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EXOGENOUS LOW-DOSE HYDROGEN PEROXIDE ENHANCES DROUGHT TOLERANCE OF SOYBEAN (*GLYCINE MAX* L.) THROUGH INDUCING ANTIOXIDANT SYSTEM

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Hydrogen peroxide (H_2O_2) functions as a signal molecule in plants under abiotic and biotic stress. In this study, the role of exogenous H_2O_2 in improving drought tolerance in two soybean cultivars (*Glycine max* L. Merrill) differing in their tolerance to drought was evaluated. Plants were grown in plastic pots with normal irrigation in a phytotron. Four weeks after radicle emergence, either 1 mM H_2O_2 or distilled water was sprayed as foliar onto the leaves of each plant, after drought stress was applied. Leaf samples were harvested on the 4th and 7th days of the drought. Antioxidant-related enzyme activity, such as the superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content was measured during the drought period. Drought stress decreased leaf water potential, relative water content and photosynthetic pigment content but enhanced lipid peroxidation and endogenous H_2O_2 concentration. By contrast, exogenous low dose H_2O_2 improved water status, pigment content and lipid peroxidation under drought stress. Endogenous H_2O_2 concentration was reduced by exogenous H_2O_2 as compared to drought treatment alone. H_2O_2 pre-treatment induced all the antioxidant enzyme activities, to a greater extent than the control leaves, during drought. H_2O_2 pre-treatment further enhanced the activities of antioxidant enzymes in the tolerant cultivar compared to the sensitive cultivar. Results suggested that low dose H_2O_2 pre-treatment alleviated water loss and H_2O_2 content and increased drought stress tolerance by inducing the antioxidant system.

Keywords: Hydrogen peroxide – *Glycine max* L. – drought tolerance – antioxidant enzymes

INTRODUCTION

Plants are constantly encountered by various abiotic stresses that negatively affect growth and productivity worldwide [15]. Drought stress is the primary cause of crop loss across the globe, reducing average yields in most major crop plants. A common effect of drought stress, as with other environmental stresses, is oxidative damage. Exposure to drought results in a loss of balance between the production of reactive oxygen species (ROS), such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot OH$) and singlet oxygen ($\cdot O_2$) and their scavenging potential [31].

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Overproduction of ROS is a common phenomenon in plants under biotic and abiotic stress conditions, which can cause oxidative damage to macromolecules and cell structures, leading to inhibition of plant growth and development, or even to death. Among all ROS, H₂O₂ acts as a central player in stress signal transduction pathways associated with tolerance to abiotic and biotic stresses [15]. Furthermore, H₂O₂ directly regulates the expression of numerous genes involved in plant defense and the related pathways such as antioxidant enzymes, defense proteins and transcription factors [16]. Several recent studies have demonstrated that exogenous H₂O₂ can enhance abiotic stress tolerance either by modulating ROS detoxification or regulating multiple stress-responsive pathways and gene expression [1, 15, 21, 33]. Ishibashi et al. [17] showed that exogenous application of H₂O₂ promotes the up-regulation of stress responsive genes and increases drought stress tolerance. Lately a micro-array study indicated that the expression level of several hundred genes altered in H₂O₂ treated *Arabidopsis* cell cultures [7]. Another effect of H₂O₂ is its capacity to induce the expression of transcription factors and genes responsible for osmolyte synthesis such as proline and betaine, and activate phosphorylation cascades using mitogen-activated protein kinases (MAPKs) [15]. Terzi et al. [33] found that exogenous H₂O₂ pre-treatment induced osmotic stress tolerance by increasing soluble sugar, proline, and polyamine levels in maize (*Zea mays* L.) seedlings. Liu et al. [23] also reported that H₂O₂ pre-treatment of two cucumber varieties improved osmotic stress resistance by activating antioxidant system. Abass and Mohamed [1] also studied the effects of H₂O₂ pre-treatment on the seeds of common bean seedlings (*Phaseolus vulgaris* L.) and analyzed drought tolerance in seedlings.

Soybean has been known as one of the major legume crops in the world, providing an abundant source of oil and protein-rich food for both human and animal consumptions. However, soybean growth, development, and yield are greatly affected by several abiotic stressors, such as flooding [19], drought [24], and salinity [32]. Particularly, in soybeans, drought stress occurring at the early stage of pod development significantly increases the rate of pod abortion, thus decreasing final seed yield [22]. As can be seen from literature although several studies report strategies to improve drought tolerance, little information is available on the antioxidative and physiological mechanism of low dose H₂O₂-induced drought stress alleviation in plants. In the present study, we hypothesize that low dose of H₂O₂ can reduce adverse effects of drought stress by inducing antioxidant systems and decreasing ROS level in plants. At the same time, this approach assumes great importance for increasing crop production in stress affected areas. Therefore, we investigated the influence of H₂O₂ as a signal molecule by examining whether exogenous H₂O₂ pre-treatment could alleviate drought stress and its relations with antioxidant systems in two soybean cultivars differing in their sensitivity to drought.

