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ORGANIC AMENDMENTS MITIGATE DROUGHT STRESS-INDUCED OXIDATIVE CHANGES IN SYNTHETIC CULTIVARS OF MAIZE

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Abstract

Drought stress is the most crucial abiotic stress. It adversely affects plant growth and increases reactive oxygen specifies. However, antioxidants biosynthesis is one of the most important defensive mechanisms of crops under drought stress. On the other hand, many scientists successfully elaborate the positive effects of organic amendments for alleviation of drought stress in cereal crops. These organic amendments, i.e., farmyard manure (FYM), poultry manure (PM) and biochar have the potential to improve soil water holding capacity. Although many scientists have worked on organic amendments to mitigate drought stress, there is still an information gap regarding organic amendment's effects on the antioxidant attributes of plants. That is why the current study was conducted on two maize varieties, "Neelum" and "Sadaf" with two irrigation levels, i.e., normal (100%FC) and drought stress (50%FC). In organic amendments, FYM, PM and biochar were applied with three replications in a randomized complete block design with a split-split plot arrangement. Results showed that antioxidants SOD, POD and CAT were significantly increased in "Neelum" and "Sadaf" under drought stress (50%FC) over control irrigation (100%FC). Adding FYM and PM significantly decreased SOD, POD and CAT over control. However, biochar remained significantly best for decreasing antioxidants under stress conditions. The addition of biochar also remained significantly better among all the treatments for the enhancement in protein and sugar contents of maize varieties. In conclusion, biochar application can provide better results for alleviating drought stress in "Neelum" and "Sadaf" maize varieties over FYM and PM. More investigations are suggested in the field to declare the best biochar application rate for drought stress alleviation in maize.

Key words: Abiotic stress, *Zea mays* L., Antioxidant, Organic amendments.

Introduction

Among crops, maize is one of the most important cereals, and has a valuable position in world production due to its important role in human and livestock nutrition. It has the highest consumer variety in addition to consumption as human food and as fodder for livestock; it is also used in the fermentation industry and the preparation of various industrial products, including ethanol (Barros-Rios *et al*., 2015). In Pakistan, due to the short growth period and rapid development of phenological stages, this crop is cultivated twice a year. According to statistics reported by the FAO, the cultivated area under maize is 1.41 million ha^{-1}, and total production and average yield have been reported 7.23 million tons and 5120 kg ha^{-1}, respectively (Anon., 2019). However changing climatic conditions and increasing drought stress are notorious factors that are adversely affecting maize production (Danish & Zafar-ul-Hye, 2019; Danish *et al*., 2019; Zafar-ul-Hye *et al*., 2020).

Water stress is a serious threat to successful maize production worldwide (Ashraf *et al*., 2011; Fiaz *et al*., 2014). Lack of nutrient supply due to drought reduces root growth and the plant will not be able to absorb water and nutrients (Ashraf *et al*., 2016; Danish & Zafar-ul-Hye, 2019; Zafar-ul-Hye *et al*., 2019, 2020; Shah *et al*., 2019). Maize is sensitive to moisture stress at different stages of growth and the pollination stage and two weeks later are the most sensitive growth period of this plant to drought stress; So that at this time, the decrease in yield is two to three times more than when the stress occurs in other stages of growth (Huang *et al*., 2006; Danish *et al*., 2020b; c; a). Therefore, to produce a product with high quality and yield, this plant needs enough water to absorb nutrients at all stages of growth. Since maize has different needs for nutrients in different stages of growth and development, the effect of drought stress on the process of accumulation of nutrients in different growth periods is different and with increasing plant needs at each stage of growth to the nutrient, the effect of water stress will also be more in that period (Ashraf *et al*., 2016).

On the other hand, farmers increase the amount of chemical fertilizers to increase crop yield to prevent a sharp decline in yield (Nehra *et al*., 2001; Rafiullah *et al*., 2020). Although the use of mineral fertilizers seems to be the fastest and safest way to compensate for soil fertility, however, the high costs of fertilizer application in the proposed committees and other issues of soil pollution and degradation are factors that have worried environmentalists in the application of these fertilizers (Ruidisch *et al*., 2013). Therefore, the use of organic fertilizers is very important for the development of sustainable agriculture.

