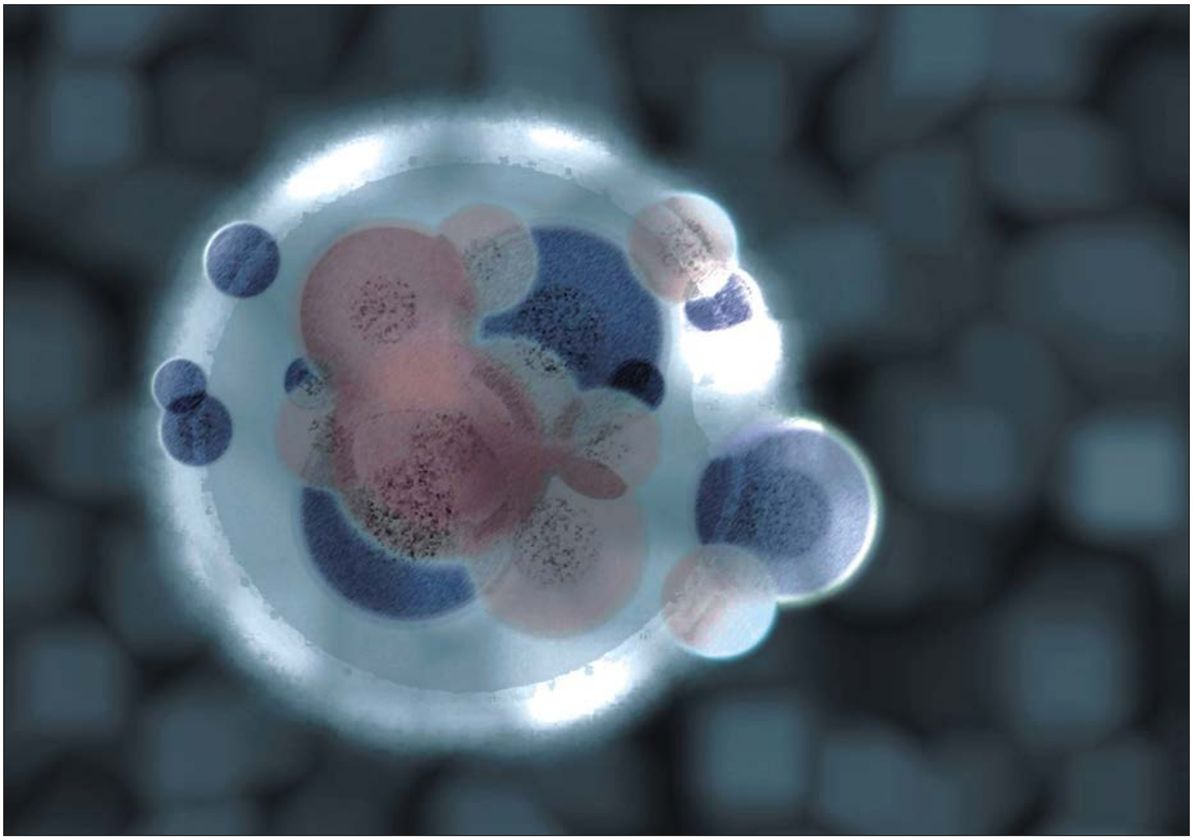

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Research Article
Changes in Antioxidant Status, Water Relations and Physiological Indices of Maize Seedlings under Drought Stress Conditions

