ASSESSMENT OF PHYSIO-BIOCHEMICAL INDICATORS FOR DROUGHT TOLERANCE IN DIFFERENT CULTIVARS OF MAIZE (*ZEA MAYS* L.)

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Abstract

　　Maize is an important cereal crop all-over the world and has been recently ranked at first among all cereal crops. However, growth of maize plants is negatively affected in arid and semi-arid areas of the globe due to scarcity of water, but genetically variable cultivars/lines differ considerably to respond to water limited conditions. To evaluate the response of some commercial cultivars of maize to water deficit conditions, a pot experiment was carried out. Eight maize cultivars (Sultan, Akbar, Pearl, MMRI, Maki Pak, Sahiwal 2002, Sadaf and Neelum) were subjected to varying [100%, 75% and 60% of field capacity (FC)] levels of water deficit conditions. Drought stress significantly decreased shoot fresh and dry weights, root fresh and dry weights and chlorophyll pigments (*a* and *b*) in all maize cultivars. However, free proline, glycinebetaine (GB), total phenolics, hydrogen peroxide (H_2O_2), malondialdehyde (MDA) contents, activities of enzymatic antioxidants (CAT, POD and SOD) and ascorbic acid (AsA) contents increased significantly under water deficit conditions. Of all maize cultivars, cv. Sadaf was superior in terms of plant growth, while, cv. Sultan proved to be inferior to the other cultivars examined in this study. It was concluded that osmoprotectants such as proline and GB can be used as stress tolerance indicators under drought stress conditions.

Key words: Maize, Drought, Antioxidants, Germination, Growth.

Introduction

　　The distribution of natural vegetation is determined by water as compared to a variety of other abiotic factors (Anjorin *et al.,* 2016). Important cereal crops in the world considerably hamper in terms of growth and yield due to scarcity of irrigation water, which in turn pose considerable challenge to food security. Plants are more susceptible to drought stress at early stages of growth than at later growth and development stages (Quan *et al*., 2016; Li *et al.,* 2017). If water stress intensity is high then germination rate and percentage as well as root and shoot length undergo considerable reduction (Fathi & Tari, 2016). Severe water stress affects many physiological and biochemical processes in plants like photosynthetic rate, stomatal regulation, cell growth, water relations, nutrient metabolism, and hormonal regulation, etc. (Anjorin *et al.,* 2016; Duan *et al.,* 2017).

　　A variety of physio-biochemical changes take place in plants exposed to drought stress. For example, stomatal closure is the instantaneous response of plants subjected to drought stress conditions and it alters many metabolic pathways by reducing nutrient and CO_2 uptake (Basu *et al.,* 2016). Furthermore, chlorophyll contents are reduced due to degradation of chlorophyll and photo- oxidation under water stress conditions (Anjum *et al.,* 2011). Production of reactive oxygen species (ROS) increases due to decrease in molecular oxygen by reducing electron transport components under water stress conditions (Basu *et al*., 2016). Lipid peroxidation, deterioration of membranes and degradation of nucleic acids and proteins are caused due to free radicals generated by drought stress conditions resulting in oxidative stress (Nair *et al.,* 2008; Fathi & Tari, 2016).

　　Resistance mechanisms in plants have been improved in many plants but the expression of such mechanisms depend upon plant species as well as plant growth stage (Basu *et al.*, 2016; Duan *et al.,* 2017). Usually, cellular homeostasis is one of the tolerance mechanisms in plants under water deficit conditions which probably takes place by raising the water absorption by the cell (Salehi-Lisar & Bakhshayeshan-Agdam, 2016). In the chloroplast, many enzymatic and non-enzymatic antioxidants are present that avert accumulation of ROS under stress conditions and safeguard the cell by controlling intracellular ROS concentration (Gapinska *et al.,* 2008; Fathi & Tari, 2016). In higher plants, some adaptive mechanisms are developed which involve the accumulation of compatible osmolytes like proline, glycinebetaine, soluble sugars and secondary metabolites under stressful cues (Shafiq *et al*., 2014; Akram *et al.,* 2018). During osmoregulation, these osmoprotectants act as radical scavengers and help in preventing the ROS-induced damages to DNA, membranes and biomolecules (Fayez & Bazaid, 2014). Of a variety of biomolecules, proline accumulates to a great extent in plants under water stress conditions. Phenolic compounds also help in scavenging free radicals and protect plants from the damaging effects of oxidative stress caused by ROS (Petridis *et al.,* 2012; Yadav & Sharma, 2016).