Organic matter is considered one of the important pillars of soil fertility (Karami *et al*., 2012). Unfortunately, the level of organic matter in Pakistani arable soils is mostly less than one percent, which is due to the excessive use of chemical fertilizers, especially nitrogen fertilizers and the lack of organic fertilizers in recent years. Among organic fertilizers, farmyard manure has a special value by providing all the nutrients needed for plant growth, even trace elements and the maximum efficiency of using this manure with the crop depends on the method of application, the time of mixing and the rate of its decomposition in the soil (Paryan *et al*., 2012).

The application of poultry manure, farmyard manure and compost improved the growth characters and yield of maize under stressful conditions (Younis *et al*., 2014, 2020; Danish *et al*., 2015; Sultan *et al*., 2020; Zafar-ul-Hye *et al*., 2021). Poultry manure is a very suitable and inexpensive organic fertilizer with high nitrogen, phosphorus, potassium and other essential elements. Compared to chemical fertilizers, the addition of poultry manure to the soil improves soil structure, nutrient retention, aeration, soil moisture retention at field capacity and water permeability (Farhad *et al*., 2009). It has also been shown that poultry manure provides phosphorus more easily than other sources of fertilizer for plants (Mahmood *et al*., 2017). Biochar, with about 40 to 80 percent carbon, is a source of organic matter for the soil and also by increasing the pH of the soil, it improves access to nutrients and by having different properties, especially water holding capacity and nutrient storage capacity in the soil, it is effective in boosting plant growth (Lehmann *et al*., 2011; Ahmed *et al*., 2021). Among them, poultry manure by saving water consumption increases water use efficiency and improves grain quality by absorbing elements (Al-Dulaimi *et al*., 2020). Biochar has played an effective role in improving nutrient uptake (Chng *et al*., 2016), induction of antioxidative capacity, osmolytes accumulation and increasing maize plant growth under drought conditions (Agegnehu *et al*., 2017; Ali *et al*., 2017; Danish *et al*., 2020a).

So far, little research has been done on the effect of different types of organic fertilizers on the physiological characteristics of crops under restricted irrigation water conditions. Therefore, this study aimed to investigate farmyard manure, poultry manure and biochar in creating drought tolerance by evaluating the activities of antioxidant enzymes and the accumulation of osmolytes. It is hypothesized that organic amendments might have the potential to improve the maize antioxidant activities under drought stress.

Material and Methods

Collection and preparation of organic amendments: For the preparation of biochar, sticks of cotton were collected from the Agronomic Research Field, Muhammad Nawaz Sharif University of Agriculture, Multan, Pakistan. After sun drying, sticks were chopped into small pieces and pyrolyzed at 450°C for two hours (Qayyum *et al*., 2014). Poultry manure (PM) was collected from Lutaf Abad Control Poultry shed, Bosan Road, Multan, and Pakistan. However, farmyard manure (FM) was collected from Cattle Farm of the Department of Veterinary Science, Bahauddin Zakariya University, and Multan, Pakistan.

Experimental site design: A field experiment was carried out in a greenhouse at Agronomic Research Farm, Department of Agronomy, Bahauddin Zakariya University, Multan, during spring 2018 and 2019. The design of the experiment was Randomized Complete Block Design (RCBD) in split-split plot arrangement with three replications.

Maize varieties collection and characteristics: There were two maize varieties, "Neelum" and "Sadaf". Both were purchased from the local certified dealer of the Government of Punjab, Pakistan. "Neelum" was drought tolerant, while "Sadaf" was drought-sensitive in characteristics.

Seeds sowing time and method: The experiment was laid out on the same date (1st February 2018 and 1st February 2019). Before sowing, the land was prepared by three ploughing, one planking and one rotavatring. A recommended seed rate of 10kg/acre was used with row to row distance of 65cm and plant to plant distance was maintained at 16 cm. Sowing was done on single-side ridges with a net plot size of 4×5 meters.