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Abstract

Background and Objectives: Continuously changing climate and drought stress is drastically affecting maize crop ecology. Drought stress at seedling stage disturbs the normal physiological plant functions and results in stunted growth. In present study the maize seedlings were tested to evaluate their stress tolerance against water limiting conditions through fluctuations in antioxidant defense mechanism, physiological responses and changes in plant water relations. **Materials and Methods:** Maize seedlings were exposed to drought stress at 3 levels (15, 25, 35%) induced by polyethylene glycol 6000 (PEG-6000), under controlled conditions with completely randomized design (CRD) having 3 replications. **Results:** Drought stress significantly inhibited the plant growth and its oxidative defense mechanism. Drought stress also affected significantly plant water relations and physiological attributes. Results showed that 35% PEG6000-induced drought stress has affected these parameters with more severity as compared to 15 and 25% stress levels and control plants. Increased quantities of reactive oxygen species (ROS) viz. hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and superoxide ($O_2^{\cdot -}$) were observed due to PEG6000-induced drought stress. Similarly, antioxidative enzymes activities were accelerated due to drought stress with high values for superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR). In contrast, leaf water contents and membrane stability values were recorded with marked decrease, with maximum membrane leakage values for 35% PEG6000 drought stress treatment. Similarly, all drought stressed plants under 3 stress levels had showed fluctuations for efficiency of dark-adapted PS-II (Fv/Fm), as compared to non-stressed control plants. **Conclusion:** In crux, drought stress at 35% PEG-6000 considerably influenced the maize seedlings in oxidative defense mechanism, water relations and chlorophyll fluorescence measurements.

Key words: Drought stress, maize physiology, antioxidants, membrane damage, growth inhibition

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Drought stress is one of the most drastic and multidimensional abiotic stress which impairs the normal plant growth, physiological processes and the agricultural productivity[1]. Drought stress hinders the normal plant functioning that lead to retarded growth and plant vigour[2]. The primary symptoms of this stress includes the fluctuations in water relations (osmotic adjustments), photosynthetic apparatus, protein denaturation and the altered enzyme activities[3,4]. Under drought stress, the perturbations in plant metabolism causes imbalance in oxidative status by generation of reactive oxygen species (ROS), which includes hydrogen peroxide (H_2O_2), hydroxyl radicals (·OH) and superoxides ($O_2{\cdot}^-$)[5]. These ROS molecules thus disrupts the enzyme functioning and cause damages to plant cellular structures, lipids and enzymes[4]. However, plants are equipped with natural defense mechanism which gets activated upon the generation of ROS and the antioxidant defense mechanism creates tolerance in plant to cope up the prevailing stress[2].

Maize is being cultivated throughout the world as an important cereal for food, feed and biofuels, but yield potential for it varies largely depending upon the geographical location. Maize crop is sensitive to climatic extremes as various abiotic stresses of drought, temperature and salinity limiting the yield by interference in physiological and morphological processes[6,7]. However, the acceleration in maize production per unit area has been largely increased in recent decade due to its increasing demand for industrial purposes[8]. Drought stress at seedling stage of maize is believed to cause the stunted growth with several physiological dysfunctions[4].

Upon exposure to drought stress, plants' ability to withstand the imposed stress alters depending upon the severity and timespan of the stress[9]. The primary tolerance responses adopted by stressed plants are the osmotic adjustments and accumulation of compatible solutes[10], however, the exacerbation of excessive ROS generation due to drought stress leads to the oxidative burst which then triggers the antioxidative defense mechanism of plant to cope with Sofo *et al.*[11]. The primary ROS products whose production get accelerated upon stress are H_2O_2 and $O_2{\cdot}^-$, which then starts damaging the protein molecules through peroxidation, however, the key defense molecules against these ROS products include SOD and CAT (catalase) as enzymatic antioxidants[12]. The SOD molecules disproportionate the $O_2{\cdot}^-$ molecules into H_2O_2 and then the CAT further reduces the H_2O_2 into H_2O, thus protecting the biological macromolecules[13].

The objectives of present study were to evaluate the maize performance for its several eco-physiological functional traits affected by different drought stress levels at seedling stage. The plant response was evaluated in terms of fluctuations in plant-water relations, production of oxidative molecules, antioxidant enzymes, membrane stability and leaf fluorescence values.

