

DOI: 10.5586/aa.1776

**Publication history**

Received: 2019-01-16

Accepted: 2019-05-31

Published: 2019-07-01

**Handling editor**

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M, PKB, MH, and MF conceived and designed the study; M, JAM, and KN executed the study; MH performed the statistical analysis of the data; MF and PKB contributed reagents, materials, and analytical tools; M and MH wrote the manuscript; MH and MF edited the manuscript; all authors contributed to improving the paper

**Funding**

The authors greatly acknowledge the Japan Student Services Organization (JASSO) Japan for providing a research fund.

**Competing interests**

MH served as guest editor of the issue; other authors: no competing interests have been declared

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**Citation**

Moumita, Mahmud JA, Biswas PK, Nahar K, Fujita M, Hasanuzzaman M. Exogenous application of gibberellic acid mitigates drought-induced damage in spring wheat. *Acta Agrobot.* 2019;72(2):1776. <https://doi.org/10.5586/aa.1776>

**ORIGINAL RESEARCH PAPER**

# Exogenous application of gibberellic acid mitigates drought-induced damage in spring wheat

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**Abstract**

Drought stress is a major problem in wheat production but it could be managed by using various exogenous protectants such as gibberellic acid (GA). Although GA is a plant growth hormone, it shows a potential to protect the plant in stress conditions. To investigate the possible role of GA in mitigating drought stress, we treated wheat (*Triticum aestivum* 'BARI Gom-21') seedlings with a GA spray under semihydroponic conditions. In the experiment, the combined effect of GA and drought stress (induced by 12% polyethylene glycol) was studied after 48 h and 72 h. In the absence of exogenous GA, drought-stressed wheat seedlings showed various physiological and biochemical changes in a time-dependent manner. Malondialdehyde (MDA), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and free proline (Pro) concentrations were increased, whereas catalase (CAT) and ascorbate peroxidase (APX) activities were reduced under drought stress. Gibberellic acid played a role in restoring the ascorbate (AsA) level, decreased the reduced/oxidized glutathione (GSH/GSSG) ratio and reduced monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) activities. Gibberellic acid significantly affected the glyoxalase system. Under drought stress, the methylglyoxal (MG) concentration was increased but GA application stimulated glyoxalase I (Gly I) and glyoxalase II (Gly II) activities to protect the wheat seedlings against stress. The study concluded that the severity of drought stress in wheat depends on the growth stage and it increases with an increase in the duration of stress, whereas exogenous GA helped the seedlings to survive by upregulating antioxidant defense mechanisms and the glyoxalase system.

**Keywords**

osmotic stress; reactive oxygen species; antioxidant; phytohormones; glyoxalase

**Introduction**

Plants are sessile organisms that have to face many unfavorable conditions due to their unstable and changing surroundings. These stressful conditions include both biotic and abiotic stresses. Due to climate change, the geographical distribution of plants may be affected which lead to decreases in plant productivity which ultimately could threaten food security. It has been reported that abiotic stress is responsible for most of the damage to plants, even as much as 50% [1]. Among the abiotic stresses, drought causes severe damage to plants in various ways, reducing growth by disrupting photosynthesis and other physiological functions, which ultimately reduce yield. Production of reactive

oxygen species (ROS) is one of the most important biochemical responses of plants to drought stress. Oxygen plays an important role in normal metabolism and in cell signaling, but during drought stress, ROS (e.g., singlet oxygen  $^1\text{O}_2$ , superoxide anion  $\text{O}_2^-$ , hydrogen peroxide  $\text{H}_2\text{O}_2$ , or hydroxyl radical  $\text{OH}^\bullet$ ) are overproduced which are very dangerous for plants [2]. Reactive oxygen species levels increase drastically resulting in oxidative damage to proteins, DNA, and lipids, and cause damage to plants by increasing lipid peroxidation, protein degradation, DNA fragmentation, and ultimately leading to cell death [1,2]. Under drought stress, stomata close and  $\text{CO}_2$  concentrations inside the leaf are reduced. Carbon fixation is therefore disrupted, and excessive excitation energy is produced in chloroplasts [3]. Under severe stress conditions, excited pigments in the thylakoid membranes may interact with  $\text{O}_2$  and form  $\text{O}_2^-$  or  $^1\text{O}_2$ , and more downstream reactions produce other ROS, such as  $\text{H}_2\text{O}_2$  and  $\text{OH}^\bullet$ . Furthermore, the reaction of  $\text{O}_2$  with other reduced components of the electron transport chain in the mitochondria can produce ROS and during photorespiration, and  $\text{H}_2\text{O}_2$  may be produced in the peroxisomes [4].

Malondialdehyde (MDA),  $\text{H}_2\text{O}_2$ , and methylglyoxal (MG) are used as indicators of oxidative stress. Under drought stress,  $\text{H}_2\text{O}_2$  concentrations, lipid peroxidation levels, ROS such as  $\text{H}_2\text{O}_2$ ,  $\text{O}_2^-$  generation rates, and MG levels are generally increased [4,5]. However, plants enable a defensive system to avoid injury to allow continued normal function. This defense system creates a balance between ROS production and activities of the antioxidative system to determine whether plants survive or they will be damaged by ROS. To minimize the effects of this damage, plants maintain the antioxidant defense system, which consists of ROS-scavenging enzymes: superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and nonenzymatic components: reduced glutathione (GSH), ascorbate (AsA), carotenoids (Car), tocopherol, phenolic compounds, alkaloids, etc., and this mechanism helps plants to cope with the stress either by avoiding and/or by removing the oxidative stress [2]. Gibberellic acid (GA; also known as gibberellin A3 and  $\text{GA}_3$ ) is a hormone that is found in both plants and fungi. It is used mainly for stimulating plant growth but it also works as a protectant under stress conditions. Various plant growth regulators have the potential to regulate water balance, stomatal closure, and are also responsible for activating secondary metabolism under drought stress [6]. Gibberellic acid is able to scavenge ROS and under drought stress, it assists plants with their more negative water potential and so maintaining photochemical efficiency of PSII [7]. It is also thought that GA assists plants under nutrient stress by increasing nutrient uptake as well as nitrogen use efficiency [8]. Pan et al. [9] showed that different growth hormones, including GA, play a similar role as antioxidant enzymes in deleting ROS. These authors also stated that GA helps to decrease lipid peroxidation and increase SOD and POD activity. Various plant growth regulators including GA improve photosynthetic ability, decrease leaf senescence, and aid in increasing seed-set under drought stress [10]. Gibberellic acid is known to increase the chlorophyll content of leaves and mineral nutrients uptake under abiotic stress [11,12], as well as mitigating the adverse effects of drought and thus improve plant growth [13,14].

The aim of the present work was to investigate the effect of GA on biochemical and physiological parameters of wheat after 48 h and 72 h of drought stress at the early seedling stage, focusing specifically on the antioxidant defense and glyoxalase systems.

## Material and methods

### Plant material and stress treatments

Uniform seeds of wheat (*Triticum aestivum* L. 'BARI Gom-21') were selected and thoroughly washed with distilled water after sterilization with 70% ethanol. They were then sown in 9-cm Petri dishes lined with six layers of filter paper moistened with 10 mL of distilled water and placed in the dark in a germinator for 48 h. Forty morphologically uniform seedlings in each Petri dish were grown on in a growth chamber (IWAKI, Asahi Techno Glass, Japan) under controlled conditions (photon flux density:  $350 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , temperature:  $25 \pm 2^\circ\text{C}$ , photoperiod: 14 h; relative humidity: 65–70%)

using 50% Hoagland's solution as a nutrient source. The full strength nutrient solution contained: 8% N, 6.43% P, 20.94% K, 11.8% Ca, 3.08% Mg, 0.07% B, 0.24% Fe, 0.03% Mn, 0.0014% Mo, 0.008% Zn, and 0.003% Cu.

