Research Article

Antioxidant Defense System is a Key Mechanism for Drought Stress Tolerance in Wheat (*Triticum aestivum* L.)

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Abstract | Drought is key abiotic stress that reduced crop growth, development and yield. While several physiological processes are played role in plant adaptations to drought stress. However, antioxidant defense system and lipid peroxidation play a key role in drought tolerance. The current study was intended to investigate physiological adaptations for drought tolerance in different wheat varieties. Three weeks old seedlings of each wheat cultivar were challenged with drought stress by skipping irrigation. After the drought stress of two weeks, plants were subjected to morphological, biochemical and physiological investigations. Drought stress reduced the biomass and enhanced the root length in all three wheat varieties. However, growth reduction due to drought stress was relatively lower in variety F23. While the shoot length of all three wheat varieties was decreased because of drought stress, whereas the root/shoot ratio was increased. The root length was higher in variety F23. The chlorophyll a and b were decreased due to water shortage. Variety Pu19 had greater chlorophyll contents in comparison to other wheat varieties in drought conditions. The total soluble proteins, proline, glycinebetaine and phenolic contents were improved in all three wheat varieties under drought stress. The variety Pu19 was exhibited a higher level of proline, glycine betaine and phenolic contents were. Similarly, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) were improved in all three wheat varieties but this increase was relatively lower in variety F23. The superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities were improved in all wheat varieties in a water shortage. The variety F23 exhibited higher antioxidants levels which are positively associated with its potential of drought stress tolerance. This study provided a helpful understanding of the significant role of the antioxidant defense system of wheat crop especially the variety F23 in drought stress tolerance.

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Introduction

Water scarcity in soil induces drought stress that leads to lower photosynthetic efficiency and reduced production of plant biomass (Chaves *et al.*, 2009). Besides, water shortage causes oxidative stress, osmotic stress and cellular damage in plants particularly in chloroplasts (Wang *et al.*, 2003).

In water deficit conditions reactive oxygen species (ROS) are induced in chloroplast and mitochondria. However, plants adopt some protective mechanisms to save themselves from the damaging effects of drought (Ashraf *et al*., 2010). For instance, plants start producing various antioxidants that are elaborate tolerance to oxidative stress. The ROS scavengers are antioxidant enzymes including superoxide dismutase, peroxidase and catalase (Mittler, 2002). Superoxide dismutase is a major scavenger of superoxides resulting in hydrogen peroxide and oxygen. CAT detoxify hydrogen peroxide (Dionisio-Sese and Tobita, 1998). Lignin biosynthesis, indole acetic acid degradation and conversion of H_2O_2 to H_2O are done by POD. In cytosol, ascorbate peroxidase is present that is an important ascorbate cycle. POD changes ascorbate into dehydroascorbate (DHA) and in this way, it is disregarded from cell in stress conditions (Hegedüs *et al*., 2001). In other mechanism, an important enzyme called 'dehydroascorbate reductase (DHAR)' plays role in ROS detoxification and recovery of ascorbate by glutathione (GSH)-dependent reduction of DHA (Do *et al*., 2016).

The production of osmoprotectants is also a protective strategy of plants in water deficit conditions. The production of compatible organic solutes is the common feedback of plants towards water shortage. The compatible organic solutes are highly soluble compounds and having low molecular weight. The higher concentration of these compounds is non-toxic to cell. In these osmoprotectants, the proline and glycine betaine are most common. The exogenous application of these two osmoprotectants helps the plants in drought stress management (Ashraf and Foolad, 2007). Various physical modifications of plant tissues, production of biomolecules, changes in physiological mechanisms and elevated levels of arsenal of ROS scavengers are responsible for drought tolerant behaviour in plants. Therefore, it is significant to make comparative study of different molecular signatures in plants growing under drought stress.

Among cereal crops, wheat (*Triticum aestivum* L.) is widely used nutritive essential food in the world (Savary *et al*., 2012). The wheat crop productivity is declining due to water scarcity in cultivated lands of Pakistan and the world. To overcome this problem, there is dire need for drought tolerant wheat cultivars screening and or raising novel varieties with drought resistance. Therefore, recent study was planned to explore the potential of drought tolerance of some selected varieties of wheat using biochemical and physiological attributes especially the antioxidative defense system.

Materials and Methods

The experiment was carried out at the Botanic Garden, Bahauddin Zakariya University Multan, Pakistan. The germplasm of the selected three varieties of wheat was taken from Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan. The plants were grown in pots (height 01 foot, diameter 09 inches) filled with garden soil (~05Kg) for three weeks under normal conditions. The drought stress was induced by skipping normal irrigation for two weeks. Afterward, the plants were harvested and various morphological, biochemical and physiological investigations were done.