MATERIALS AND METHODS

Plant material and growth conditions: Seeds of local maize hybrid (Tri Hybrid-352) in Riyadh were acquired and were used as plant material for a pot experiment conducted under controlled conditions, firstly in an automatic incubator for 48 h to allow the seeds to germinate and then transferred in plastic pots (5 plants/pot) in glasshouse till the plants attained three leaves. After three-leaf stage (20 days), the uniform seedlings were shifted to plastic containers (20 L capacity) having media as Hoagland solution. The external temperature was maintained throughout at 25-27°C and the relative humidity of ~65%. However, the Hoagland solution was renewed every 4 days in 20 L plastic containers. Peat moss used as growth medium along with Hoagland solution in the glasshouse plastic pots. The experiment was conducted in the month of April, 2018 at the Department of Biology of Princess Nourah Bint Abdulrahman University, Saudi Arabia, which lasted for about 30 days in total and after that the plant material was harvested for dry mass calculation.

Drought stress treatment setup: At the 4-leaf stage of maize seedlings, the drought stress (DS) treatments were created in the 20 L containers. Each plastic container had 18 seedlings and four treatments were set, with each treatment having three containers considered as 3 experimental replications. Drought stress was imposed as: (i) 0% polyethylene glycol 6000 (PEG6000) which was taken as control treatment, (ii) 15% polyethylene glycol 6000, (iii) 25% polyethylene glycol 6000 and (iii) 35% polyethylene glycol 6000. Hereafter, the 4 treatments will be referred to as control, 15, 25 and 35% DS, respectively. The utilized PEG6000 was obtained from Sigma Chemicals Co. USA. The experiment was laid out in completely randomized design, 3 replicates per treatment. Plant leaf sampling was done for each replicate after 72 h of drought stress treatment and the growth attributes including fluorescence measurements (5 plants each replication) were done at the final harvest after 96 h of treatment setup. The sampled leafs (3rd leaf from bottom) were immediately stored at -80°C.

Growth measurements: Harvested samples were immediately processed for measuring growth indices. Each sample was first rinsed with distilled water and then sample was wiped out for excessive water before measuring the fresh weight (FW). After that the samples were oven-dried (70°C) to measure the dry weight (DW).

Biochemical measurements: Fresh leaf samples (0.5) were utilized for the estimation of H_2O_2, MDA and $O_2{\cdot}^-$ contents. Standard protocols were followed as described by Talaat *et al.*[14] to estimate the H_2O_2 contents using spectrophotometer absorbance (410 nm) readings. Similarly, the MDA contents were determined by centrifugation and absorbance following the method published by Yang *et al.*[15]. While, the $O_2{\cdot}^-$ contents were measured by absorbance method as described by Maia *et al.*[16]. Homogenized leaf samples (0.5 g) were used for extraction of antioxidant enzymes and their assays. SOD activity was determined following method by Tambussi *et al.*[17], similarly the membrane electrolyte leakage (%) was estimated by computing the conductivity of leachates due to injured plasma membrane as reported by Tambussi *et al.*[17]. However, the leaf water contents were determined following the method described by Guo *et al.*[18]. Ascorbate peroxidase (APX) and glutathione reductase (GR) activities were estimated according to the methods of Pyngrope *et al.*[19] and Li *et al.*[20], respectively.

Fluorescence measurements: During the experimental duration, at 3-leaf stage IMAGING-PAM (M-Series-Walz, Germany) was used to measure chlorophyll fluorescence for the maize seedlings. Under dark-adaptation conditions for 2 min, the efficiency of Photosystem II (PS-II) was recorded for each replicate under various drought treatments. The PS-II efficiency (Fv/Fm) was calculated by working out the minimum fluorescence (Fo') and maximum fluorescence (Fm') of dark-adapted samples:

$$\frac{Fv}{Fm} = \frac{Fm' - Fo'}{Fm'}$$

Statistical analysis: All the data were statistically analyzed using SPSS statistical package (version 20.0). The comparisons were made between the treatments means using Tukey's *post hoc* honest significance test at 0.05 significance level. Values indicate the mean±standard errors (SE) from the three experimental replications. Pearson correlation coefficients were calculated to determine the relationship between all studied traits and correlation plots were generated using R Statistical Software (Performance Analytics R package).