Seven-day-old seedlings were subjected to drought stress by using 12% of polyethylene glycol (PEG-6000) in 50% Hoagland's solution and plants grown under the above conditions for 48 h or 72 h. Gibberellic acid ( $100 \text{ mg L}^{-1}$ ) or water (as the control) was sprayed while the drought stress was imposed. Approximately 5 mL of spray was used as this volume did not cause excessive loss of the solution from the leaves.

The study was arranged as a completely randomized design with 12 treatments and three replicates. The treatments were: control (C), water spraying (C+W), GA spraying (C+G), drought (D), water spraying during drought (D+W), and GA spraying during drought (D+G), for both 48 h and 72 h. Seedlings were selected after 48 h or 72 h of treatment and used for the study of various growth and physiological parameters.

### Measurement of growth parameters

Plant heights were measured and fresh weights (FW) and dry weights (DW) determined. For FW, 10 randomly selected seedlings were weighed. These 10 seedlings were then dried in oven at  $80^\circ\text{C}$  for 48 h and again weighed for DW determination.

### Measurement of leaf relative water content

Relative water content (RWC) was measured according to the method of Alam et al. [5]. Ten randomly selected seedlings were chosen and the leaf laminae weighed to determine their FW. These leaves were then floated on distilled water in a Petri dish and kept in the dark for 8 h. After this, excess surface water was removed with a paper towel and the leaves were weighed again and to give turgid weights (TW). The leaves were then placed in an oven at  $80^\circ\text{C}$  for 48 h and the DW were measured. RWC values were calculated using the formula:  $RWC (\%) = [(FW - DW)/(TW - DW)] \times 100$ .

### Estimation of lipid peroxidation

Malondialdehyde concentration is an indicator of lipid peroxidation. To estimate this, the methodology of Heath and Packer [15] was followed with a slight modification by Hasanuzzaman et al. [16]. Leaf samples (0.5 g) were ground in 5% (w/v) trichloroacetic acid (TCA). The homogenates were collected in centrifuge tubes and centrifuged at  $11,500 \text{ g}$  for 15 min. The supernatant was collected and mixed with thiobarbituric acid (TBA) and heated at  $95^\circ\text{C}$  in a hot water bath. After 30 min, it was allowed to cool and the absorbance was then read at 532 nm. Malondialdehyde concentration was calculated by using the extinction coefficient  $155 \text{ mM}^{-1} \text{ cm}^{-1}$  and expressed as nmol of MDA  $\text{g}^{-1}$  FW.

### Determination of methylglyoxal concentration

The method of Nahar et al. [4] was employed to estimate the MG content. Perchloric acid (5%) was mixed to homogenize leaf samples which were then centrifuged at  $4^\circ\text{C}$  for 10 min at  $11,000 \text{ g}$ . Charcoal was added to decolorize the supernatant and a saturated solution of sodium carbonate was added to neutralize it. Sodium dihydrogen phosphate and *N*-acetyl-L-cysteine (20  $\mu\text{L}$ ) were added to the supernatant and the final volume was 1 mL, where *N*- $\alpha$ -acetyl-S-(1-hydroxy-2-oxoprop-1-yl) cysteine was formed. After 10 min, the absorbance at 288 nm was recorded, and the MG concentration was calculated by using a standard curve.

### Measurement of hydrogen peroxide

Hydrogen peroxide was measured according to the methodology of Yu et al. [17]. 0.5 g leaf sample was homogenized with 3 mL of 50 mM potassium-phosphate (K-P) buffer (pH 6.5) at 4°C and then centrifuged at 11,500 g for 15 min. One mL of 0.1% TiCl<sub>4</sub> in 20% H<sub>2</sub>SO<sub>4</sub> (v/v) was mixed with 3 mL of supernatant. After 10 min at room temperature, supernatants were again centrifuged at 11,500 g for 12 min. To determine H<sub>2</sub>O<sub>2</sub> the absorption was read at 410 nm. An extinction coefficient of 0.28 μM<sup>-1</sup> cm<sup>-1</sup> was used to calculate H<sub>2</sub>O<sub>2</sub> concentration which was then expressed as nmol g<sup>-1</sup> FW.

### Determination of free proline (Pro) concentration

The method of Alam et al. [5] was used whereby a 0.25 g leaf sample was homogenized with 5 mL of 3% sulfosalicylic acid and then centrifuged at 11,500 g for 15 min. Two mL supernatant was mixed with 1 mL of acid ninhydrin (1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL 6 M phosphoric acid) and 1 mL of glacial acetic acid. The mixture was placed in a water bath at 100°C. After 1 h, it was transferred to a test tube and kept on ice. Then, 2 mL of toluene was added and mixed thoroughly using a vortex mixer. The upper layer was transferred and measured spectrophotometrically at 520 nm where toluene was used as the blank. Pro concentration was determined from a standards curve.

### Determination of chlorophyll (chl) concentration

Fresh leaf material (0.25 g) was taken from randomly selected seedlings to measure the chl concentrations. Ten mL of acetone (80% v/v) was used as the homogenizing reagent and then the samples were centrifuged at 10,000 g for 10 min. The absorbance of the supernatants was measured with a spectrophotometer at 663 and 645 nm for chl *a* and *b*, respectively. Chl concentrations were calculated according to the formula of Hasanuzzaman et al. [16].

### Histochemical detection of H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>-</sup>

Histochemical analysis was performed mainly to detect the O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub> in leaves using a method according to Nahar et al. [4] with slight modification. Here, 0.1% nitrobluetetrazolium chloride (NBT) solution and 0.1% 3-diaminobenzidine (DAB) were used to stain the leaf samples. Leaves were stained in these solutions for 24 h in the dark and next, they were blanched by immersing in boiling ethanol. H<sub>2</sub>O<sub>2</sub> reacted with DAB and brown spots were detected. Blue spots were due to the reaction of O<sub>2</sub><sup>-</sup> with NBT [18].

### Determination of ascorbate and glutathione

Fresh leaf samples were homogenized with meta-phosphoric acid and ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged and analyzed for AsA and GSH. The AsA content was measured according to the method of Alam et al. [5]. The oxidized fraction was reduced by 0.1 M dithiothreitol and AsA was determined spectrophotometrically using a standard curve. The GSH pool was determined by the method of Yu et al. [17] using 2-nitro-5-thiobenzoic acid (NTB). Oxidized glutathione (GSSG) was measured after removing GSH by 2-vinylpyridine derivatization. The absorbance was read at 412 nm. Standard curves with known concentrations of GSH and GSSG were used and GSH was calculated by subtracting GSSG from total GSH.

## Enzyme extraction and assays

Leaf samples were taken in a precooled mortar and pestle and homogenized. The homogenates were centrifuged and the supernatant was used for determination of enzyme activities at 0–4°C.

APX (EC: 1.11.1.11) activity was measured according to the methodology of Nakano and Asada [19]. The enzyme extract was mixed with a reaction buffer [50 mM K-P buffer (pH 7.0), 0.5 mM AsA, 0.1 mM H<sub>2</sub>O<sub>2</sub>, 0.1 mM EDTA] and the final volume was 700 µL. The absorbance reading was in a decreasing trend and it was performed at 290 nm for 1 min. An extinction coefficient of 2.8 mM<sup>-1</sup> cm<sup>-1</sup> was used to calculate the APX activity.