　　The production of maize is greatly hampered under drought stress (Zhang *et al.,* 2011), which is further reduced due to the current climatic change scenario (Lobell *et al.,* 2014). There are some reports which show that water stress induced reduction in yield of maize and other crops occurs by the stress-induced adverse effects on photosynthetic potential, radiation-use-efficiency, harvest index, reproductive activities and plant growth (Xu *et al.,* 2008; Song *et al.,* 2018). Due to increasing global population, it is essential to improve the yield and production of important crops under normal and water deficit conditions (Basu *et al.,* 2016). So, the primary objective of the present study was to explore key physio-biochemical indicators for discriminating some potential

commercial maize cultivars for drought tolerance, because drought tolerant cultivars can be recommended for cultivation on drought-hit areas as they can certainly produce more yield on drought-hit areas compared to drought sensitive ones.

Materials and Methods

The present investigation was carried out to evaluate the effect of varying water limited regimes on different maize cultivars. A two-factor factorial CRD experiment was performed at the Botanical Garden of Government College University (GCUF), Faisalabad. The seeds (caryopses) of eight cultivars (Sultan, Akbar, Pearl, MMRI, Sahiwal 2002, Neelum, Sadaf and Maki Pak) of maize were obtained from the Maize and Millet Research Institute, Yusafwala, Sahiwal, Punjab. In each pot, 8 kg sandy loam soil was filled and 10 seeds of each cultivar were sown in each pot. Later on, after germination, in each pot five seedlings were maintained. Different water deficit regimes (100%, 75% and 60% field capacity) were applied after two weeks of germination. After 21 days of drought stress treatment, two plants from each pot were harvested to measure shoot fresh and dry weights as well as root fresh and dry weights. During the study, following biochemical attributes were determined:

Chlorophyll pigments: Chlorophyll contents were determined following Arnon (1949).

Leaf free proline: Sulfosalicylic acid (3% w/v) was used to homogenize the fresh leaf sample (0.5 g). Free proline contents in the leaves were recorded following the protocol of Bates *et al.*, (1973).

Glycinebetaine (GB): Leaf sample (500 mg each) was ground in 0.5% of toluene (10 mL) and filtered the mixture. The method of Grieve & Grattan (1983) was employed to determine the GB contents in the extracts of fresh leaf samples.

Total phenolics: The method of Julkunen-Titto (1985) was employed to estimate the total phenolics in fresh leaf samples of maize plants. Then, total phenolics were calculated using a gallic acid calibration curve ranging from 10-80 mg L^{-1}.

Malondialdehyde (MDA): MDA contents were measured following the protocol of Cakmak & Horst (1991).

Hydrogen peroxide (H_2O_2): Trichloroacetic acid (0.1% w/v) was used to determine H_2O_2 contents following the method of Velikova *et al.*, (2000).

Ascorbic acid (AsA): To measure the AsA contents in fresh leaf samples, the protocol of Mukherjee & Choudhuri (1983) was employed.

Enzymatic antioxidants (SOD, POD & CAT): Leaf sample (500 mg each) was extracted in 10 mL of potassium phosphate buffer. The protocol of Chance &

Maehly (1955) was employed to determine the activities of POD and CAT enzymes, whereas the method of Giannopolitis & Ries (1977) was employed to estimate the activity of SOD enzyme in the leaf samples.

Statistical analysis: A two-factor factorial CRD was used to organize the experimental units. The data for each parameter were subjected to analysis of variance (ANOVA) using the statistical software CoStat (Version 6.2).