RESULTS

Growth attributes: Drought stress severely influenced the growth parameters of maize seedlings exposed to different levels of PEG-6000 (Table 1). Results showed that the maximum significant decrease in fresh and dry weights of maize seedlings was exhibited by the 35% DS treatment, which was followed by the subsequent 25 and 15% DS treatments as compared to normal control plants (Table 1). The impact of decrease in dry weight was more severe for the stress levels of 25 and 35% PEG-6000 with a significant lower values of 0.41 and 0.28 g compared with control treatment, respectively. Leaf water contents were also significantly disturbed by different PEG-6000 levels in maize seedlings (Table 1). The maximum decrease in the total leaf water contents (69.13%) was observed in 35% DS treatment as compared to the control plants.

Chlorophyll fluorescence readings: Through chlorophyll fluorescence measurements, Fv/Fm value provides the robust indicator of maximum quantum yield of PS-II. Drought stress treatments have significantly lowered the value of Fv/Fm, thus depicting the photoinhibition effect in stressed plants as compared to control un-stressed plants (Table 1). Results

Table 1: Perturbations in the fresh weight, dry weight, leaf water contents and the efficiency of PS-II in the drought stressed leaves of maize

Treatments	Fresh weight (g)	Dry weight (g)	Leaf water contents (%)	Fv/Fm
Control	7.86±0.060^{a}	1.20±0.021^{a}	85.94±0.99^{a}	0.81±0.010^{a}
15% DS	5.99±0.070^{b}	0.69±0.041^{b}	76.48±1.86^{b}	0.68±0.017^{b}
25% DS	4.66±0.080^{c}	0.41±0.038^{c}	74.33±0.51^{bc}	0.58±0.018^{c}
35% DS	4.09±0.075^{d}	0.28±0.015^{c}	69.13±1.10^{c}	0.44±0.018^{d}
HSD value ($p \le 0.05$)	0.3544	0.1399	5.8936	0.0822

Data represent the mean values±SE (n = 3), different alphabetic letters in a column represent significant difference at $p \le 0.05$, DS: Drought stress