CAT (EC: 1.11.1.6) activity was determined using the method according to Hasanuzzaman et al. [16]. The enzyme solution was mixed with the 50 mM K-P buffer (pH 7.0), 15 mM H<sub>2</sub>O<sub>2</sub> and the final volume was 700 µL. The CAT activity was measured by using the decreasing trend of absorbance at 240 nm and an extinction coefficient of 39.4 M<sup>-1</sup> cm<sup>-1</sup>.

Monodehydroascorbate reductase (MDHAR; EC: 1.6.5.4) activity was measured by the method of Hossain et al. [20]. The change of absorbance was measured at 340 nm for 1 min and MDHAR activity was calculated using an extinction coefficient of 6.2 mM<sup>-1</sup> cm<sup>-1</sup>. It was expressed as nmol min<sup>-1</sup> mg<sup>-1</sup> protein.

Dehydroascorbate reductase (DHAR; EC: 1.8.5.1) activity was measured according to Nakano and Asada [19]. In brief, the crude enzyme solution was mixed with the reaction buffer and distilled water. The absorbance was read at 265 nm for 1 min and DHAR activity was calculated using an extinction coefficient of 14 mM<sup>-1</sup> cm<sup>-1</sup> and expressed as nmol min<sup>-1</sup> mg<sup>-1</sup> protein.

Glutathione reductase (GR; EC: 1.6.4.2) activity was measured by the procedure of Hasanuzzaman et al. [21]. The absorbance reading was taken at 340 nm for 1 min and the activity was calculated using an extinction coefficient of 6.2 mM<sup>-1</sup> cm<sup>-1</sup>.

Glyoxalase I (Gly I; EC: 4.4.1.5) activity was measured according to the method of Hasanuzzaman et al. [22] using the increasing trend of absorbance at 240 nm for 1 min. The activity was calculated using an extinction coefficient of 3.37 mM<sup>-1</sup> cm<sup>-1</sup>.

Glyoxalase II (Gly II; EC: 3.1.2.6) activity was assayed using the procedure of Principato et al. [23]. The absorbance was recorded at 412 nm for 1 min. Gly II activity was calculated using an extinction coefficient of 13.6 mM<sup>-1</sup> cm<sup>-1</sup> and was expressed as µmol min<sup>-1</sup> mg<sup>-1</sup> protein.

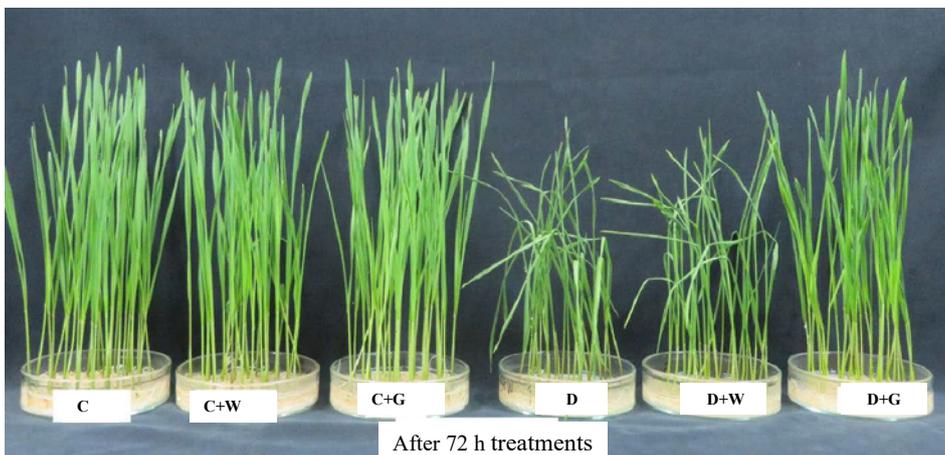
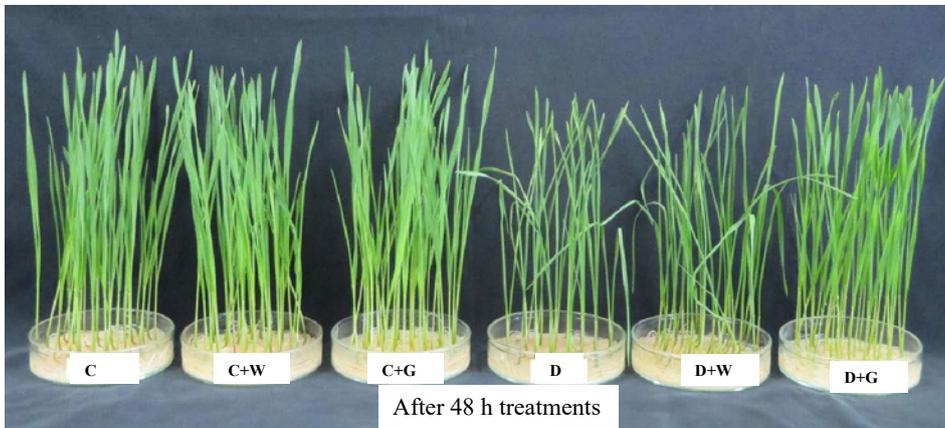
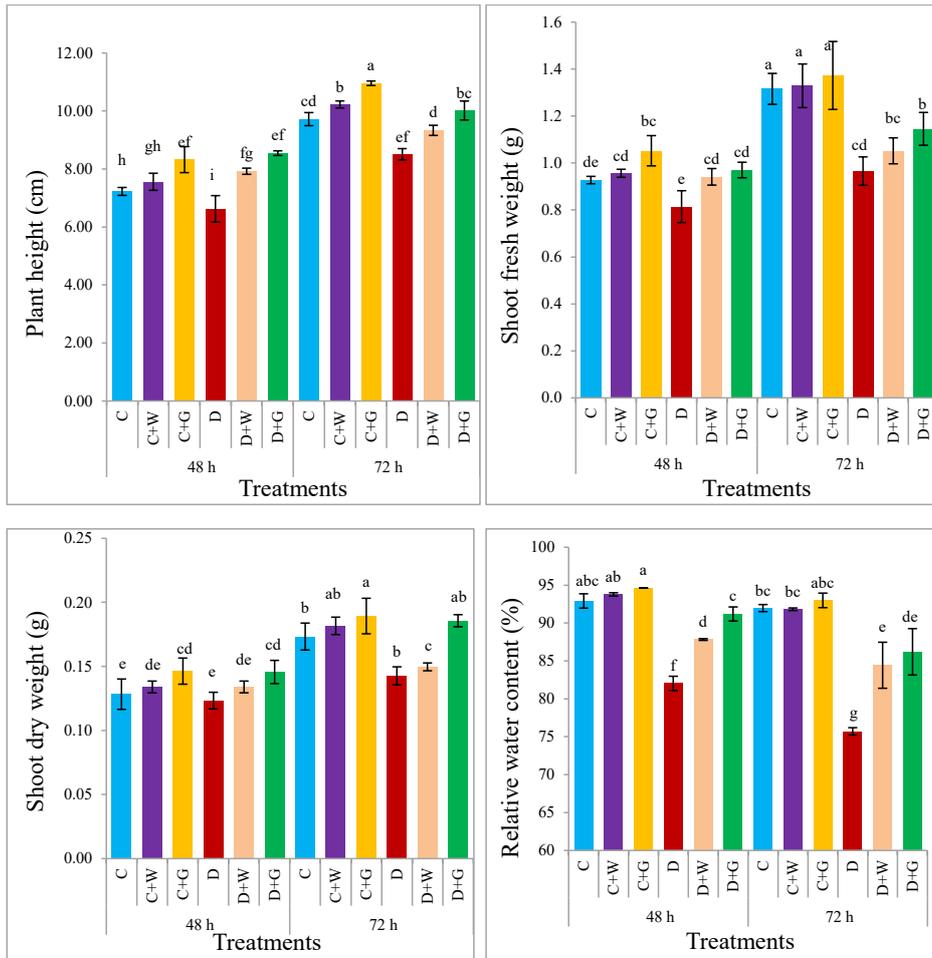
## Statistical analysis

The data were analyzed with the computer-based software XLSTAT 2016 [24] following analysis of variance (ANOVA). Mean separations were tested by Duncan's multiple range test (DMRT) where  $p < 0.05$  was considered as significant.