MATERIALS AND METHODS

Growth of the plants and stress applications

Two *Glycine max* L. cultivars sensitive to drought deficit in different levels (cv. *Glycine max* L. Merrill 537 (drought tolerant) and cv. *Glycine max* L. Merrill 520 (drought sensitive)) were used as the plant material. Seeds were surface sterilized with 0.1% HgCl₂ for 3 min followed by repeated washings with sterilized distilled water. Seeds were sown in plastic pots (14 cm height, 16 cm top and 11 cm bottom diameter) containing soil and peat (5:1). Seedlings were grown in a phytotron (temperature: 23 °C±2, relative humidity: 60%±5 and light intensity: 400 μmol·m⁻²·s⁻¹) for 30 days. Four weeks after radicle emergence, either 1 mM H₂O₂ or distilled water was sprayed as foliar once daily for three days onto the leaves of each plant, and then irrigation was stopped. Treatments with H₂O₂ or distilled water were always followed by drought stress. Plants with no spray treatment and maintained irrigated throughout the experiment were used as control treatment. For measurements of all parameters, the leaves were collected at 4th and 7th days of drought after application and stored at -80 °C.

Measurement of plant water status

Leaf water potential (Ψ_{leaf}) was measured with a thermocouple psychrometer at 27±1 °C (PSYPRO, Wescor, Inc., Logan, UT USA). Relative water content (RWC) in the leaves was determined according to Castillo [6]. Discs approximately 6 mm in diameter were cut from the youngest fully expanded leaves of plants and sealed in the C-52 psychrometer chamber. Samples were equilibrated for 60 min before the readings were recorded by a water potential data logger in the psychrometric mode. Values of leaf water potential were measured as MPa.

Photosynthetic pigments

Total chlorophyll (Chl) and carotenoid (Car) contents were determined following the method of Arnon [3]. Fresh leaf samples were selected randomly and homogenized in a mortar in 80% acetone. The extract was centrifuged at 5,000 g for 5 min. Absorbance of the supernatant was recorded at 663, 645, and 450 nm by spectrophotometer.

Antioxidant enzyme assays

Enzyme extraction was carried out at 0–4 °C. Frozen leaf segments (0.5 g) were crushed into fine powder in a mortar and under liquid N₂. The leaf powder was homogenized in 5 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinylpyrrolidone (PVP), with the addition of 5 mM ascorbic acid (ASC) in the

case of the APX assay. The homogenate was centrifuged at 20,000 g for 20 min at 4 °C and the supernatant was used for the following enzyme assays.

Superoxide dismutase (SOD, EC 1.15.1.1) specific enzyme activity was measured (25 °C) based on the method of Beauchamp and Fridovich [4], as modified by Dhindsa and Matowe [8]. Catalase (CAT, EC 1.11.1.6) specific enzyme activity was determined by following the consumption of H₂O₂ (25 °C, $e = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$) at 240 nm [2]. Ascorbate peroxidase (APX, EC 1.11.1.11) specific enzyme activity was estimated by monitoring ascorbate oxidation at 290 nm (25 °C, $e = 2.8 \text{ mM}^{-1} \text{ cm}^{-1}$) [25]. Glutathione reductase (GR, EC 1.6.4.2) specific enzyme activity was determined by measuring the decrease in absorbance at 340 nm (25 °C, $e = 6.22 \text{ mM}^{-1} \text{ cm}^{-1}$) according to Foyer and Halliwell [9]. Monodehydroascorbate reductase (MDHAR, EC 1.6.5.4) specific enzyme activity was determined by following the oxidation of NADH at 340 nm [13]. Dehydroascorbate reductase (DHAR, EC 1.8.5.1) specific enzyme activity was assayed essentially as described before by Hossain and Asada [12]. The activity of each enzyme was expressed on a protein basis. Protein content was determined according to Bradford [5], using bovine serum albumin as standard.

Lipid peroxidation

Lipid peroxidation was measured in terms of malondialdehyde content ($e = 155 \text{ mM}^{-1} \text{ cm}^{-1}$), a product of lipid peroxidation following the method of Heath and Packer [11]. Leaf samples (0.5 g) were homogenized in 10 ml of 0.1% (w/v) trichloroacetic acid (TCA). 4 ml of 0.5% (w/v) thiobarbituric acid containing 20% (w/v) TCA was added to 1 ml aliquot of supernatant. The absorbance of the supernatant was recorded at 532 and 600 nm and the malondialdehyde (MDA) content was expressed as nmol MDA g⁻¹ FW.

Hydrogen peroxide concentration

Hydrogen peroxide concentration was determined according to a modified method of Velikova et al. [34]. Leaves (0.25 g) were ground in 3 ml of 5% trichloroacetic acid with 0.1 g of activated charcoal at 0 °C. 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 0.75 ml of 1 M KI were added to 0.5 ml aliquot of the supernatant. The absorbance was measured at 390 nm and H₂O₂ concentration was expressed as $\mu\text{mol g}^{-1}$ FW.

Statistical analysis

All analysis was repeated three times on a mixture of leaves from three individual plants with three technical replicates. Variance analysis of mean values was performed with the Duncan multiple comparison test using SPSS software for Microsoft Windows (Ver. 15.0, SPSS Inc., USA) and a significance level was determined at the 5% ($P < 0.05$) level.