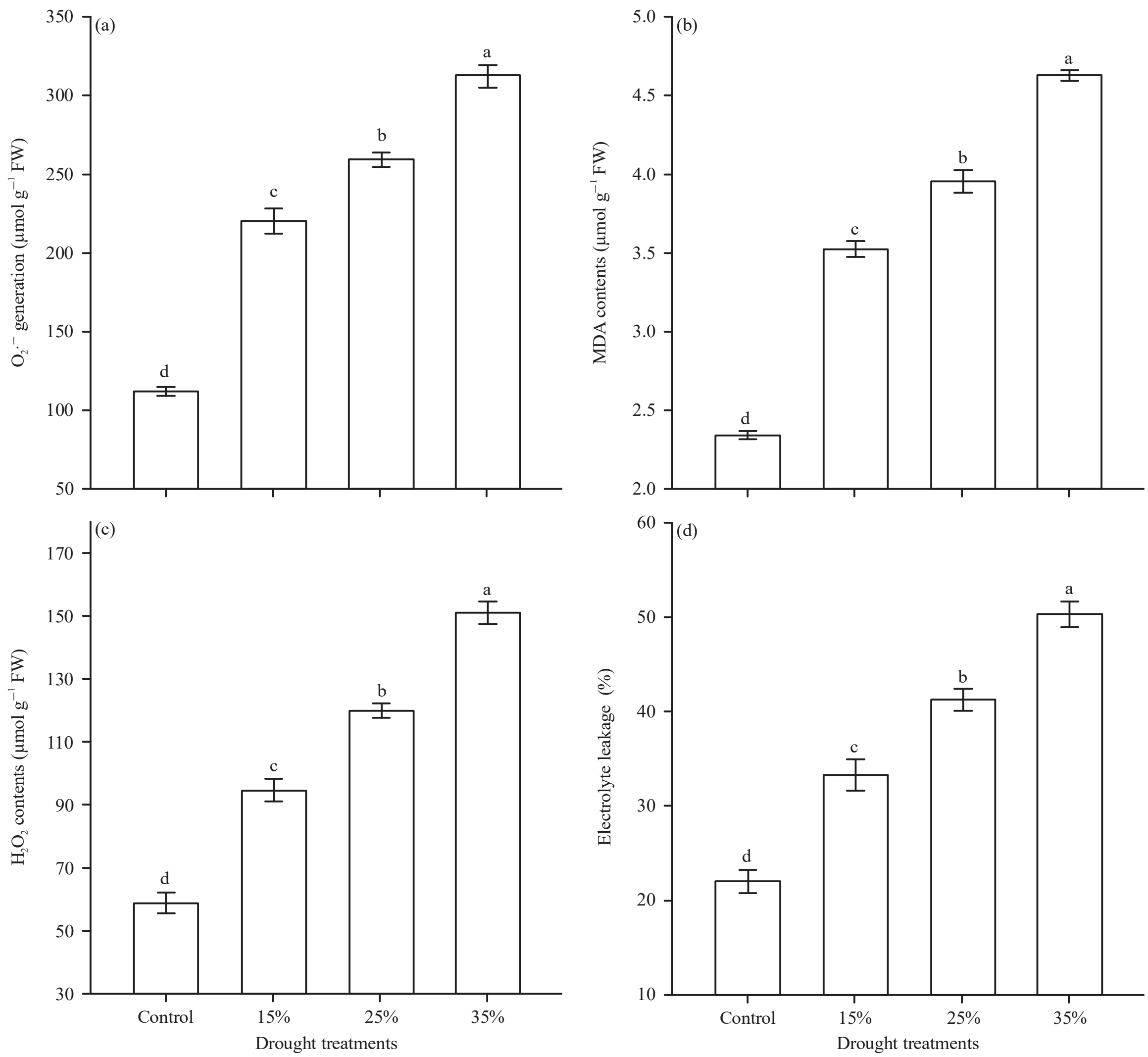

Fig. 1(a-d): Perturbations in the (a) $O_2{\cdot}^-$ generation, (b) MDA (malondialdehyde) contents, (c) H_2O_2 contents and (d) Electrolyte leakage in the drought stressed leaves of maize

Data represent the mean values±SE (n = 3), different letters on bars represent significant difference at $p \leq 0.05$

described that the Fv/Fm value was lowered from 0.81-0.44 at progressive drought levels, with the maximum photoinhibition effect observed in 35% DS treatment having value of 0.44. However, a mild stress effect was observed for 15% DS treatment with Fv/Fm value of 0.68.

ROS molecules generation: In this study, the drought stress triggered the huge production of ROS molecules i.e., $O_2{\cdot}^-$, H_2O_2 and MDA contents in the plants treated with different levels of PEG-6000 (Fig. 1). The maximum generation of these active oxygen species ($O_2{\cdot}^-$, H_2O_2 and MDA) was recorded for the treatment 35% DS compared to all other treatments including control. The maximum $O_2{\cdot}^-$ generation rate was recorded for the treatment 35% DS, which was 312 µmol g^{-1} FW (Fig. 1). The maximum increase in H_2O_2 contents was recorded for 35% DS treatment (150.99 µmol g^{-1} FW), followed by 25 and 15% DS treatments, respectively, as compared to control un-stressed plants (Fig. 1).

Due to the over production of ROS molecules, results showed a significant increase in the electrolyte leakage of membranes for the plants under drought stress conditions (Fig. 1). The maximum increase in electrolyte leakage with value 50.25% was recorded for the treatment of 35% DS imposition, as compared to control. However, the other 2 DS treatments of 15 and 25% PEG-6000 showed less electrolyte leakage (Fig. 1).