## Results

### Growth and biomass

The shortest plants were those grown under drought stress, whereas the maximum plant heights were found in the C+G treatment after 48 h and 72 h (Fig. 1). Plant height increased by 15% after 48 h and by 11% after 72 h in the C+G treatment in comparison to the C treatment. It was reduced by 8% and 12% after 48 h and 72 h, respectively, due to the drought stress. Exogenous GA increased the height of plants exposed to the drought stress by about 20% and 12% after 48 h and 72 h, respectively (Fig. 1). Fresh weight was reduced by 12% and 27% in the D treatment compared to the control after 48 h and 72 h, respectively. After GA spraying, 16% and 19% increases in FW were noted after 48 h and 72 h, respectively, as compared to the drought-stressed plants (Fig. 1). After 48 h, the D+W and D+G treatments gave similar results for increasing FW, but after 72 h, the D+W did not show any significant difference, whereas D+G further increased FW.



**Fig. 1** Effect of drought stress and exogenous GA on plant height, fresh weight, dry weight, and relative water content in wheat seedlings. C – control; W – water; D – drought induced by 12% PEG; G – GA and C+G, C+W, D+G, D+W denotes only GA spraying, only water spraying, GA spraying during drought, and water spraying during drought, respectively. Means ( $\pm$ SD) was calculated from three replicates for each treatment. Means with different letters on top of the bars are significantly different at  $p \leq 0.05$  from LSD tests.

Drought stress also reduced the DW by 4% and 18% after 48 h and 72 h, respectively. However, in the D+G treatment, 18% and 30% increases in DW were recorded after 48 h and 72 h, respectively, as compared to the drought-stressed plants (Fig. 1).

#### Relative water content

Relative water content decreased with drought stress by 12% and 18% after 48 h and 72 h, respectively, as compared to the control (C). The D+G treatment increased RWC by 11% after 48 h and by 14% after 72 h in comparison to the D treatment. It was also found that RWC was the greatest in the C, C+W, and C+G plants. In the D+W treatment, the RWC increased by about 7% and 12% after 48 h and 72 h, respectively, as compared to drought treatments (Fig. 1).

#### Chlorophyll

Drought stress significantly reduced chl content. The percentage reductions in chl *a* and *b* and total chl were 12%, 15%, and 12%, respectively. However, the exogenous application of GA partially reduced the inhibitory effects of drought and significantly enhanced the chl *b* concentration by 17% in plants harvested after 72 h after drought treatment, as compared to untreated, drought stressed plants (D). Under our experimental conditions, water spray (D+W) played a similar role to GA (D+G) and helped to maintain chl *b* concentration during drought stress (Tab. 1).

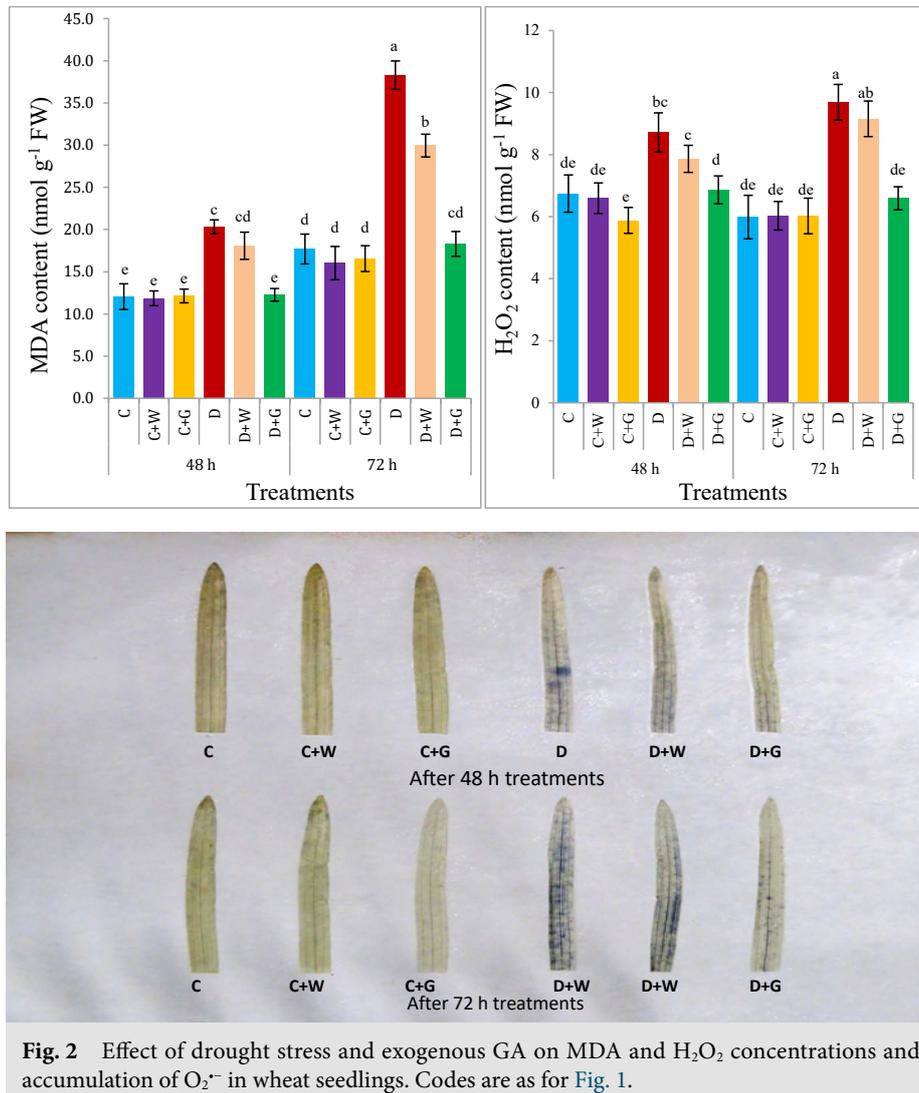
**Tab. 1** Effect of exogenous GA on chlorophyll concentrations in wheat seedlings under drought stress.