Maintenance of plant population: Thinning was done after 15 days after emergence (DAE) and gap-filling was done to maintain plant population till harvest.

Fertilizer application rate and time: Organic amendments (Biochar, poultry manure and Farmyard manure) were amended at the rate of 10 tonnes per hectare as per individual treatment. A recommended dose of nitrogen, phosphorous and potash at the rate of 250kh/ha, 125kg/ha, 125kg/ha was also applied in each experimental unit. A whole dose of potash and phosphorous was applied at the time of sowing.

Drought stress development: The development of drought stress was done based on soil field capacity (FC). Moisture required to produce maize was kept as control (100%FC). However, half of the recommended irrigation (50%FC) was applied for the development of artificial drought stress (Danish *et al*., 2020c; a). The field capacity of the experiment was controlled by using a cut-throat flume.

Harvesting and data collection: After 120 days, plants become physiological mature and harvested for antioxidants data collection in leaves tissues. Fully functional and healthy, two leaf samples from each treatment were selected and washed using distilled water.

Determination of antioxidant enzymatic activities: Peroxidase (POD) activity in leaves was determined by Sakharov and Ardila (Sakharov & Ardila, 1999) method using guaiacol substrate. 3 mL of sample solution was prepared having 0.05 mL of enzyme extract, 2.75 mL of 50 mM concentrated phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2, and 0.1 mL of 4% guaiacol solution. Absorbance was noted at 470 nm wavelength. The unit enzyme activity was accounted for as the amount of POD enzyme present. Catalase (CAT) concentration in cells was assayed by following Aebi (Aebi, 1984) method. For this, 3.0 mL of a reaction mixture comprising 100 L enzyme extract, 100 L 300 mM concentrated H_2O_2 and 2.8 mL 50 mM concentrated phosphate buffer with 2 mM ETDA of pH 7.0. CAT enzyme activity was estimated at 240 by a decline in absorbance as by H_2O_2 loss. Finally, the superoxide dismutase (SOD) contents were determined by following the protocol of Beauchamp and Fridovich (Beauchamp & Fridovich, 1971). The activity of the ascorbate peroxidase (APx) enzyme was evaluated by following Nakano and Asada method (Nakano & Asada, 1987).

Statistical analysis

A standard statistical procedure was used for the statistical analysis of collected data (Steel *et al*., 1997). Two factorial ANOVA was applied for the assessment of the significance of treatments. Each treatment was compared by using the Fisher LSD test at $p \leq 0.05$. For the preparation of pair comparison graphs, Pearson correlation and principal component analysis statistical tool Origin 2021Pro software was used (OriginLab Corporation, 2021).

Results

CAT and APx: The effect of treatments was significant on CAT and APx in maize plant tissues under normal irrigation (100% FC) and drought stress (50% FC). N+control showed significantly highest CAT under 50% FC compared to all other treatments. A significant decrease in CAT was noted in N+FYM, N+PM and N+Biochar than N+control at 50% FC. A similar trend was also noted among S+control, S+FYM, S+PM and S+Biochar at 50% FC for CAT. No significant change was noted where N+control and N+FYM were applied at 100%FC. Similarly, N+PM and N+Biochar were statistically alike to each other but showed a significant decrease in CAT over N+control and N+FYM under 100% FC. Application of S+control, S+FYM, S+PM and S+Biochar at 100% FC did not differ significantly for CAT (Fig. 1A). Treatments N+Biochar, N+PM and N+FYM showed a significant decline in APx when applied at 100% FC over N+control. A similar trend was also noted among S+control, S+FYM, S+PM and S+Biochar at 100% FC for APx. N+FYM and N+control remained statistically alike to each other but were significantly different from N+PM and N+Biochar under 50% FC. However, treatments S+Biochar, S+PM and S+FYM showed significantly lower APx compared to S+control (Fig. 1B). The highest decrease in CAT and APx was noted in Neelum (N) and Sadaf (S) varieties where biochar was applied over control at 50% FC generated drought stress.