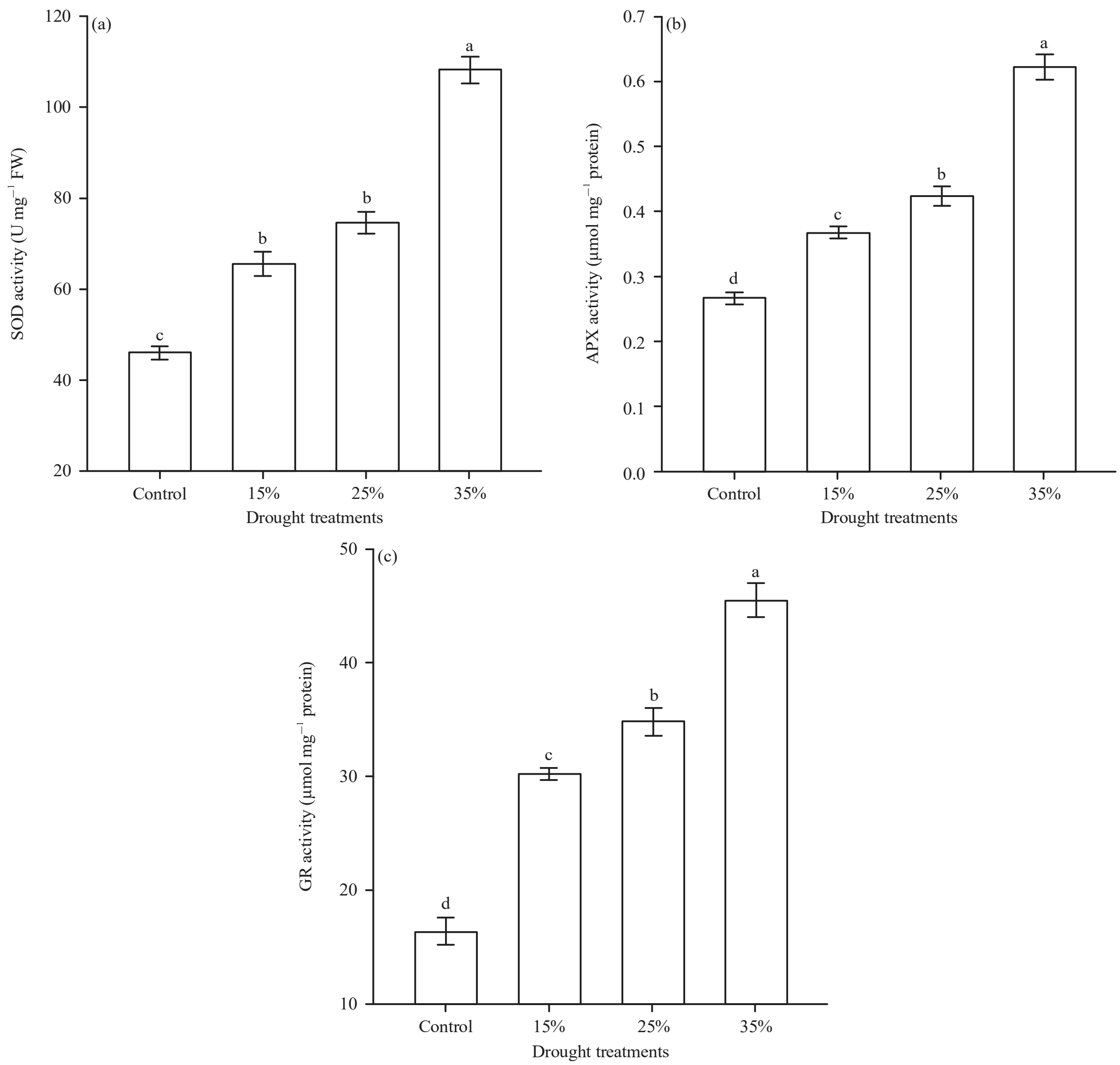

Fig. 2(a-c): Perturbations in the activities of (a) Superoxide dismutase (SOD), (b) Ascorbate peroxidase (APX) and (c) Glutathione reductase (GR) in the drought stressed leaves of maize