Treatment	Chl <i>a</i> (nmol/g DW)	Chl <i>b</i> (nmol/g DW)	Chl ( <i>a</i> + <i>b</i> ) (nmol/g DW)
C 48 h	12.49 $\pm$ 0.66 <sup>abc</sup>	3.47 $\pm$ 0.20 <sup>a</sup>	15.96 $\pm$ 0.84 <sup>ab</sup>
C+W 48 h	12.18 $\pm$ 0.64 <sup>abc</sup>	3.19 $\pm$ 0.26 <sup>ab</sup>	15.37 $\pm$ 0.83 <sup>abc</sup>
C+G 48 h	12.33 $\pm$ 0.65 <sup>abc</sup>	3.28 $\pm$ 0.17 <sup>ab</sup>	15.61 $\pm$ 0.82 <sup>abc</sup>
D 48 h	11.02 $\pm$ 0.58 <sup>d</sup>	2.95 $\pm$ 0.31 <sup>bc</sup>	13.97 $\pm$ 0.78 <sup>d</sup>
D+W 48 h	11.69 $\pm$ 0.62 <sup>cd</sup>	3.17 $\pm$ 0.32 <sup>ab</sup>	14.86 $\pm$ 0.83 <sup>bcd</sup>
D+G 48 h	11.52 $\pm$ 0.61 <sup>cd</sup>	3.33 $\pm$ 0.20 <sup>ab</sup>	14.85 $\pm$ 0.79 <sup>bcd</sup>
C 72 h	13.02 $\pm$ 0.69 <sup>a</sup>	3.48 $\pm$ 0.21 <sup>a</sup>	16.49 $\pm$ 0.87 <sup>a</sup>
C+W 72 h	12.80 $\pm$ 0.68 <sup>ab</sup>	3.30 $\pm$ 0.22 <sup>ab</sup>	16.10 $\pm$ 0.85 <sup>ab</sup>
C+G 72 h	12.47 $\pm$ 0.67 <sup>abc</sup>	3.43 $\pm$ 0.20 <sup>a</sup>	15.90 $\pm$ 0.84 <sup>ab</sup>
D 72 h	11.76 $\pm$ 0.62 <sup>bcd</sup>	2.73 $\pm$ 0.34 <sup>c</sup>	14.49 $\pm$ 0.81 <sup>cd</sup>
D+W 72 h	12.55 $\pm$ 0.69 <sup>abc</sup>	3.23 $\pm$ 0.31 <sup>ab</sup>	15.78 $\pm$ 0.83 <sup>abc</sup>
D+G 72 h	12.47 $\pm$ 0.66 <sup>abc</sup>	3.19 $\pm$ 0.19 <sup>ab</sup>	15.66 $\pm$ 0.83 <sup>abc</sup>

Treatment codes: C – control; W – water spraying; D – drought induced by 12% PEG; GA spraying and C+G, C+W, D+G, D+W denote only GA spraying, only water spraying, GA spraying during drought, and water spraying during drought, respectively. Means ( $\pm$ SD) were calculated from three replicates for each treatment. Means in a column with different letters are significantly different at  $p \leq 0.05$  from the LSD test.

### Markers of oxidative damage

Our results showed that MDA concentration, as a product of lipid peroxidation, was increased under drought stress in a time-dependent manner. Malondialdehyde concentration increased by 68% and 116% after 48 h and 72 h of drought stress, respectively. The D+G treatment significantly reduced MDA accumulation by 40% and 52% after 48 h and 72 h, respectively, in comparison to the drought condition (Fig. 2). The D+W treatment showed no significant influence after 48 h, but after 72 h, a 23% decrease in MDA concentration was found. As drought increased the oxidative damage of lipids, the  $H_2O_2$  concentration also increased about 29% and 62% in stressed plants after 48 h and 72 h, respectively. Gibberellic acid did play a protective role under drought stress by reducing  $H_2O_2$  accumulation. As well for D, in the D+G treatment, there were 21% and 32% concentrations of  $H_2O_2$  after 48 h and 72 h, respectively, (Fig. 2) but treatments D and D+W did not show any differences between them after 48 h and 72 h.

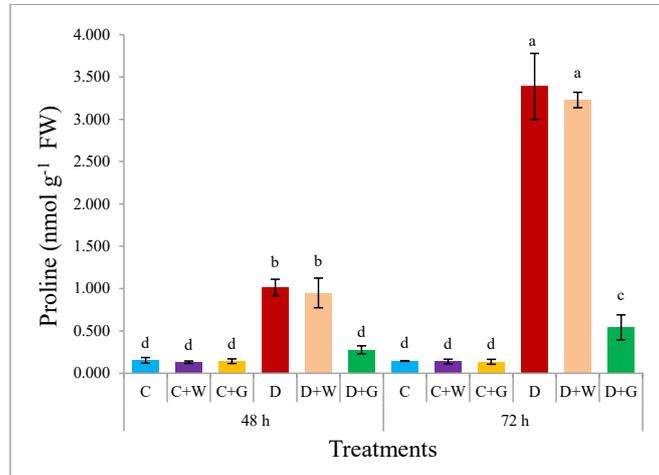


### Histochemical detection of $H_2O_2$ and $O_2^{\cdot-}$

Leaves from each treatment were collected and stained with DAB and NBT to observe ROS accumulation. Brown spots due to the production of  $H_2O_2$  were formed due to the DAB staining and dark blue spots were formed after NBT staining as a result of  $O_2^{\cdot-}$  generation. In drought-stressed plants, the spots on leaves were very prominent and were more frequent than in the GA-treated and drought exposed plants (Fig. 2).

### Proline concentration

Free proline accumulation increased several-fold under drought stress, especially after 72 h. Conversely, the D+G treatment caused a decrease in its concentrations to 16% and 27% of the values recorded under drought stress conditions (D) (Fig. 3).



**Fig. 3** Effect of drought stress and exogenous GA on free proline content in wheat seedlings. Codes are as for Fig. 1.

### Nonenzymatic antioxidants

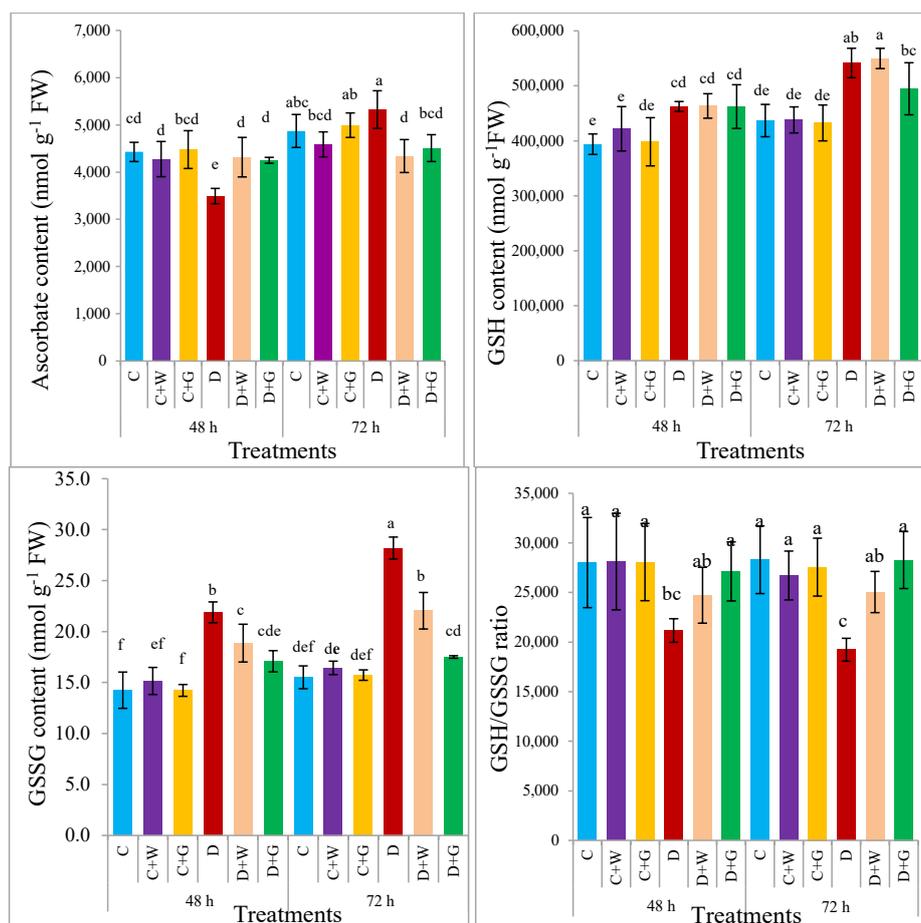
It was found that after 48 h of drought stress, AsA concentration was reduced by 27% and D+W and D+G increased its level about 12% and 24%, respectively, by comparison to the respective controls. The opposite trend was noted after drought stress at 72 h, where AsA concentration increased. Under these conditions both D+W and D+G treatments decreased the AsA concentration by 23% and 19%, respectively, in comparison to the D treatment (Fig. 4). Moreover, drought stress increased the GSH concentration after 48 h and 72 h by 17% and 24%, respectively. However, the D+G treatment did not cause significant changes in the GSH pool both after 48 h and 72 h (Fig. 4).