The absorbance in all assays was measured by using UV-VIS double beam spectrophotometer (Hitachi U-2900). The shoot and root length were measured in cm by a regular ruler. The freshly harvested plant was separated into shoots and roots and was weighed by digital balance (Shimadzu). After that, shoots and roots were incubated in oven at 80°C for one week to measure the dry biomass. The chlorophyll a and b contents were estimated by adopting the Arnon protocol (Arnon *et al*., 1956). Fresh leaves (2.0g) were ground in 80% acetone. The absorbance was observed at 645nm and 663nm on spectrophotometer. For the estimation of total soluble proteins (TSPs), fresh leaf samples (2.0g) were ground in sodium phosphate buffer (pH 6.8, 10ml) followed by centrifugation at 13000 rpm for 15 min. After centrifugation, the supernatant was separated into an Eppendorf tube. This extract was diluted with the same buffer and mixed with Bradford's reagent before absorbance measurements at 595nm (Bradford, 1976). For the measurement of proline, 200mg fresh leaf sample was ground in 3% (w/v) sulfosalicylic acid. Then filtered with Whatman filter paper. Acidic Ninhydrin and glacial acetic acid (1ml each) were added into filtrate and reaction mixture was heated in a water bath at 70°C for 30 min. After 30 min, it was cooled on ice. After cooling 2ml organic toluene was added. The toluene phase containing the chromophore was measured at 520nm (Bates *et al*., 1973).

For the measurement of superoxide dismutase (SOD)

activity, 3 ml reaction mixture was prepared by mixing 1.5 M Na_2CO_3 (0.1ml), 200mM methionine (0.2ml), 3 mM EDTA (0.1ml), 2.25mM *p*-NitroBlue Tetrazolium chloride (NBT, 0.1ml), 100mM potassium phosphate buffer (1.5ml), distilled water (1ml). The extracted enzyme samples (0.05ml) were added to reaction mixture. The mixture of the above solutions without enzyme sample was used as negative control (Giannopolitis and Ries, 1977). The POD and CAT were measured according to the protocol of Chance and Maehly (1955). For the estimation of MDA contents, fresh leaf samples were ground in trichloroacetic acid (TCA 0.1%, 0.5% thiobarbituric acid). After that, the samples were heated for 10 min in water bath. After 10 min, the samples were cooled and centrifuged at 10000 rpm for 20 min. The reading of supernatant was measured at 532nm and 600nm (Heath and Packer, 1968). The glycine betaine contents were measured by of Grieve and Grattan (1983) method. The concentration of hydrogen peroxide was estimated by following Sergiev *et al.* (1997).

The phenolic contents of leaves were measured according to protocol of Ainsworth and Gillespie (2007) by using Folin-Ciocalteu solution. The amount of ascorbic acid was measured by using the protocol of Moeslinger *et al.* (1995). The MS-Excel 2013 was used to record the data and to calculate mean values, standard deviation and standard error. The two ways analysis of variance (ANOVA) and LSD at 5 % level of probability ($p<0.05$) was calculated by using software Statistix v 8.1.

Results

Drought has a drastic effect on the growth and yield of wheat plants. The current study revealed that drought stress has significantly reduced shoot length, biomass production and chlorophyll a and b contents in wheat plants. While the drought stress has significantly increased the root length, chlorophyll a/b ratio, hydrogen peroxide, malondialdehyde, peroxidase, superoxide dismutase, catalase, total soluble proteins, proline, glycine betaine and phenolic contents. The variety Galaxy showed maximum growth under control and drought stress while the variety F23 showed a greater decrease in shoot length in drought stress. The reduction in shoot length in variety Pu19 was less than that of variety F23 as shown in Figure 1A. In drought stress, root length of all varieties was increased. The highest root length was measured in Galaxy under drought stress. Among three varieties, F23 showed minimum growth under control conditions and minimum enhancement in root length in drought conditions. The variety Pu19 showed little increase in root length under drought stress as shown in Figure 1B. The plant biomass production was reduced in water shortage as shown in Figure 2.

The chlorophyll contents were reduced in water deficit conditions. The highest chlorophyll a contents were recorded in variety Galaxy and lowest in F23 under control condition as shown in Figure 3A. The chlorophyll b contents were highest in Galaxy under control condition. The F23 showed the lowest