RESULTS

Water status parameters

We measured the leaf water potential of both cultivars during the drought period after H₂O₂ to understand how water status was affected by the treatment. Ψ_{leaf} and RWC decreased in both cultivars during drought stress. However, H₂O₂ pre-treatment sustained the water potential and RWC of stressed plants at a higher level than drought

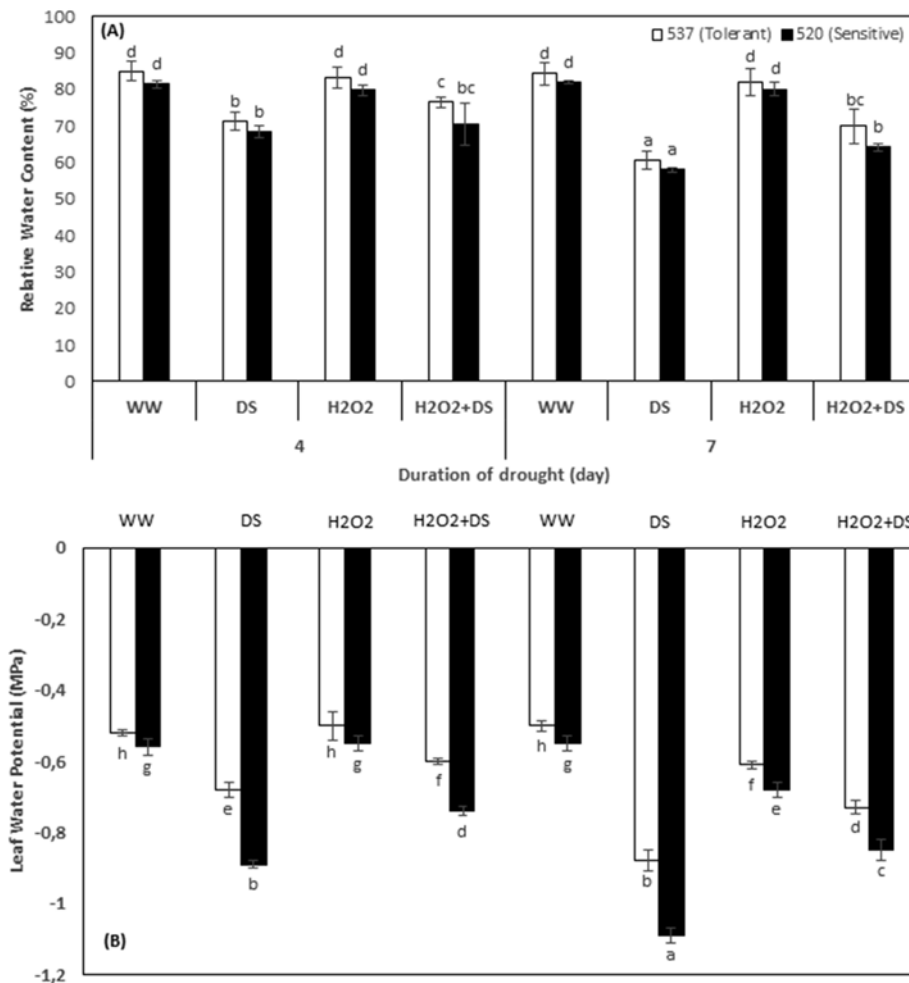


Fig. 1. Relative water content (A) and leaf water potential (B) and in two soybean cultivars [537 (tolerant-open square), 520 (sensitive-filled square)] treated with H₂O₂ during drought stress. WW, DS, H₂O₂ and H₂O₂+DS are abbreviations of well-watered, drought stress hydrogen peroxide and hydrogen peroxide and drought stress. Vertical bars represent standard deviation. Different letters denote significant differences between the two cultivars along with different treatments at P<0.05

treatment alone. In tolerant cultivar, Ψ_{leaf} of drought treated plants were down from -0.68 MPa on the 4th day to -0.88 MPa on the 7th day of the drought period. Water potential of H_2O_2 treated plants were also down from -0.60 MPa to -0.75 MPa on the 4th and 7th days of the drought period (Fig. 1B). After pre-treatment with H_2O_2 , RWC of tolerant cultivar were down from 85% to 76% and 69% on the 4th and 7th days of the drought period compared to the control plants, respectively (Fig. 1A). A similar trend of the Ψ_{leaf} and RWC was also recorded in sensitive cultivar.