Results

Mean squares from analysis of variance (ANOVA) of data showed that shoot fresh and dry weights as well as root fresh and dry weights of eight different maize cultivarsdecreased significantly ($p \leq 0.001$) under drought stress conditions (75% and 60% of FC). However, the response of cultivars to water deficit conditions varied significantly and the maximum reduction was recorded in cv. Sultan followed by cv. Sadafunder drought stress (Fig. 1).

Chlorophyll contents (*a* and *b*) considerably ($p \leq 0.001$) decreased in all maize cultivars at different stress conditions (Fig. 1). Although, drought stress significantly reduced the total chlorophyll contents in all maize cultivars, cv. Pearl was highest in chlorophyll *a* contents whereas, cv. MMRI in chlorophyll *b* contents of all cultivars under stress conditions.

Varying watering regimes significantly ($p \leq 0.001$) improved leaf free proline as well as glycinebetaine (GB) contents inall maize cultivars. Maximum increase in proline and GB contents was observed in cvs. Sadaf and Pearl under drought stress conditions (Figs. 1-2).

It was observed that water deficit conditions enhanced ($p \leq 0.01$) malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents in all maize cultivars. However, cv. MMRI was the lowest in MDA contents and cv. Sultan in H_2O_2 contents of all cultivars under varying water stress regimes (Fig. 2).

Water deficit conditions considerably ($p \leq 0.001$) increased the total phenolics in all maize cultivars (Fig. 2). Cultivars, Sadaf, Akbar, Maki Pak and Neelum accumulated lower amount of phenolics as compared to the other cultivars under stress conditions. The interaction between the cultivars and drought stress regimes was considerably significant.

Water deficit conditions considerably ($p \leq 0.001$) enhanced the ascorbic acid contents in all maize cultivars. However, the interaction between the drought stress regimes and maize cultivars wasnon-significant. Of all maize cultivars, cvs. Sultan and Sahiwal 2002 were better than the other cultivars in ascorbic acid contents under varying water regimes (Fig. 2).

