stress promoted the effective removal of H_2O_2 by GSH and maintained a high GSH/GSSG ratio [60]. In soybean [43], H_2O_2 and GSH have interacted with stress signals. This can explain why, in this present experiment, the application of exogenous ABA inhibited the accumulation of H_2O_2 by increasing GR activity and GR1 levels to increase GSH content. Analysis of enzyme gene expression revealed that the levels of DHAR1 and GR1 mRNAs were highly consistent with the physiological activity trends of DHAR and GR, respectively. Therefore, it is concluded that DHAR1 and GR1 regulate the two enzymes more closely in this study.

5. Conclusions

Through the water stress test of ZD958 maize at the pustulation period, it was found that ABA enhanced the scavenging ability of H_2O_2 and MDA by regulating the physiological process of the AsA-GSH cycle. In addition, under water deficit conditions, ABA-induced the upregulation of GR activity and transcription maintained the stability of the GSH/GSSG ratio and helped to maintain the intracellular redox state. The GR1 and DHAR1 expression level changes were consistent with those in GR and DHAR enzyme activity levels. ABA promotes the regeneration of AsA by stabilizing the activity and transcriptional expression of MDHAR and DHAR. Therefore, the ABA-mediated AsA-GSH cycle improves the antioxidant capacity, alleviates the damage ofROS, reduces growth inhibition, and enhances the tolerance of plants to drought stress.

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