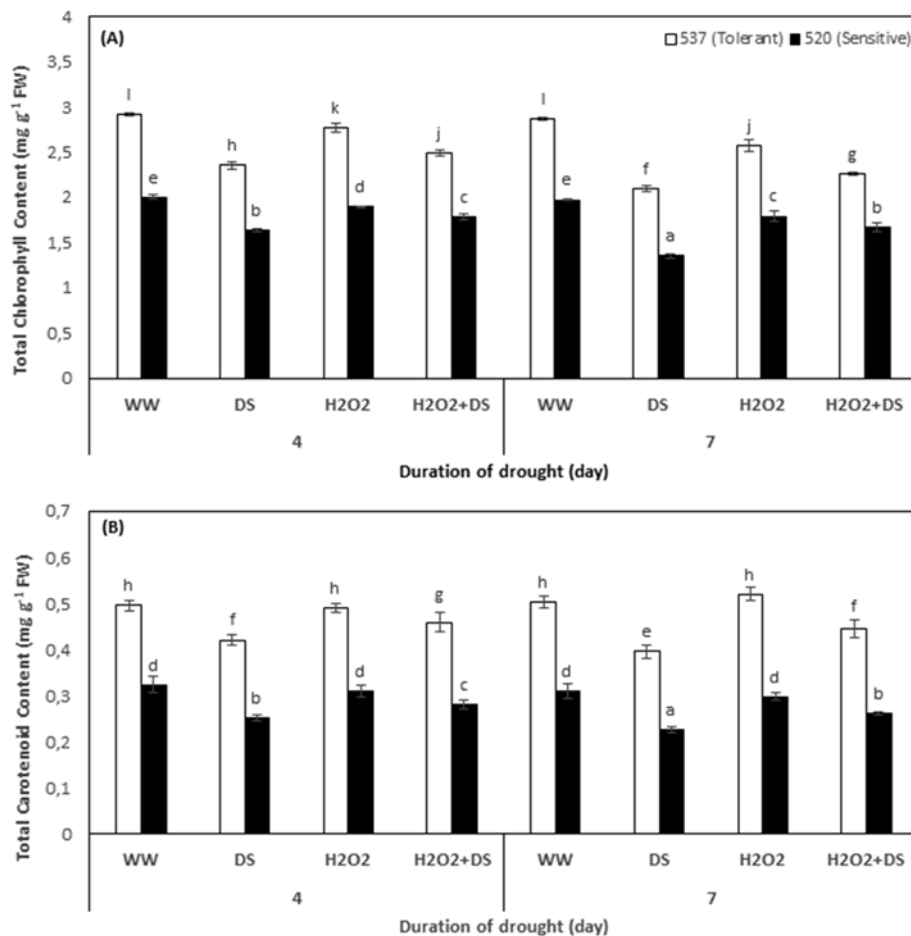


Fig. 2. Total chlorophyll (A) and total carotenoid (B) in two soybean cultivars [537 (tolerant-open square), 520 (sensitive-filled square)] treated with H_2O_2 during drought stress. WW, DS, H_2O_2 and H_2O_2 +DS are abbreviations of well-watered, drought stress hydrogen peroxide and hydrogen peroxide and drought stress. Vertical bars represent standard deviation. Different letters denote significant differences between the two cultivars along with different treatments at $P < 0.05$

Photosynthetic pigments

Drought stress significantly decreased total Chl content in both cultivars as compared to the control, which was greater in tolerant cultivar than in sensitive one. Exogenous H_2O_2 alone decreased total Chl content in both cultivars as compared to the control but the effect was not as high as in drought treatment alone. However, decrease in total Chl content was lower in H_2O_2 -treated plants than drought treatment alone for both cultivars. After exogenous applications of H_2O_2 , total Chl content of tolerant cultivar decreased by 15% and 27% on the 4th and 7th days of drought as compared to the control, but the reductions were 24% and 43% in drought treatment alone, respectively. A similar trend of the total Chl was also recorded in sensitive cultivar (Fig. 2A).

Compared to the control, total carotenoid (Car) in both cultivars was decreased at drought treatment alone and, this reduction was lower in the H_2O_2 pre-treated plants. Exogenous H_2O_2 alone did not change the total Car content in both cultivars as compared to the control. After exogenous applications of H_2O_2 , total Car content of the tolerant cultivar decreased by 8% and 13% on the 4th and 7th days of drought as compared to the control but the decrements were 15% and 21% in drought treatment alone, respectively. A similar phenomenon was observed in sensitive cultivar, but the decrements were higher pronounced in that of tolerant cultivar (Fig. 2B).

Antioxidant enzymes

All antioxidant enzyme activities were increased during drought treatments in both cultivars, and this increment was higher in the H_2O_2 pre-treated plants. The degree of increase related to H_2O_2 pre-treatment was higher in the tolerant compared to the sensitive cultivar. In plants pre-treated with H_2O_2 , SOD activity of tolerant cultivar increased by 93% and 190% on day 4 and 7 of the drought period compared to the control, respectively. In sensitive cultivar, the activity increased by 66% and 65% on the 4th and 7th days of the drought period, respectively. Moreover, exogenous H_2O_2 alone increased SOD activity in both cultivars as compared to the control but the effect was not as high as in drought treatment alone (Fig. 3A).

CAT activity in H_2O_2 treated plants increased during the drought period. After exogenous applications of H_2O_2 , the CAT activity was increased by 49% and 120% in tolerant cultivar on the 4th and 7th days of the drought period compared to the control, respectively. In sensitive cultivar, the activity in H_2O_2 treated plants was increased by 79% and 98% (Fig. 3B).

The combination of H_2O_2 pre-treatment and drought stress resulted in an increase in APX activity in both cultivars. After H_2O_2 pre-treatment, APX activity increased by 106% and 194% in tolerant cultivar on the 4th and 7th days of the drought compared to the control, respectively. The activity increased by 41% and 99% in sensitive cultivar. On 7th day of drought, exogenous H_2O_2 alone increased APX activity in both cultivars as compared to the control but did not change APX activity of sensitive cultivar on 4th day of drought (Fig. 3C).