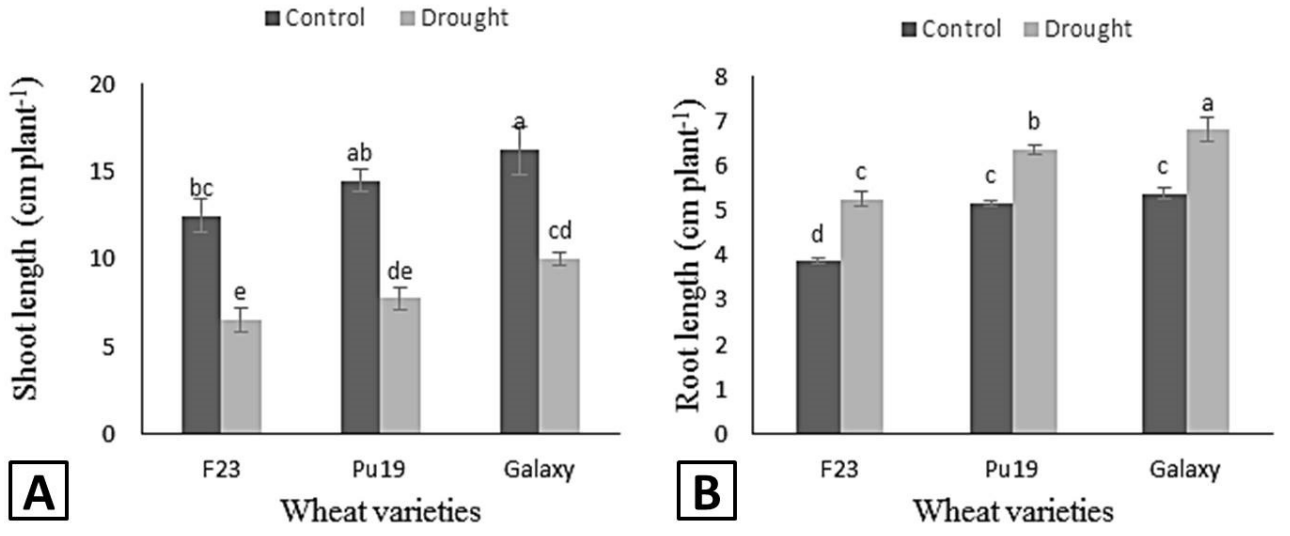

Figure 1: *Shoot length (A) and root length (B) of wheat varieties grown under control and drought condition. Different letters show the significant differences calculated by LSD at p<0.05. The data presented are mean values ± standard error of four replicates.*

chlorophyll b contents as shown in Figure 3B. The chlorophyll a to b ratio was enhanced under drought stress. The major decrease was observed in Galaxy as shown in Figure 3C. The total chlorophyll contents in Galaxy were greater in both conditions. In variety Pu19, the total chlorophyll contents were slightly increased under drought conditions. The maximum decrease in total chlorophyll contents due to drought stress was recorded in variety F23 as shown in Figure 3D.

The hydrogen peroxide (H_2O_2) contents were enhanced in all varieties in drought stress. In variety Pu19, the H_2O_2 contents were lowest as shown in Figure 4A. The malondialdehyde contents were increased in all varieties in water shortage. In variety Galaxy, the MDA contents were maximum in drought conditions. While the variety F23 had the lowest MDA in both conditions as shown in Figure 4B.

The peroxide contents were enhanced in all varieties due to drought stress. The highest increase was recorded in variety Pu19 as shown in Figure 5A. The superoxide dismutase contents were enhanced in drought stress. The variety Galaxy showed the highest SOD in drought conditions as shown in Figure 5B. The total soluble proteins were improved in all varieties in drought conditions. The highest accumulation was observed in Galaxy in drought stress as shown in Figure 5C. The catalytic activity was improved in all varieties in drought stress in relation to control. The variety Pu19 had the highest CAT activity under drought conditions in comparison to all varieties in both conditions as given in Figure 5D.