The content of GSSG increased under drought stress and with the time of its duration (Fig. 4). About 53% and 46% increases in GSSG concentration were noted after 48 h and 72 h of drought stress, in comparison to the respective control. Under D+G treatment, decreases in the GSSG concentration by 22% and 38% were found after 48 h and 72 h, respectively. Water spray (D+W) caused about a 22% decrease in GSSG concentration after 72 h (Fig. 4).

Drought stress reduced the GSH/GSSG ratio. After 48 h, this reduction was about 32%, whereas after 72 h, it was 37%. Gibberellic acid played a role in restoring the GSH/GSSG ratio as approximately 28% (after 48 h) and 35% (72 h) increases in this ratio were found in the D+G plants as compared to that in those that were drought-stressed (D) (Fig. 4).

### Enzymatic antioxidants

Drought stress increased the APX activity by about 54% and 61% after 48 h and 72 h, respectively. After application of GA it was found that the activity of APX was 33% (48 h) and 37% (72 h) lower than under stress conditions, which was statistically similar to the control (Fig. 5). Catalase activity increased under drought stress by about 17% and 22% after 48 h and 72 h, respectively. Gibberellic acid treated plants showed 10% and 7% lower CAT activity than drought-stressed plants not supplied with GA (Fig. 5). Compared to the control, MDHAR activity increased by 16% and 10% under drought stress after 48 h and 72 h, respectively. Exogenous GA applied under drought

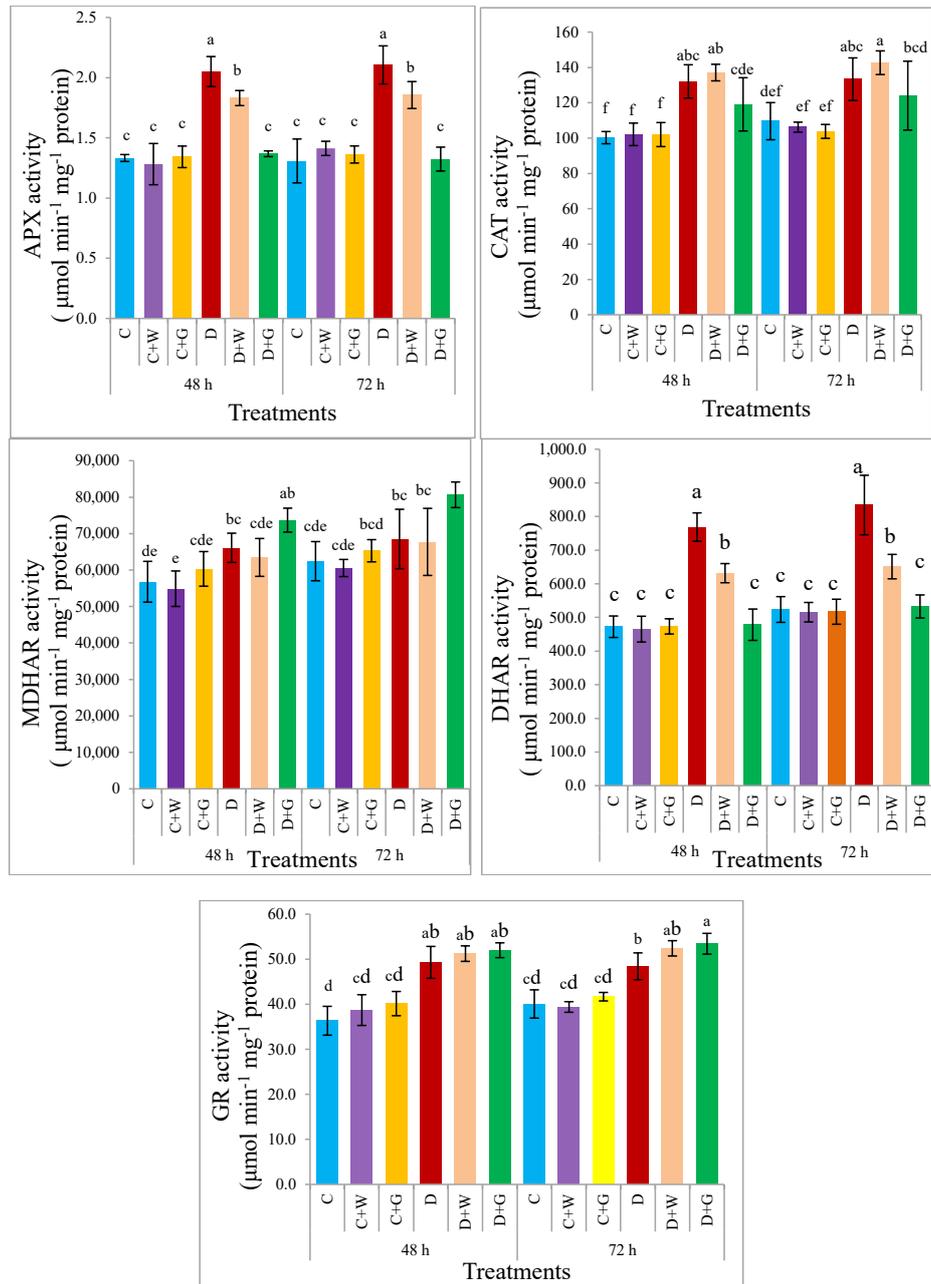


**Fig. 4** Effect of drought stress and exogenous GA on ascorbate-glutathione pool in wheat seedlings. Codes are as for Fig. 1.

stress reduced the damage to plants as it was found that the MDHAR activity was 11% (48 h) and 18% (72 h) higher than the stressed plants. Drought stress reduced DHAR activity by 63% and 59% after 48 h and 72 h, respectively, whereas the GA-treated seedlings showed 38% and 36% lower DHAR activity after 48 h and 72 h, respectively, as compared to those drought-stressed. The D+W treatment reduced DHAR activity by 18% and 22%. Under drought stress, GR activity increased about 26% and 21% after 48 h and 72 h, respectively, compared to the control. Exogenous GA did not show any significant influence on GR activity in drought exposed plants after 48 h, but when the stress lasted for 72 h, its activity was 9% higher in GA supplied plants (Fig. 5).