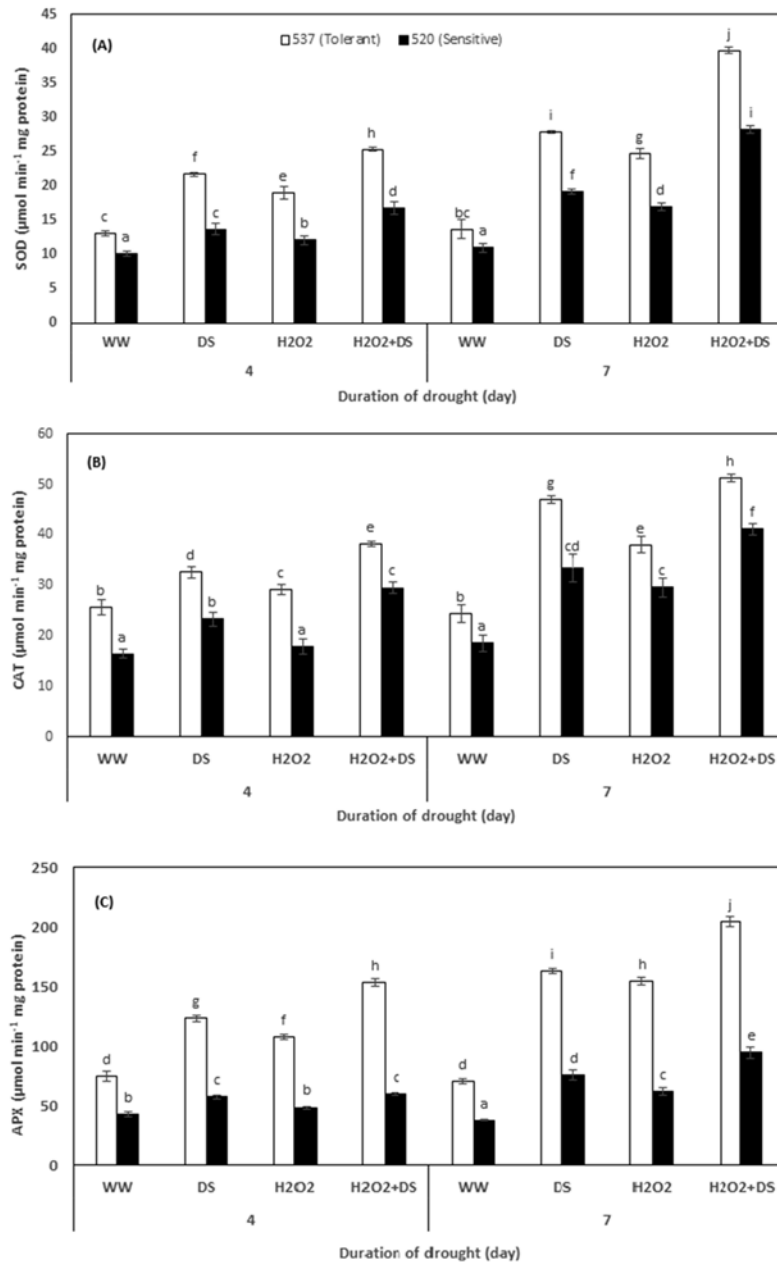


Fig. 3. Specific activities of antioxidant enzymes of superoxide dismutase (A), catalase (B), ascorbate peroxidase (C), glutathione reductase (D), dehydroascorbate reductase (E) and monodehydroascorbate reductase (F) in two soybean cultivars [537 (tolerant-open square), 520 (sensitive-filled square)] treated with H_2O_2 during drought stress. WW, DS, H_2O_2 and H_2O_2 +DS are abbreviations of well-watered, drought stress hydrogen peroxide and hydrogen peroxide and drought stress. Vertical bars represent standard deviation. Different letters denote significant differences between the two cultivars along with different treatments at $P < 0.05$.