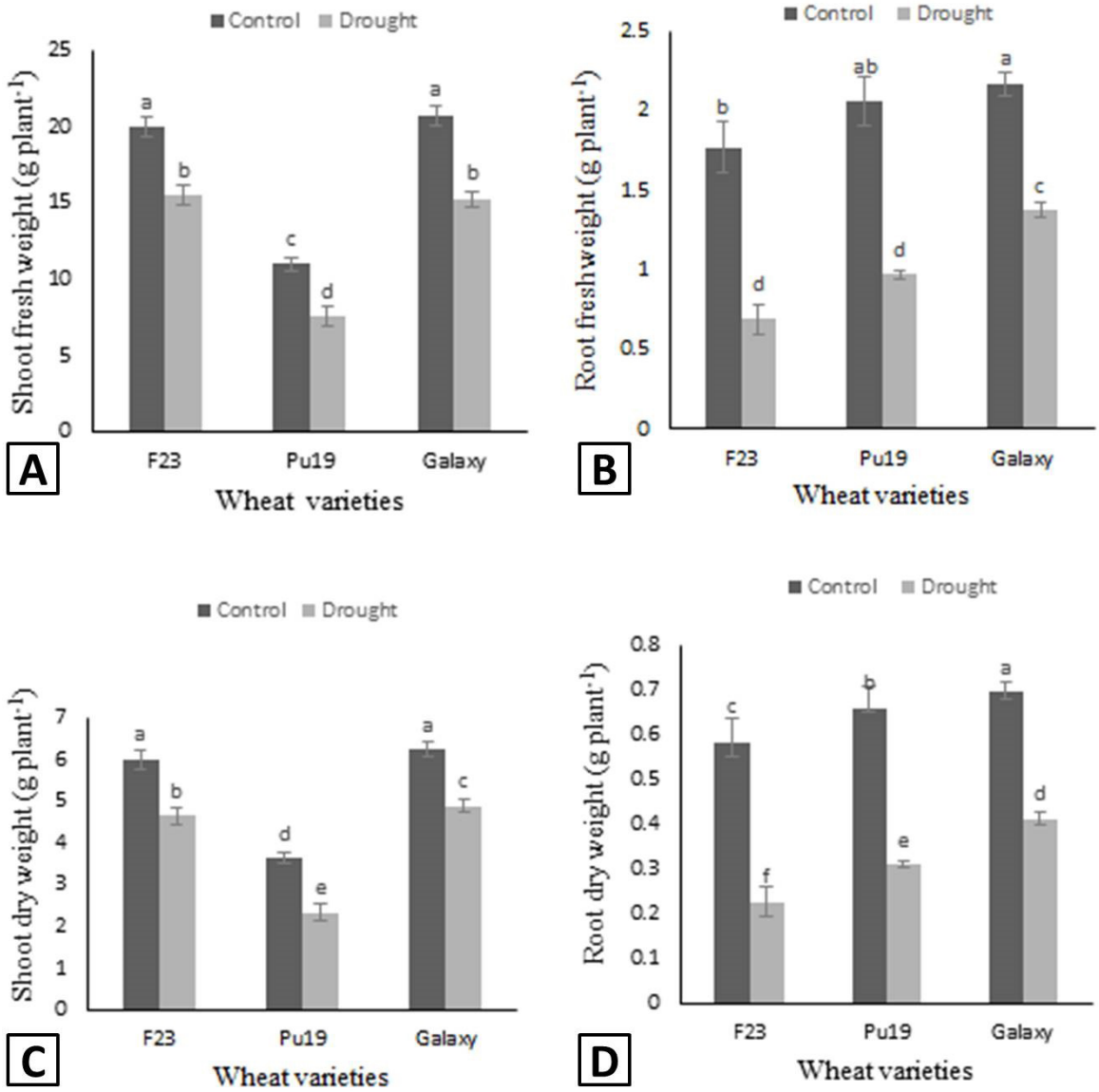

Figure 2: *Fresh weight of shoot (A), fresh weight of root (B), dry weight of shoot (C) and dry weight of root (D) of wheat varieties grown under control and drought condition. Different letters show the significant differences calculated by LSD at p<0.05. The data presented are mean values ± standard error of four replicates.*