Data represent the mean values±SE (n = 3), different letters on bars represent significant difference at $p \leq 0.05$

Enzymatic antioxidants: Drought stress triggered the production of antioxidant molecules in response to huge ROS production in maize seedlings under stress (Fig. 2). Results showed a maximum SOD activity (108.17 U mg^{-1} FW) for 35% PEG-6000 treatment as compared to control (Fig. 2). However, a significant increase in SOD activity was also observed for 15 and 25% DS treatments, but the differences among these 2 treatments were not significant.

Amongst the other enzymatic antioxidants, GR and APX activity was recorded for plants under stress and non-stress conditions. Results showed that drought stress has triggered a rapid increase in GR activity, with maximum value of 48.2 μmol mg^{-1} protein exhibited by maize seedlings under 35% DS treatment as compared with control plants (Fig. 2). Almost the same trend was recorded for APX activity in stressed plants, as for GR activity, in response to different drought treatments. Drought stress caused a significant increase in APX activity, with the maximum value recorded for 35% DS treatment (0.62 μmol mg^{-1} protein) as compared to non-stressed control plants (Fig. 2).

DISCUSSION

Drought stress is believed to limit the plant growth and normal functioning through interventions in various morphophysiological and biochemical traits[21,22]. This study

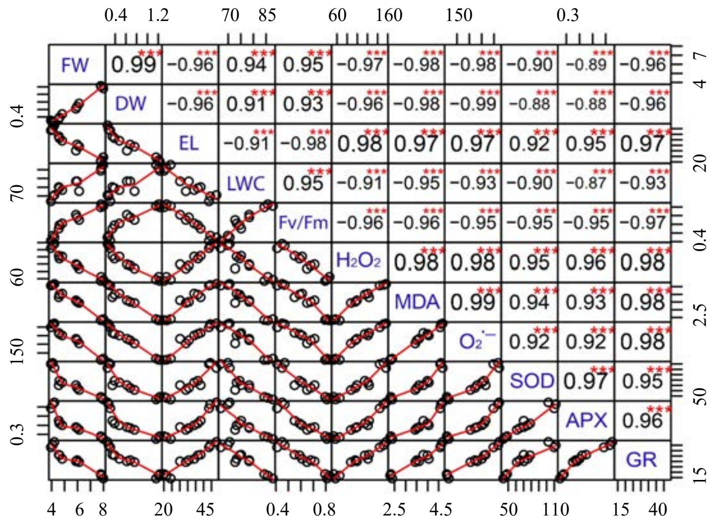

Fig. 3: Correlation plots for analyzed parameters in maize hybrid under different levels of drought stress (PEG-6000) during early seedling growth stage

evaluated a maize hybrid for its oxidative defense mechanism at early seedling stage. Although, all the drought stress treatments had greatly influenced the studied plant traits, but 35% PEG-6000 treatment affected the oxidative balance with great extent. Results showed that 35% PEG-6000 caused a significant decrease of about ~75-100% in plant dry and fresh weights. This decreasing effect was explained by the reduced leaf water contents (Table 1), which is considered as the primary accelerator of molecular and oxidative damage in plants under stress[23,24]. The similar phenomenon of decreased biomass weights under drought stress is well established[25,26].