Activities of peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) enzymes increased considerably ($p \leq 0.001$; 0.05) in all maize cultivars. Of all maize cultivars, cv. Pearl was better in the activities of CAT and POD enzymes, while, cv. Sadaf showed higher activity of SOD enzyme at 60% of field capacity (Fig. 2). The activity of CAT enzyme was lowest in cvs. Neelum and Maki Pak under water stress conditions.
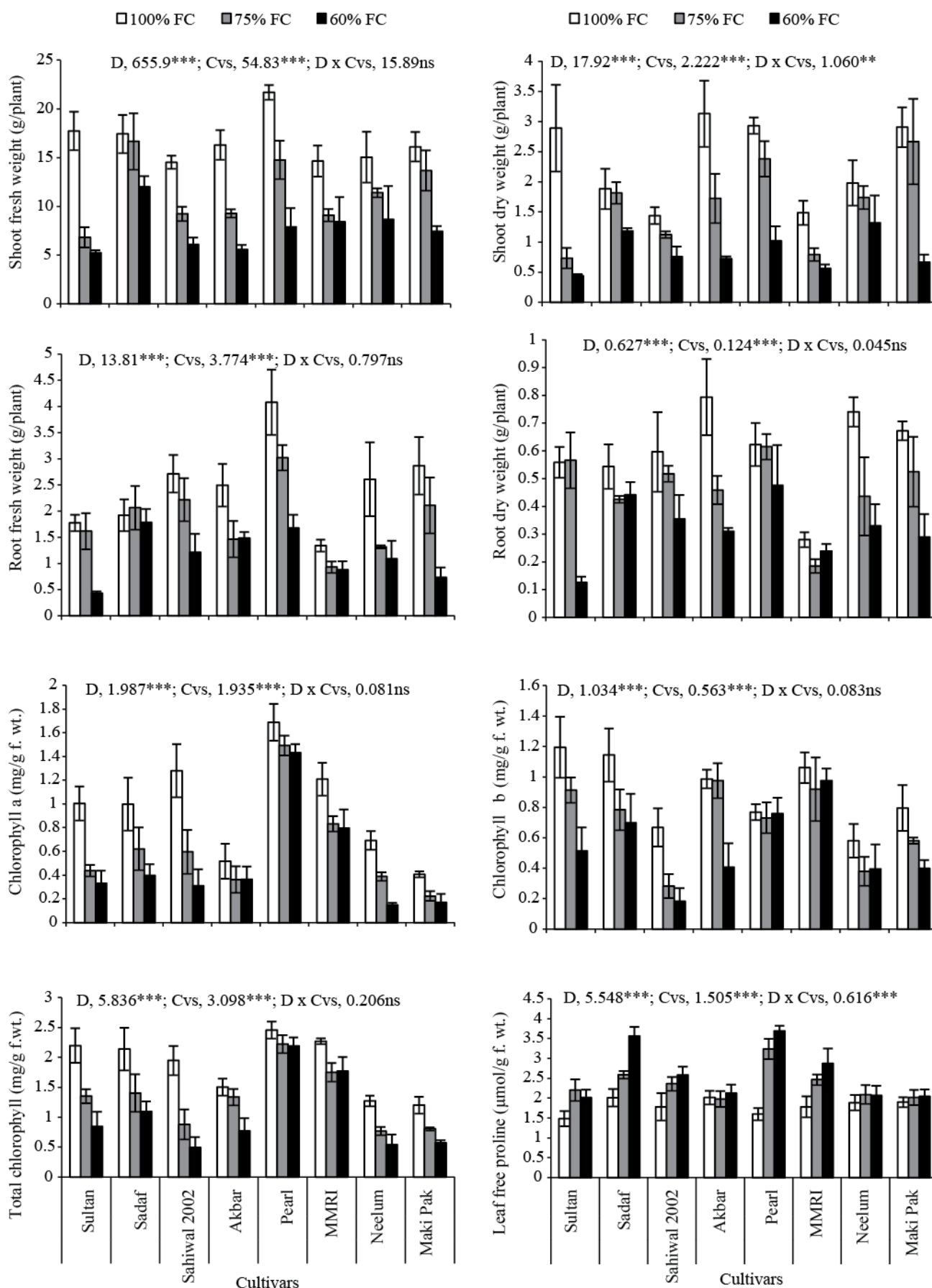
Fig. 1. Shoot and root fresh and dry weights, chlorophyll a and b contents, total chlorophyll and proline contents of eight different maize (*Zea mays* L.) cultivars subjected to varying (100%, 75% and 60% of field capacities) levels of drought stress (Mean ± S.E.).
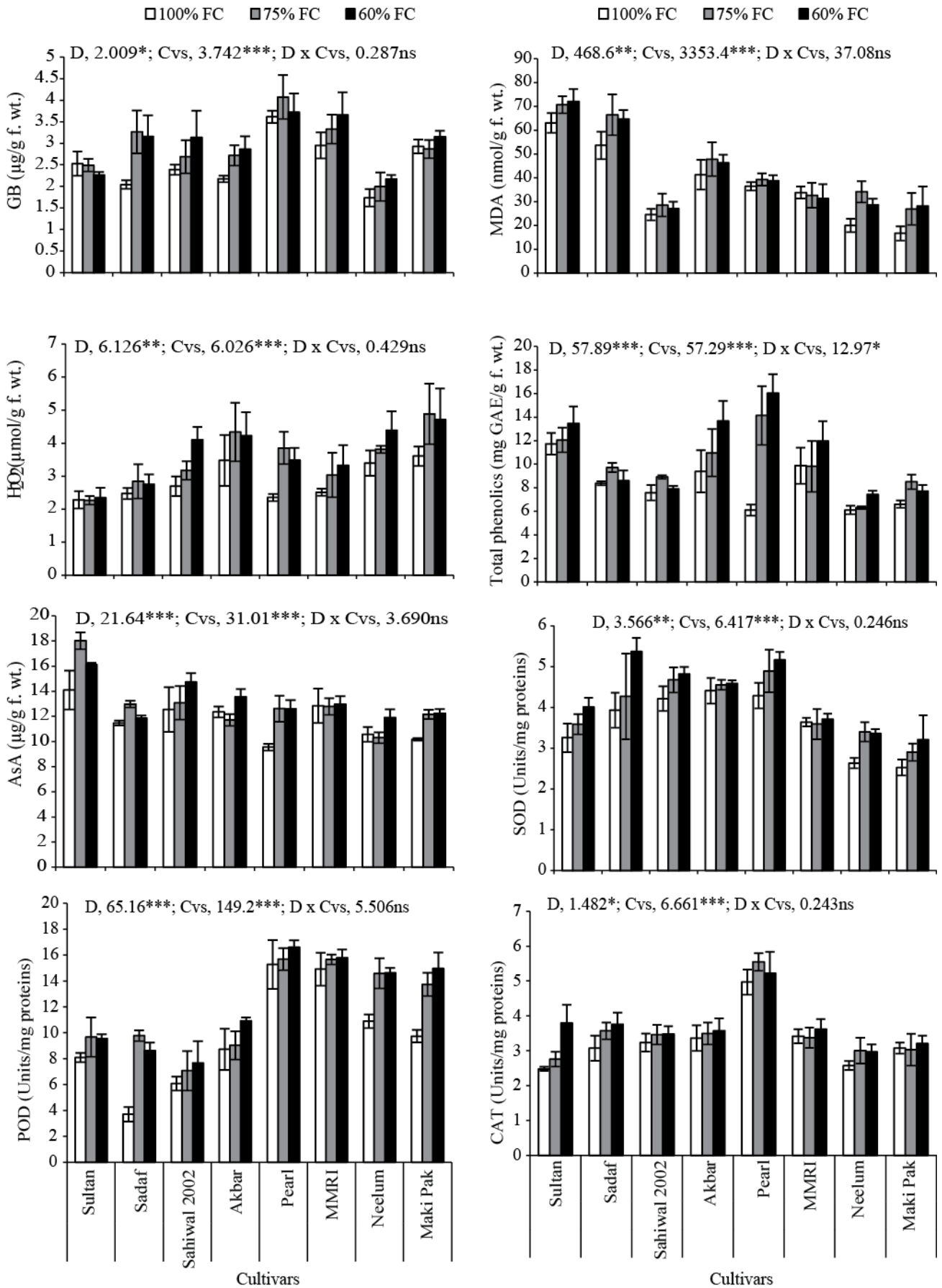
Fig. 2. Glycinebetaine (GB), malondialdehyde (MDA), hydrogen peroxide (H2O2), total phenolics, ascorbic acid (AsA) contents amd activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) enzymes of eight different maize (*Zea mays* L.) cultivars subjected to varying (100%, 75% and 60% of field capacities) levels of drought stress (Mean ± S.E.).