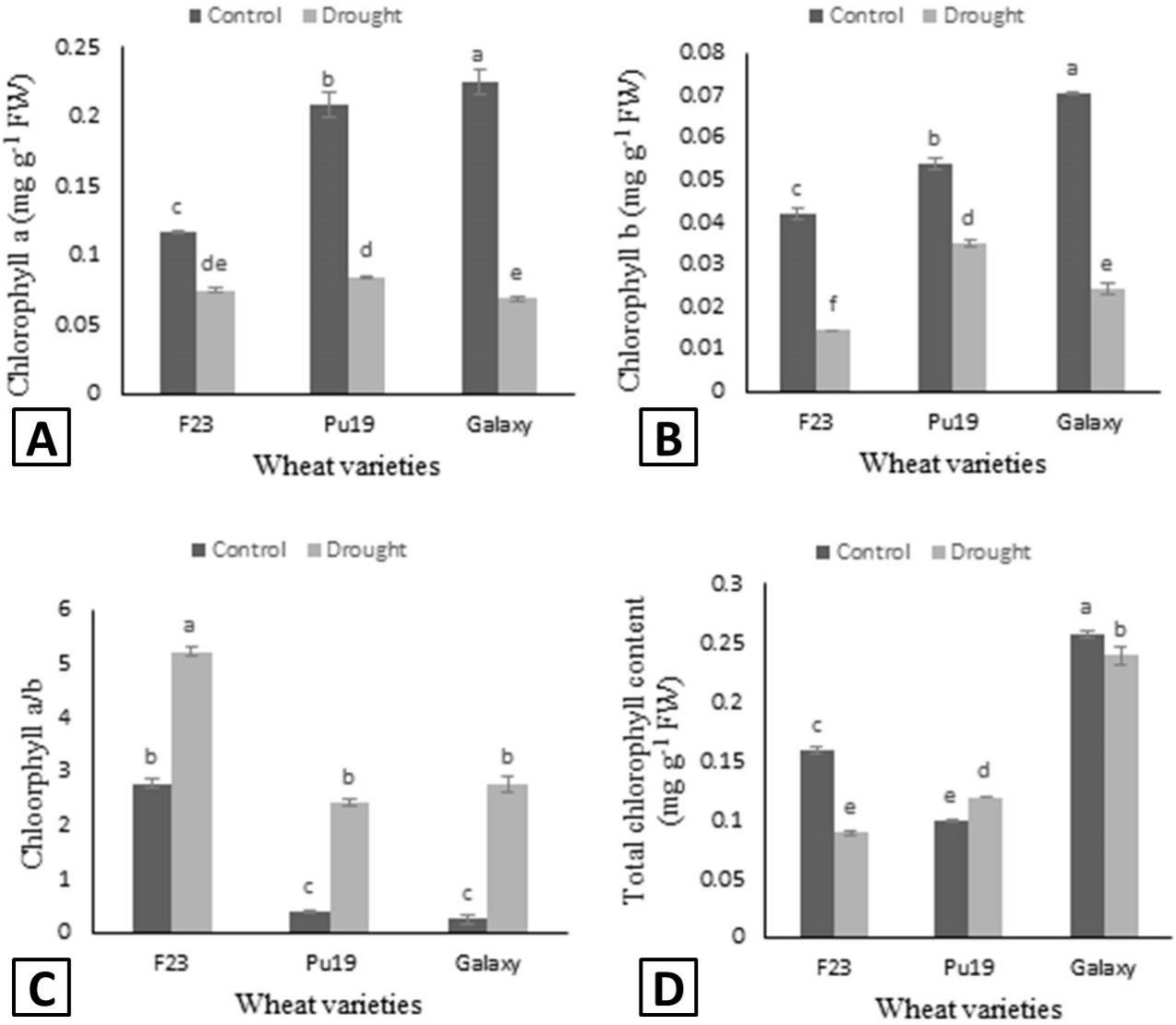

Figure 3: *Chlorophyll a (A), chlorophyll b (B), chlorophyll a/b (C) and total chlorophyll contents (D) of wheat varieties grown under control and drought condition. Different letters show the significant differences calculated by LSD at p<0.05. The data presented are mean values ± standard error of four replicates.*

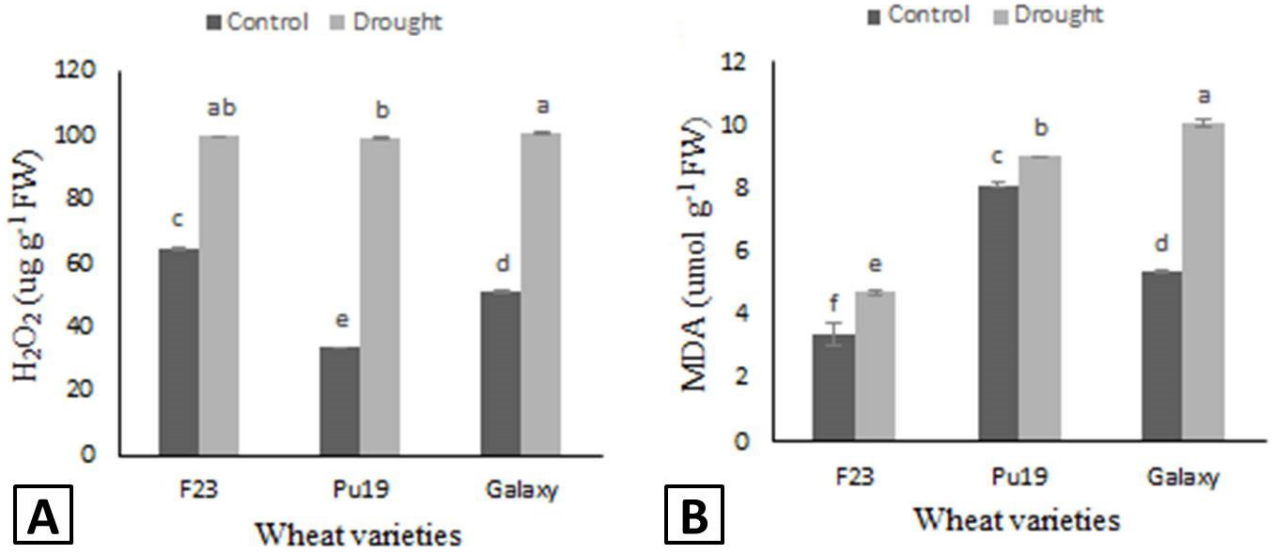

Figure 4: *H_2O_2 contents of wheat leaves grown under control and drought condition. Different letters show the significant differences calculated by LSD at p<0.05. The data presented are mean values ± standard error of four replicates.*