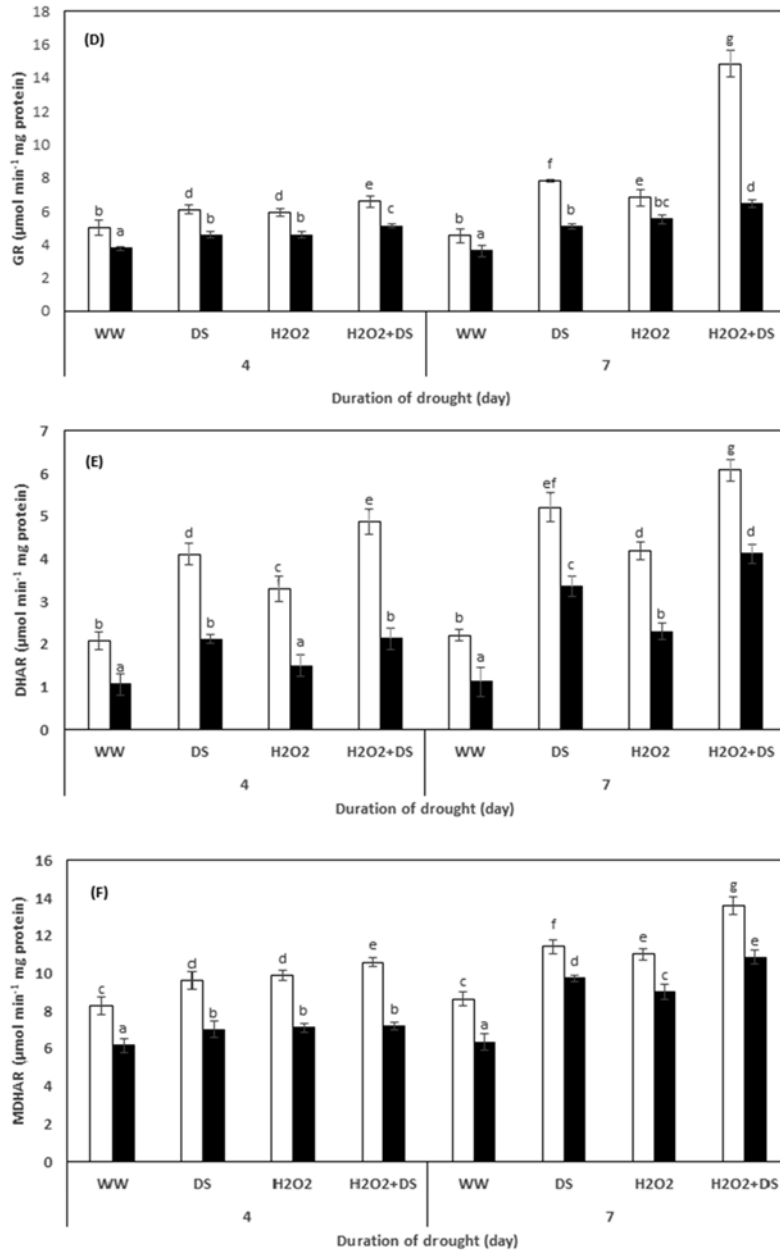


Fig. 3. (cont.)

When compared with corresponding control, H_2O_2 pre-treatment significantly increased GR, DHAR and MDHAR activities in both cultivars under drought stress. In H_2O_2 treated drought stressed plants, all three enzyme activities were higher than those in the drought treatment alone (Fig. 3D, 3E, 3F). On the 4th and 7th days of the drought period, GR activity in H_2O_2 -treated tolerant cultivar was increased by 31% and 229% but this increment was by 36% and 79% in sensitive cultivar. DHAR and MDHAR activities also showed a trend which is similar to GR activity (Fig. 3E, 3F).