Discussion

In the present study, plant growth measured using shoot and root fresh and dry weights decreased in all eight maize cultivars under water deficit conditions. The decrease in plant biomass under stress conditions can be attributed to inefficient uptake of water and nutrients, poor stomatal regulations, perturbed root architecture as well as low availability of water to plant metabolic processes (Shafiq et al., 2014; Kosar et al., 2015; Akram et al., 2018). It is believed that considerable water use efficiency, nutrient acquisition, stimulation of defense system (oxidative), maintenance of hormonal balance and structural integrity of metabolites (primary or secondary) are positively associated with stress tolerance capacity of different plant species (Ashraf, 2010; Mwadzingeni et al., 2016; Schmidthoffer et al., 2018).

Chlorophyll pigments play an essential role in dissipation of energy and harvesting of light under water deficit conditions (Akram et al., 2018). In the present study, chlorophyll pigments decreased in all maize cultivars under drought stress conditions. Decline in pigments under drought stress is a common response of plants to drought stress, which has already been observed in different crops, e.g. canola (Akram et al., 2018), carrot (Razzaq et al., 2017), chickpea (Mafakheri et al., 2010), Vigna radiata (Batra et al., 2014), and potato (Arabshahi & Mobasser, 2017). They relate this chlorophyll reduction to over-production of reactive oxygen species (ROS), nutritional imbalance and disruption in enzyme activities to deficiency of water at cellular or plant levels.