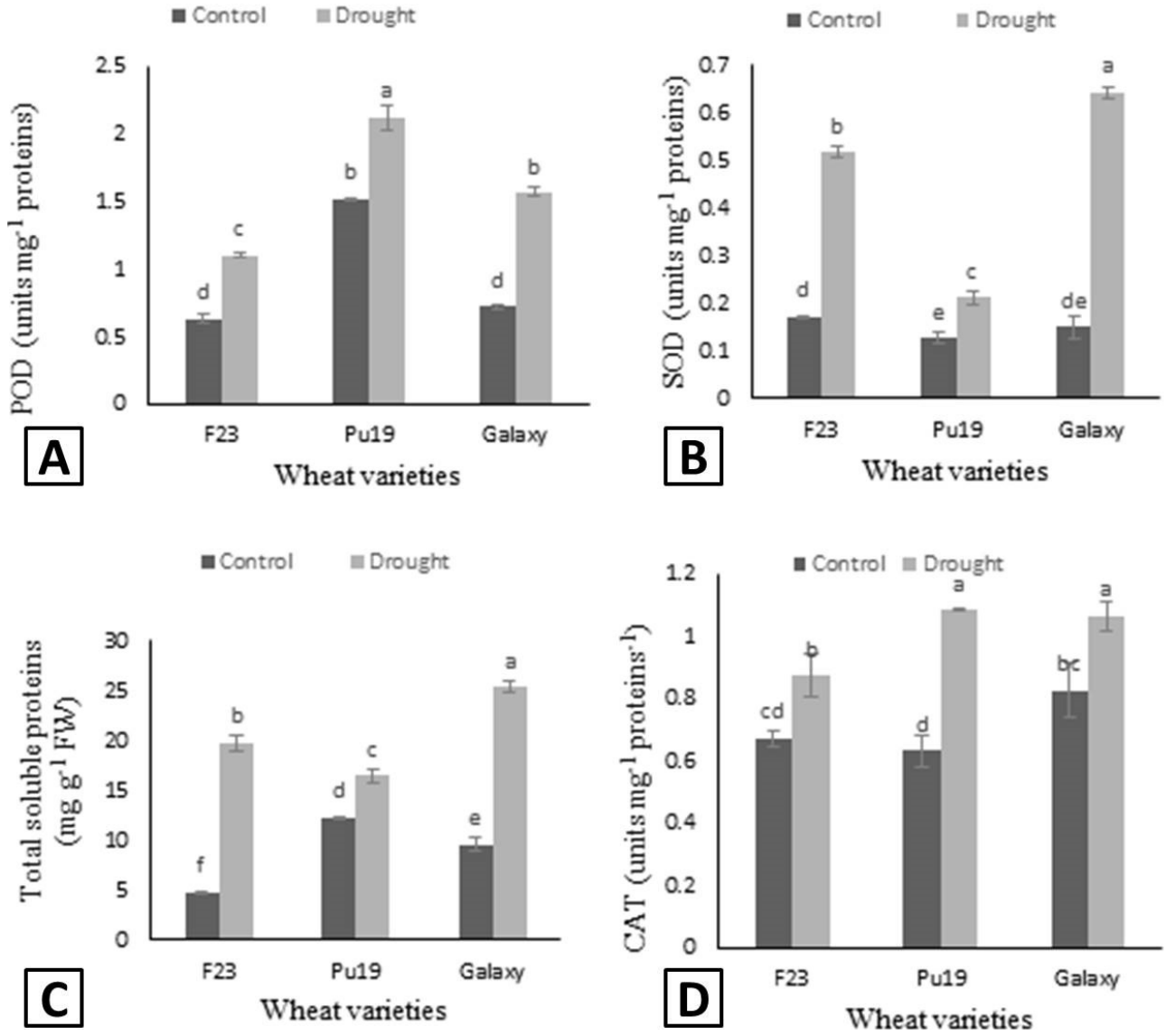

Figure 5: *POD (A), SOD (B) total soluble proteins (C) and CAT (D) measured in three different wheat varieties grown under control and drought condition. Different letters show the significant differences calculated by LSD at p<0.05. The data presented are mean values ± standard error of four replicates.*

The enhanced level of proline was observed in drought stress. In variety F23 the proline concentration was highest in drought conditions as shown in Figure 6A. Glycine betaine (GB) was increased in all varieties in water shortage. The maximum concentration of GB was estimated in variety Galaxy under drought conditions. The accumulation of GB in variety Pu19 under drought conditions was the minimum as shown in Figure 6B. The phenolic contents were increased because of drought stress. The highest elevation was noted in variety Galaxy in drought stress. The lowest phenolic contents were observed in F23 in control condition as shown in Figure 6C. The ascorbic acid was increased in two varieties of wheat F23 and Pu19 in drought stress. While it was reduced in variety Galaxy. This variety had a better accumulation of ascorbic acid in normal conditions as shown in Figure 6D.

Discussion

Drought stress reduces plant growth and development and limits plant production (Shao *et al.*, 2008). The current study showed that in water shortage, the shoot length was decreased (44%) in all varieties of wheat while the root length (28.7%) and root to shoot ratio were increased (Figure 7). Aldesuquy *et al.* (2012) and (2014) have reported the reduction of shoot length and enhancement in root length and root shoot ratio in drought stress. Plants in water deficit conditions trigger root growth and produce longer roots to search water in the soil at relatively deeper levels.

This adaptation leads to their potential for drought stress tolerance. Generally, drought stress causes a reduction in biomass therefore, reduction in shoot length was observed in all varieties of wheat when grown in water deficit. However, levels of reduction in different varieties were different. Similar reports were submitted by Ghebremariam *et al*. (2013). In another study which was carried out on *P. acutifolius* and *P. vulgaris,* the shoot length was decreased in drought stress (Türkan *et al*., 2005). Similarly, in maize, the shoot length was decreased under drought stress (Khan *et al*., 2004). In the present study it was observed that biomass production decreased under water deficit conditions (Figure 2). The decrease in biomass production was described in maize Rashid *et al*. (2018) and common beans/ green gram Webber *et al*. (2008).