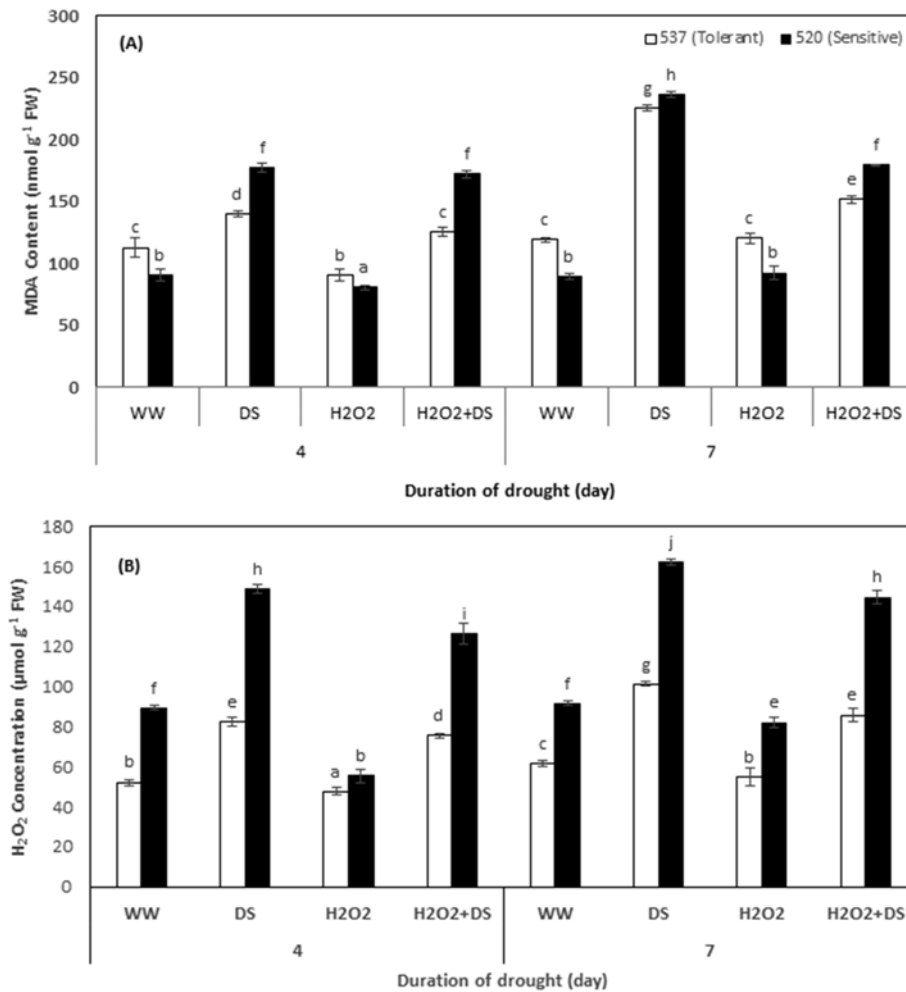


Fig. 4. Lipid peroxidation (A) and hydrogen peroxide (B) concentration in two soybean cultivars [537 (tolerant-open square), 520 (sensitive-filled square)] treated with H_2O_2 during drought stress. WW, DS, H_2O_2 and H_2O_2+DS are abbreviations of well-watered, drought stress hydrogen peroxide and hydrogen peroxide and drought stress. Vertical bars represent standard deviation. Different letters denote significant differences between the two cultivars along with different treatments at $P < 0.05$

Lipid peroxidation and H₂O₂ concentration

As measured on 4th and 7th days of drought, lipid peroxidation increased during drought stress in both cultivars but sensitive cultivar showed a higher degree of peroxidation. Exogenous H₂O₂ alone decreased MDA content as compared to control on the 4th day of drought but did not change on the 7th day of drought. H₂O₂ pre-treatment also alleviated lipid peroxidation under drought stress (Fig. 4A).

Drought treatments resulted in higher H₂O₂ concentration in both cultivars as compared to well-watered plants, both on day 4 and 7. Exogenous H₂O₂ alone decreased endogenous H₂O₂ concentration in both cultivars as compared to the control. Exogenous application of H₂O₂ reduced H₂O₂ production caused by drought exposure in both cultivars, and this effect was more apparent in tolerant cultivar than in sensitive cultivar (Fig. 4B).

DISCUSSION

As signaling molecule in plants, H₂O₂ has been well reported to be involved in plant response to various environmental stresses. The present investigation suggests that exogenous application of H₂O₂ may help reduce adverse effects of drought in soybean cultivars. Maintenance of plant water status is a fundamental phenomenon for normal growth of plants under harsh environmental conditions. Relative water content and leaf water potential are the most important indicators of drought stress in plants and can be considered as an index for drought tolerance [29]. It is believed that H₂O₂ has the ability to improve plant water status [17, 33]. The results of the present study showed that RWC and Ψ_{leaf} declined in response to drought stress. On the other hand, H₂O₂ treated plants exhibited higher RWC and Ψ_{leaf} during drought stress in compare with control plants. In other words, exogenous H₂O₂ treatment recovered leaf water status. Our findings demonstrated that improvement of water status following the exogenous application of H₂O₂ may be a result of alleviation of the detrimental effects of drought stress. Therefore, such an ameliorative effect on RWC and Ψ_{leaf} may have been due to the role of H₂O₂ in osmotic adjustment and ensuring the accumulation of compatible solutes under drought stress conditions. Thus it could be deduced that H₂O₂ contributed to the stress acclimation of soybean cultivars. In a similar study, Ishibashi et al. [17] showed that RWC of drought-stressed leaves pre-treated with H₂O₂ were higher than that of leaves pre-treated with distilled water. In addition, the same authors reported that exogenous application of H₂O₂ caused an increase in the mRNA levels of key enzymes for the biosynthesis of oligosaccharides, which are known to help plants tolerate drought stress. Gene expression changes in this manner enable soybean plant to become adapted and avoid drought stress through the maintenance of leaf water content. In another study, Terzi et al. [33] indicated that H₂O₂ pre-treated seedlings showed improved water status under osmotic stress, which induces osmotic stress tolerance by increasing soluble sugar, proline, and polyamine levels. On the other hand, exogenous H₂O₂ may be a stimulation of stoma-

tal closure to reduce water loss. Indeed, literature contains numerous evidence that H_2O_2 is an essential signal in mediating stomatal closure induced by ABA under a variety of abiotic stresses, such as drought and salinity [18, 26, 27]. However, Ishibashi et al. [17] reported that leaf water retention by H_2O_2 spraying was probably due to the increased oligosaccharide biosynthesis rather than rapid stomatal closure.

In our experiment, drought stress led to a sharp decrease in the amount of photosynthetic pigments. Treatment with H_2O_2 alleviates the drought stress by improving pigment contents in both cultivars. Similar to our results, Abass and Mohamed [1] found that H_2O_2 treatment enhanced plant growth parameters and photosynthetic pigment content in common bean seedlings grown under drought conditions when compared with the seedlings of water treated seeds. Additionally, Liu et al. [23] showed that exogenous H_2O_2 protects the chloroplast ultrastructure under osmotic stress induced by PEG in two cucumber (*Cucumis sativus* L.) varieties. These findings demonstrate that H_2O_2 pre-treatment is a potential inducer to tolerate drought in plants by a great number of physiological processes and that this leads to better growth and development.