We observed that exposure to drought stress increased MDA and H_2O_2 contents in all maize cultivars reflecting that lipid peroxidation had taken place in the maize plants under drought stress. Furthermore, since H_2O_2 increased in maize cultivars so it is possible that oxidants other than this might have been produced if drought had triggered oxidative stress in the maize cultivars. Similar to our results, drought-induced increased accumulation of MDA and H_2O_2 has already been observed in wheat (Hamurcu et al., 2014), cucumber (Li et al., 2011), and canola (Akram et al., 2018) plants under water stress conditions.

Osmotic adjustment in plants is common physiological phenomenon to maintain the photosynthesis, stomatal conductance and leaf water status in plants under water scarcity. Accumulation of compatible osmolytes like proline and GB by osmoregulation help protect the plants in stabilizing enzymes, membranes and detoxification of ROS (Ashraf & Foolad, 2007; Basu et al., 2016). In the present study, these osmolytes accumulated significantly by drought stress conditions. Recently, Anjorin et al., (2016) reported that water stress significantly improved the proline contents in drought tolerant maize variety DTESYN. The tolerance of plants may be associated with the accumulation of osmolytes like proline and GB, which may also be involved in scavenging ROS (Kaya et al., 2013). In another study, increase in proline accumulation was observed in drought tolerant cotton plants which exhibited osmotic adjustment under drought stress conditions (Wu et al., 2015). Similarly, Zhang et al., (2015) reported that water stress improved the GB in maize plants that was proved to be effective in improving water relation parameters and dry biomass production.

Non-enzymatic antioxidants including total phenolics and ascorbic acid are the antioxidants that help detoxify ROS under oxidative stress (Shafiq et al., 2014, 2015). We observed that in the maize cultivars, total phenolics increased under drought stress. In contrast to these findings, drought stress reduced phenolics in cotton (Ahmad et al., 2008), canola (Shafiq et al., 2014), corn (Ali et al., 2011), while in contrast, an increase was observed in carrot (Razzaq et al., 2017) plants under stress conditions. In a previous study, an increase in AsA was observed in canola (Shafiq et al., 2014) plants that is analogous to what was observed in the present study under water deficit conditions.

Oxidative stress in plants can be minimized by upregulation of antioxidants (enzymatic and non-enzymatic) including catalase, peroxidase, superoxide dismutase, ascorbate peroxidase, ascorbic acid, carotenes, reduced glutathione, polyphenol oxidase and glutathione reductase (Das & Roychoudhury, 2014; Ahmad et al., 2017). Improved activities or levels of enzymatic and non-enzymatic antioxidants are important under water stress conditions (Yadav & Sharma, 2016). Of all, SOD enzyme plays an essential role in catalyzing the dissociation of two molecules of superoxide into H_2O_2 and O_2 while, POD and CAT enzymes take part in counteracting a number of latent oxidants and recover stress tolerance against drought stress in crops (Ashraf, 2009; Akram et al., 2018). It has been proposed that tolerance against drought stress of any species can be connected to improved activity of antioxidant enzymes (Lima et al., 2002; Yadav & Sharma, 2016). All 8 maize cultivars under drought stress showed higher activities of SOD, CAT and POD enzymes in the present study. Similarly, canola (Akram et al., 2018) and radish (Shafiq et al., 2015) cultivars showed high activities of enzymes under stress conditions and were categorized as relatively tolerant on the basis of these biochemical attributes.

Conclusion

Overall, drought stress significantly decreased plant growth (shoot and root fresh and dry biomass) and chlorophyll contents in all maize cultivars. Whereas, increase in the H_2O_2, MDA, proline, GB, AsA contents, activities of SOD, CAT and POD enzymes and total phenolics was observed under water stress conditions. However, of all maize cultivars, cv. Sultan was the lowest and cvs. Sadaf and Pearl the highest in plant growth under stress conditions. Overall, better growth of relatively tolerant maize cultivars, cv. Sadaf and Pearl under water deficit conditions can be associated with high level of proline, GB and oxidative defense system.

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