In chloroplast, the chlorophyll is a major component that plays an important role in photosynthesis. In the current study, the chlorophyll a and b contents were declined in drought stress (54.9%; Figure 7). The total chlorophyll was decreased under drought stress in Galaxy and F23 (reduction 6.9% and 43.7%; Figure 7). These results were similar to report by Valifard *et al*. (2012) but in other cultivars. The chlorophyll a to b ratio in wheat was augmented under stress conditions in all varieties.

In the present study, the proline contents in all varieties were greater under drought as compared to control (66.1%; Figure 7). In rice varieties the proline contents were increased in water deficit conditions (Lum *et al*., 2014). The proline is an important amino acid that is accommodating in stress because it provided energy for plant development and growth (Brunken, 1977). It was indicated that the total soluble proteins were increased under low moisture contents in wheat (Figure 7).

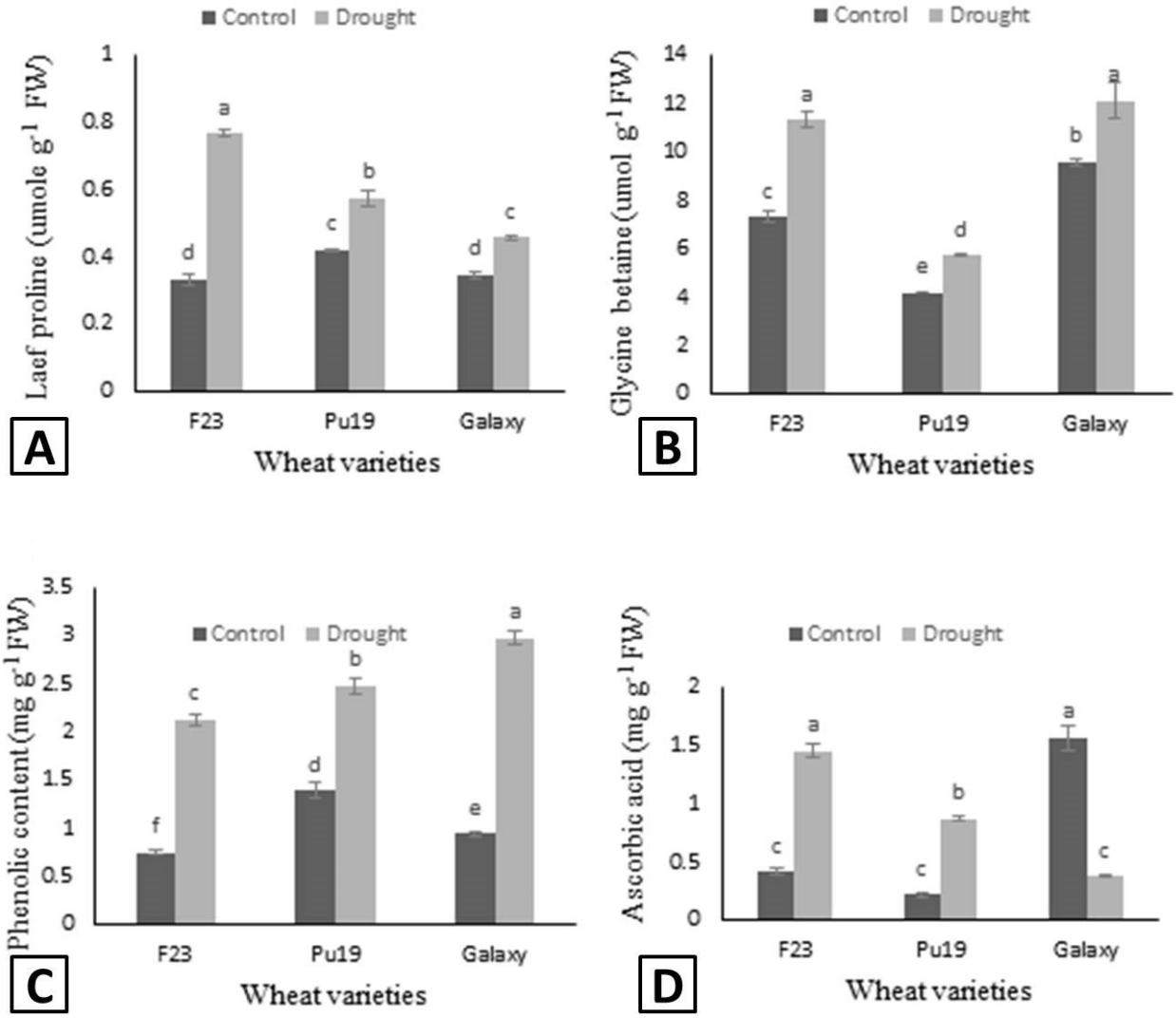

Figure 6: *Leaf proline (A), glycine betaine (B), phenolic Contents (C) and ascorbic acid (D) of wheat varieties grown under control and drought condition. Different letters show the significant differences calculated by LSD at p<0.05. The data presented are mean values ± standard error of four replicates.*