Lipid peroxidation is used to assess the extent of oxidative stress in plants, and its level increased under stress conditions [23]. In the present study, H_2O_2 pre-treatment prevented lipid peroxidation while the peroxidation increased in both cultivars under drought stress. According to presented results, exogenous low-dose H_2O_2 lowered level of lipid peroxidation and protected seedlings from oxidative damage. On the other hand, decrease in MDA content may be associated with the stimulation of antioxidant enzymes by low doses of H_2O_2 . There were other reports suggesting exogenous H_2O_2 applications avoided the rising of MDA and endogenous H_2O_2 concentration in plants [21, 33, 35].

Under stress conditions, the generation of ROS is a common phenomenon observed in most plants. Increasing ROS level causes oxidative damage to biomolecules such as lipids, proteins and nucleic acids [30]. In our work, drought stress caused accumulation of H_2O_2 , but H_2O_2 concentration was effectively reduced by H_2O_2 pre-treatment in both cultivars. Similarly, Hossain and Fujita [14] examined the potential biochemical mechanisms of H_2O_2 treatment induced drought tolerance in mustard (*Brassica juncea* L.) seedlings and found that the concentration of endogenous H_2O_2 were markedly lower than that of seedlings subjected to drought stress without H_2O_2 pre-treatment in H_2O_2 pre-treated drought stressed-seedlings. A strong negative relationship of antioxidant enzymes with H_2O_2 concentration proved that H_2O_2 applications enhanced antioxidant enzymes activity, which resulted in scavenging effectiveness of H_2O_2 . The data suggested that exogenous H_2O_2 might modulate plant defense responses to drought stress through the regulation of H_2O_2 production.

The activation of antioxidant system is an important adaptive strategy to reduce ROS production and minimize oxidative damage under stress conditions. In most cases, H_2O_2 seems to be positively used by plants to activate multiple stress-responsive genes that help plants cope with environmental challenges. Genes encoding antioxidant enzymes are central players in this network and their function has pro-

found effects in controlling excessive ROS accumulation and cellular redox balance [15]. In this work, some antioxidant enzymes (SOD, APX, CAT, GR, MDHAR, DHAR) were studied in soybean cultivars in order to explain the role of exogenous low-dose H_2O_2 on the regulation of antioxidant defense in plants under drought stress. Based on the results, it has been clear that H_2O_2 induces antioxidant enzyme activities under drought stress more than that of the other groups did. Additionally, exogenous H_2O_2 pre-treatment further enhanced the activities of antioxidant enzymes in the tolerant cultivar compared to the sensitive cultivar. There are data supporting the claim that H_2O_2 increases the activity of antioxidant enzymes such as CAT, APX and SOD in cucumber seedlings and *Ctenanthe setosa* plants under osmotic stress [23, 28]. According to our results, the higher activities of antioxidant enzymes in the H_2O_2 pre-treated plants coincide with a decrease in the concentration of endogenous H_2O_2 , suggesting that H_2O_2 pre-treatment increases the ability of soybean cultivars to scavenge H_2O_2 via antioxidant enzymes under drought stress.

APX, MDHAR, DHAR and GR are also involved in the ASC–GSH cycle, which is an efficient antioxidant system for detoxifying H_2O_2 in the cell. Based on our data, there is also a trend in activities similar to that of the enzymes above in ASC–GSH cycle in both cultivars. These antioxidant enzyme activities in plants usually depend on antioxidant metabolites such as ascorbate, glutathione, tocopherol and polyphenols [20], especially ascorbate and glutathione play a key role in the redox signal transduction in higher plants under stress conditions [20]. Therefore, this metabolism might be partly responsible for higher antioxidant capacity. Our results showed that ASC–GSH cycle enzymes may act to remove produced H_2O_2 under drought stress. Thus, H_2O_2 may increase drought stress tolerance of soybean cultivars by promoting the activity of ASC–GSH cycle. At the same time, higher MDHAR, DHAR and GR activities were also observed in H_2O_2 treatments under osmotic stress, and increase in GR activity could promote the recycle of GSSG to GSH [10]. It is well known that ascorbate function greatly depends on its redox status which is closely related with MDHAR and DHAR specific enzyme activities, and increasing MDHAR and DHAR specific enzyme activities by exogenous H_2O_2 under osmotic stress has been important for the efficient antioxidant activity. Similarly, Hossain and Fujita [14] reported that H_2O_2 pre-treated seedlings maintained significantly higher APX, GR, CAT and GST activities, as well as a higher GSH/GSSG ratio compared with seedlings under drought only, which can activate ROS detoxification pathways and modulate the tolerance of seedlings to water deficit under drought stress.

In conclusion, water status and antioxidant enzymes activities were significantly enhanced by exogenous low-dose H_2O_2 pre-treatment in soybean cultivars under drought stress. When H_2O_2 treatment was combined with drought stress the level of endogenous H_2O_2 and MDA was reduced. However, exogenous H_2O_2 increased the antioxidant enzyme activities even more. An increased antioxidant capacity may, in turn, decrease the level of H_2O_2 in the cells. These findings indicate that application of H_2O_2 can help reduce the negative effects of drought stress by inducing antioxidant activity and decreasing H_2O_2 level and water loss to some extent and may have a key role in providing tolerance to soybean.

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