Changes in leaf photosynthesis in response to any external stress is mainly reflected as the perturbations in the efficiency of PS-II[27]. Results showed a wide range of variability for all the drought treatments which differed significantly in PS-II efficiency, measured as Fv/Fm. Upon exposure of unstressed leaves to any kind of stress, the changes in PS-II efficiency result in lower Fv/Fm[28]. A 35% PEG-6000 has drastically influenced the photosynthetic machinery and resulted in minimum (0.44) Fv/Fm value (Table 1). These results indicate that under drought stress condition plants swiftly tend to adjust their water loss by lowering the transpiration and CO_2 absorption through stomatal down-regulation[18].

Perturbations in the oxidative balance, characterized by ROS generation and antioxidants production, is one of the initial damaging stage in drought stress[29,30]. If the ROS generation exceeds the scavenging potential of plant's oxidative defense mechanism, it causes a condition of oxidative stress in which these oxygen species cause peroxidation of protein molecules[30,31]. Drought stress has induced the ROS generation which is reflected by the huge production of $O_2^{\cdot -}$ molecules, H_2O_2 contents and MDA contents, which then caused the enhanced electrolyte leakage (Fig. 1). This oxidative damage was more pronounced for 35% PEG-6000. All the drought stress levels had significantly accelerated the ROS molecules generation, thus depicting the adverse water limiting effects. The damaging effects of ROS molecules such as $O_2^{\cdot -}$, H_2O_2 and MDA are reported widely[32,33].

Among the ROS-scavenging enzymatic molecules, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are the key enzymatic components of plant defense system which may directly scavenge the ROS molecule or these may produce the non-enzymatic antioxidants[10,34,35]. Whereas, the ascorbate peroxidase (APX) and glutathione reductase (GR) are considered as catalysts for non-enzymatic antioxidants[20]. Results showed that with the production of ROS such as $O_2^{\cdot -}$, H_2O_2 and MDA contents, the SOD, APX and GR activity significantly increased in all the drought stress treatments in comparison with control plants (Fig. 2). This increased generation of enzymatic antioxidants in drought stressed plants showed the sufficient tolerance in studied maize hybrid[9]. However, it is believed that the initial growth stage in maize is much sensitive to drought stress as compared to later reproductive stages[32]. Furthermore, the exposure of stressed plants for a prolonged period might result with reduced tolerance[29], which ultimately damage the plant cell membranes (Fig. 3) and the protein structures[25]. Therefore, the negative effects of drought stress were reflected as reduced fresh and dry weights, decreased leaf water contents and with low PS-II quantum yield (Table 1). The correlation analysis also showed the strong relationship among the parameters studied, as oxidative stress has caused increased production of antioxidants and decreased negatively the plant growth traits with strong negative correlation values (Fig. 3). Present study established that the maize seedlings can withstand the drought stress until a certain limit and after that the damage due to ROS molecules productions is irreversible and disrupts the redox balance. Therefore, future studies should be focused to explore the maize performance under severe drought stress conditions in the field to uncover the stress impacts at the final grain yields and the yield penalty at the expense of plant physiological processes.

CONCLUSION

In present study, drought stress of 35% PEG-6000 has greatly reduced the plant biomass, leaf water contents and most importantly it lowered the efficiency of PS-II (measured as Fv/Fm) to a great extent. Similarly, drought stress has triggered the ROS production and caused damage to membrane permeability, which then activated the oxidative defense system of plant by producing more enzymatic antioxidants. Although, the plant's ability to tolerate the imposed drought stress was significant, but the excessive oxidative damage was verified by hampered plant growth and the chlorophyll fluorescence measurements.

SIGNIFICANCE STATEMENTS

Present study aimed at evaluating the oxidative defense mechanism of maize under water stress conditions has discovered the fact that maize plant at seedling stage cannot withstand the severe water stress condition, as high stress had led to a great burst of ROS molecules. Meanwhile, the redox imbalance has also affected badly the photosynthetic performance of maize plants. Therefore, a better understanding of maize oxidative defense status and the physiological perturbations under stressful environment may help researchers to devise management strategies and breeding programs aiming to improve the maize performance under water limiting conditions.

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