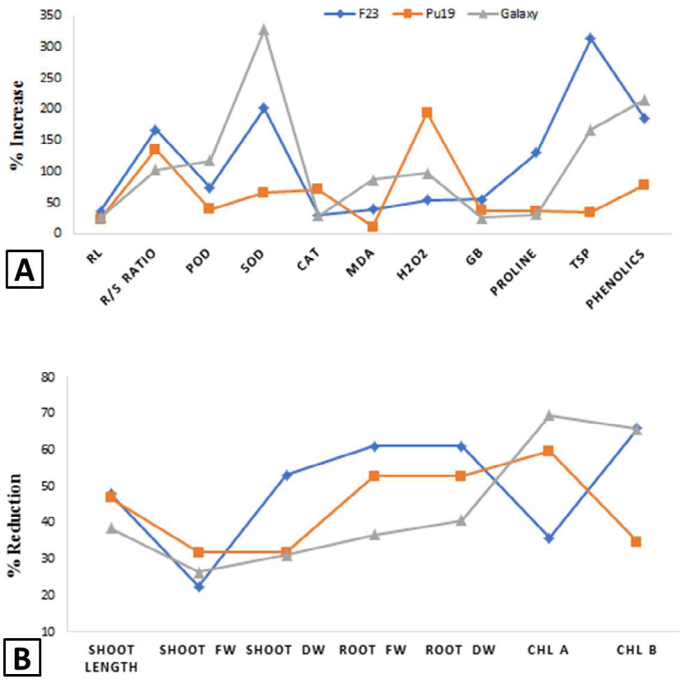

Figure 7: *Percentage of reduction (A) and increase (B) in biomass, chlorophyll contents, antioxidants and osmoprotectants in wheat varieties grown under control and drought condition. RL, root length; R/S ratio, root and shoot ratio; TSP, total soluble proteins; GB, glycine betaine; POD, peroxidase; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde.*

Lipid peroxidation indicates the presence of free radical reactions in tissues. The contents of malondialdehyde are often used as an indicator of the level of lipid peroxidation that occurs due to oxidative stress. In the extant study, the malondialdehyde contents were enlarged in all varieties in drought stress (45.8%; Figure 7). The increase in MDA contents under drought stress was also observed by Labudda (2013).

Peroxidase enzyme catalyzes H_2O_2-dependent oxidation of the substrate. We found that the peroxidase was increased in drought stress in all varieties of wheat (76.9%; Figure 7). Superoxide dismutase is a key antioxidant enzyme which defends the plant against extreme superoxide radicals formed in plants in stress condition. The superoxide dismutase contents of all varieties were improved in drought stress (199%; Figure 7). Another finding on wheat exhibited that SOD contents were increased in early water deficit conditions, while in prolonged exposure to water deficit conditions these were diminished (Shao *et al.*, 2008). The CAT activity was improved (43.18%; Figure 7) in all variety in drought stress that may be helpful in stress management as studied by Zhang and Kirkham (1994).

We found that the accumulation of Glycine betaine was improved in wheat in drought stress (39%; Figure 7). These results were related to a previous study in *Helianthus annuus* L. (Baloğlu *et al.*, 2012). The GB was improved in barley (Havir and McHale, 1987). Similarly, the proline concentration also increased in drought stress (Hussain *et al.*, 2008).

In the recent study, it was noted that in all varieties, the hydrogen peroxide (H_2O_2) contents were higher in drought stress as compared to control (115%; Figure 7). In an alternative study, the H_2O_2 contents were improved under drought stress in wheat. The role of H_2O_2 had been recognized in cell signaling cascade (Sairam *et al.*, 1998; Sairam and Srivastava, 2001).

The increased phenolic contents (159.8%; Figure 7) under drought stress in all wheat varieties were similar to the results found by Bolat *et al.* (2014). The ascorbic acid accumulation in two varieties F23 and Pu19 of wheat was increased in drought stress (Figure 6). These results were similar to the previous study in Akria and Mobil tomato cultivars. Ascorbic acid is an important non-enzymatic antioxidant that plays important role in oxidation-reduction system of the cell and prevents the cell from damage. It decreases the oxygen radicals and produces α-tocopherol (Akram *et al.*, 2017).

Conclusions

Basis on the above findings, it was concluded that among three varieties of wheat, the variety F23 exhibited better potential of drought stress management by utilizing elevated levels of antioxidants as a key defense mechanism and it may further be studied to investigate the molecular mechanism of drought tolerance.

Novelty Statement

This research indicated that drought plays a negative impact on wheat plants by decreasing growth and development. The antioxidants play an effective role against this drought stress and increase the plant growth and development.

Authors' Contribution

Muhammad Rashid and Mahmood Ahmad Sajid performed experiments and wrote the manuscript; Nosheen Noor Elahi and Sibgha Noreen helped in

data analysis and manuscript writing; Kausar Hussain Shah designed the experiments, analyzed the data and corrected/edited the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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