#### Methylglyoxal concentration and glyoxalase system activity

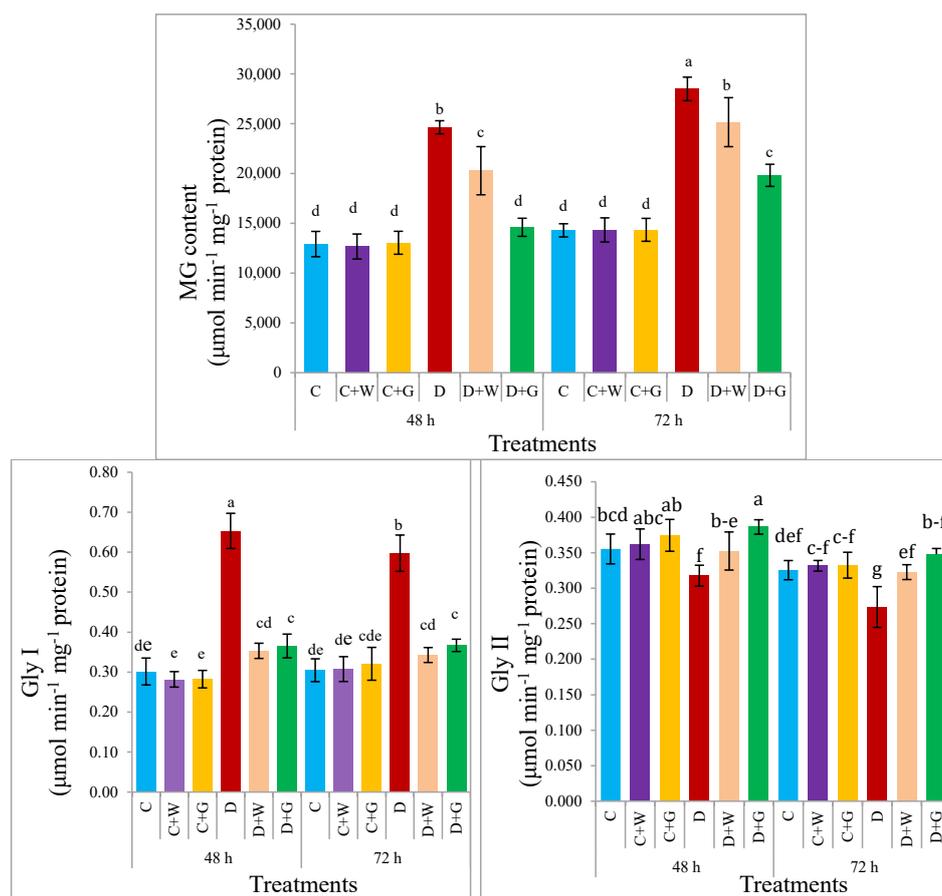
Drought stress increased MG concentration by 91% and 99% after 48 h and 72 h, respectively. Application of GA under drought stress reduced the MG concentration by about 41% and 30% after 48 h and 72 h, respectively, in comparison to drought alone. The D+W treatment played a less significant role than exogenous GA as it reduced MG concentration by 18% and 12% after 48 h and 72 h, respectively (Fig. 6). It was found that Gly I activity was increased by about twice due to drought stress, as compared to the control. Both GA and water application under drought reduced the Gly I activity almost to the level of the control (C). Under drought stress, the activity of Gly II decreased by about 11% and 16% after 48 h and 72 h, respectively, in comparison to the control. Exogenous GA increased Gly II activity by 22% and 27% under drought stress after 48 h and 72 h, respectively (Fig. 6).



**Fig. 5** Effect of drought stress and exogenous GA on AsA-GSH cycle enzymes activity in wheat seedlings. Codes are as for Fig. 1.

## Discussion

Drought is one of the most hazardous abiotic stresses for plants. It reduces plant height, FW, DW, RWC, and other growth parameters. Gill and Tuteja [2] stated that the initial response of plants under drought stress is to close the stomata, so reducing  $\text{CO}_2$  uptake and photosynthesis which ultimately reduces growth and development. Alam et al. [5] stated that under drought conditions plant height may be decreased due to damage by ROS, cell disruption, and reduced photosynthetic activity. In the present experiment, similar findings were found to those that have been reported for mungbean [4], corn [25], maize [26], and chickpea [6]. Siddique et al. [27] supported the theory that drought stressed plants are unable to take up adequate nutrients to sustain normal growth, which explains why shoot DW was lower. Ahmadizadeh et al. [28] and Clua et al. [29] also found similar results. Relative water content (RWC) is widely used to assess plant water status, and also as an indicator of metabolic activity and an index for dehydration tolerance. Plants under drought stress substantially decrease the RWC



**Fig. 6** Effect of drought stress and exogenous GA on MG content and glyoxalase system activity in wheat. Codes are as for Fig. 1.

and transpiration rate, due to increases of leaf temperature [27]. A reduction of RWC under drought conditions was found in mungbean [4], wheat [30], and peanut [31]. In our experiment, plant height, FW, DW, and RWC were all reduced both after 48 h and 72 h of stress, which could be regained by applying GA (Fig. 1). As GA is a plant growth hormone, it increased the plant FWs, DWs, and plant heights [32]. The work of Schwechheimer [33] also supported the present findings as GA application was found to increase plant biomass under environmentally stressed conditions.

A reduction in chl concentration is another effect of drought stress. According to Farooq et al. [34] the decrease in total chl concentration is the result of pigment photo-oxidation and chl degradation. The chl content may be also reduced due to damage to chloroplast membranes causing excessive swelling, distortion of the lamellae, vesiculation, and the appearance of lipid droplets [35]. Similar findings to the present work were reported in wheat [36], *Brassica* spp. [5], sunflower [37], barley [38], canola [39], and chickpea [40]. Our findings also confirm that chl *a*, chl *b* and total chl concentrations were all reduced in drought-stressed plants. However, exogenous application of GA maintained the chl levels by protecting the plant from oxidative stress (Tab. 1). Shah [41] and Turkyilmaz [42] reported that GA application can restore normal chl content. Keyvan [30] also made a similar observation in the case of chl *a* and *b* thus supporting the present findings.

Abiotic stresses such as drought cause serious injury to cell organelles and cell membranes which is frequently the first effect of damage by drought [5]. Under drought stress, ROS are overaccumulated and they damage cell membranes by lipid peroxidation as well as by protein degradation [29]. Hasanuzzaman and Fujita [43] stated that ROS production and an increase in the amount of MDA and H<sub>2</sub>O<sub>2</sub> are clear evidence of plant damage [5,44]. An increase in the production of H<sub>2</sub>O<sub>2</sub> under drought stress is a sign of oxidative stress caused by cell damage [45]. Abedi and Pakniyat [46] also reported that growth and yield are reduced due to the increase of oxidative stress because of

overaccumulation of ROS in chloroplasts, mitochondria, and peroxisomes. Kachout et al. [47] reported that although  $H_2O_2$  is toxic to plants it can be detoxified by CAT and SOD activity. Tatar and Gevrek [48] found that under drought stress wheat was indeed affected by various oxidative stresses. They showed that under drought stress lipid peroxidation increased. Both MDA and  $H_2O_2$  concentrations are the stress markers and in present study it was found that there was an increase in their amounts under drought. However, the GA-treated plants showed a reduced amount of these compounds which is evidence that exogenous GA might work as a drought-protectant [49]. Fath et al. [50] presented a contradictory theory as they found that GA increased plant cell death by increasing  $H_2O_2$  production during germination. Khan et al. [6] have confirmed that plant growth regulators can surely help to increase drought resistance.

When a plant is in a stressed condition one of the most important metabolic responses is the accumulation of free proline as it has the capability to maintain the redox balance in the cell. Moreover, it is an osmo-protectant that assists in the maintenance of water balance and works against the damage caused by ROS, thus aiding plants in adapting to adverse conditions [51]. Tatar and Gevrek [48] also stated that proline is an osmo-protectant that assists plants to survive by reducing ROS levels. In the present study, we detected higher concentrations of free proline under drought stress, but it was also found that exogenous GA-treated plants showed a reduced amount of proline (Fig. 3). Although Li et al. [10] found that free proline increased by about 63% after applying GA in rapeseed plants under drought stress, the findings of Ahmad [52] supported the present study in that GA application decreased proline content by 13% and 21% at different levels of salt stress.

Various enzymatic and nonenzymatic interactions as well as others regulatory mechanisms of plants help to increase resistance to stress. In this study, AsA reduced after 48 h of drought stress as it is a key scavenger of ROS; after applying GA, it was increased. However, after 72 h, the AsA concentration increased and was reduced after GA application (Fig. 4). Hasanuzzaman and Fujita [43] stated that in case of mild drought stress the concentration of AsA increased, but under severe stress AsA levels decrease [53]. A reduction of AsA may be caused due to the reduced form of AsA which is the result of MDHAR and DHAR activities [16]. Here, MDHAR activity was higher under drought than in the control, and exogenous GA further increased its activity (Fig. 5), indicating that GA plays a protective role in stressed plants. As with MDHAR activity under drought stress, DHAR activity also increased which is a sign of plant stress, but it was shown that DHAR activity was reduced in the GA-treated plants (Fig. 5). However, according to Gill and Tuteja [2], under drought stress no significant changes in MDHAR activity and a lower activity of DHAR may be found due to the reduced AsA concentrations.

In the AsA-GSH cycle, the concentration of GSH depends on the activity of GR. In drought conditions, when GSH increases, the GR activity may also increase [54], as we found in our present results. Our study clearly showed higher concentrations of GSH under drought stress, but exogenous application of GA reduced its concentration (Fig. 4), which is in accordance with the findings of Müller et al. [55]. Abedi and Pakniyat [46] and Ahmadizadeh et al. [56] reported the same findings and stated that GA plays a role in decreasing GSH concentrations under drought stress. Increases in the amount of GSH under drought and its decrease after using protectants was also found by Alam et al. [5]. The reduction of GSH may be due to the scavenging of ROS and conversion to GSSG, which implies reduced oxidative damage [16]. In our experiment, GSSG concentration was significantly increased in drought-stressed plants and application of GA decreased the GSSG compared to the stressed condition both after 48 h and 72 h (Fig. 4). These results are supported by other studies (e.g., [5,36]) where under drought stress, when ROS levels increase, GSH is oxidized to GSSG and the GSSG levels and GR activity also increase (Fig. 5). According to Nahar et al. [51], the GSH/GSSG ratio plays a balancing role between the normal and the stressed conditions. When the ratio is high it implies the plant has a stress tolerance capacity. Results from our experiment show that a reduction in the GSH/GSSG ratio in drought conditions and that the addition of GA augmented this ratio (Fig. 4), which was a comparable conclusion to other studies [4,36,54].

Ahmadizadeh et al. [28] showed that in wheat during drought stress various enzymatic and nonenzymatic mechanisms are activated to protect the plants, including

the AsA-GSH pathway and SOD and CAT activities. Under drought stress, the CAT activity increases [47]. In our experiment, both CAT and APX activities increased under drought stress and the use of exogenous GA caused a reduction in them (Fig. 5). In some reported cases, CAT activity decreases, whereas APX increases both under drought stress and after using treatment with protectants [36,51,54]. Not only for drought stress but also for any environmental stress, CAT activity is increased and plays a critical role against the oxidative damage as well complementing SOD, APX, and other enzymes in detoxifying ROS [2]. It has also been found that under stress conditions GA decreases the APX activity and increases CAT activity [52].

In plant systems, MG is often produced simultaneously with the increase of ROS [57]. MG is detoxified through the activity of Gly I and Gly II [4]. In the present experiment, it was found that MG increased under drought stress (Fig. 6), but after applying exogenous GA its level was reduced. This implies that GA has a protective role and can detoxify MG. When a plant is under stress, it is protected from MG accumulation by the glyoxalase system, i.e., increasing the Gly I and Gly II activities [57].

## Conclusion

This study suggests that although GA is a growth hormone, it can also work as drought protectant in wheat seedlings. Exogenous application of GA improved plant growth and physiological parameters under drought stress conditions. Although the plant has its own defense mechanisms, exogenous GA can help to mitigate drought-induced oxidative damage through maintaining RWC, upregulating the enzymatic and nonenzymatic mechanisms balancing the antioxidant system. It detoxifies the MG through the glyoxalase system, eradicates the ROS such as H<sub>2</sub>O<sub>2</sub>, and reduces lipid peroxidation. Foliar application of GA also protected the wheat seedlings against drought by conserving the chlorophyll concentrations, maintaining the AsA-GSH cycle, and increasing MDHAR, DHAR, and GR activities. Both after 48 h or 72 h duration of drought stress when oxidative stress appears, exogenous GA helped to mitigate it. Although the effectiveness of GA as a phyto-protectant has been studied extensively in the case of salt stress, very little work has done for on drought stress. The present study supports the role of GA as a phyto-protectant, but further work regarding the use of exogenous GA under drought stress is needed

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### **Egzogenna aplikacja kwasu giberelinowego łagodzi uszkodzenia pszenicy jarej wywołane suszą**

#### **Streszczenie**

Stres spowodowany suszą jest głównym problemem w produkcji pszenicy, ale można mu zapobiegać przy pomocy różnych egzogennych środków ochronnych, takich jak kwas giberelinowy (GA). Chociaż GA jest fitohormonem, wykazuje potencjalne działanie ochronne w stosunku do

roślin rosnących w warunkach stresowych. W celu zbadania ewentualnego oddziaływania GA w łagodzeniu stresu suszy, sadzonki pszenicy (*Triticum aestivum* 'BARI Gom-21') traktowaliśmy GA w formie oprysku w warunkach pół-hydroponicznych. W przeprowadzonym doświadczeniu badano łączny wpływ GA i stresu suszy (indukowanego przez 12% glikol polietylenowy) po 48 godzinach i 72 godzinach. W siewkach pszenicy poddanych działaniu suszy, pod nieobecność egzogennej GA, stwierdzono różnorodne zmiany fizjologiczne i biochemiczne, uzależnione od czasu ekspozycji. Pod wpływem suszy stężenia dialdehydu malonowego (MDA), nadtlenu wodoru ( $H_2O_2$ ) oraz wolnej proliny (Pro) zwiększały się, podczas gdy aktywność katalazy (CAT) i peroksydazy askorbinianowej (APX) uległa zmniejszeniu. Kwas giberelinowy odgrywał rolę w przywracaniu prawidłowego poziomu askorbinianu (AsA), zmniejszał stosunek glutationu zredukowanego/utlenionego (GSH/GSSG) oraz obniżał aktywności reduktazy monodehydroaskorbinianowej (MDHAR) i reduktazy dehydroaskorbinianowej (DHAR). Kwas giberelinowy istotnie wpłynął na układ glioksalazy. Pod wpływem stresu suszy stężenie metyloglioksalu (MG) wzrosło, ale aplikacja GA stymulowała aktywność glioksalazy I (Gly I) i glioksalazy II (Gly II) chroniąc siewki pszenicy przed stresem. W badaniach wykazano, że natężenie stresu suszy u pszenicy zależy od etapu wzrostu i nasila się wraz z wydłużeniem czasu trwania stresu, podczas gdy egzogenne GA zwiększa możliwość przetrwania siewek poprzez wzmocnienie mechanizmów obrony antyoksydacyjnej i regulację systemu glioksalazy.