SOD and POD: Treatments remained significantly different for SOD and POD in maize plant tissues under 50 and 100%FC. N+PM and N+Biochar showed significantly low SOD under 100%FC than N+control and N+FYM. A significantly low SOD was observed in S+FYM, S+PM and S+Biochar over S+control at 100% FC. Similar kinds of results were also observed in S+control, S+FYM, S+PM and S+Biochar at 50% FC for SOD. No significant change in SOD was noted where N+control and N+FYM were applied at 50% FC. Similarly, N+PM and N+Biochar showed a significant decline in SOD over N+control and N+FYM under 50% FC (Fig. 2A). Treatments N+Biochar, N+PM showed a significant decline in POD than N+FYM and N+control at 50 and 100% FC. Treatments S+control, S+FYM and S+PM were non-significant to each other, but S+Biochar differed significantly over S+control, S+FYM and S+PM at 100% FC for POD. Application of S+Biochar, S+PM and S+FYM showed significantly lower POD over S+control (Fig. 2B). A maximum decline in SOD and POD was noted in Neelum (N) and Sadaf (S) varieties in biochar treatment over control at 50% FC generated drought stress.

Soluble sugar and phenolic contents: The effect of treatments was significant on soluble sugar and phenolic contents of maize plant tissues under normal irrigation (100% FC) and water stress (50% FC). The treatment N+control had significantly the lowest sugar contents under 50% FC compared to other applied treatments. A significant increase in soluble sugars was noted in N+FYM, N+PM and N+Biochar than N+control in a water stress environment. A similar trend was also noted among S+control, S+FYM, S+PM and S+Biochar at 50% FC for soluble sugar contents. No significant difference was observed where S+control and S+FYM were applied at 100% FC. Similarly, S+PM and S+Biochar were statistically alike to each other and showed a significant increase in soluble sugars over S+control and S+FYM under 100% FC. Application of S+control, S+FYM, S+PM and S+Biochar at 100% FC did not differ significantly for leaf sugar concentrations (Fig. 3A). Treatments N+Biochar, N+PM and N+FYM showed a significant decline in maize phenolic contents when applied at normal irrigation conditions over N+control. A similar trend was also noted among S+control, S+FYM, S+PM and S+Biochar at 100% FC for phenolic contents. N+FYM and N+control remained statistically alike to each other but were significantly different from N+PM and N+Biochar under 50% FC. However, treatments S+Biochar, S+PM and S+FYM showed significantly lesser phenolic contents as compared to S+control (Fig. 3B). The highest increase in soluble sugars and decrease in phenolic content was noted in Neelum (N) and Sadaf (S) varieties where biochar was applied over control at 50% FC generated drought stress.

Protein contents and soluble proteins: A significant effect of treatments was observed on protein contents and soluble proteins of maize under normal irrigation (100% FC) and drought stress (50% FC). The treatment N+control had significantly lowest protein contents under 50% FC compared to other applied treatments. A significant increase in protein contents was noted in N+FYM, N+PM and N+Biochar than N+control in water stress conditions. A similar effect was also observed among the treatments S+control, S+FYM, S+PM and S+Biochar at 50% FC for protein contents. No significant change was noted where S+control and S+FYM were given at normal irrigation. Similarly, S+PM and S+Biochar were both statistically similar to each other and showed a significant increase in protein contents over S+control and S+FYM under 100% FC. Application of S+control, S+FYM, S+PM and S+Biochar at 100% FC did not differ significantly for leaf protein concentrations (Fig. 4A). Treatments N+Biochar, N+PM and N+FYM showed a significant increase in maize soluble protein contents when applied at normal irrigation conditions over the N+control treatment. A similar difference was also noted among S+control, S+FYM, S+PM and S+Biochar at 100% FC irrigation levels for soluble protein contents. N+FYM and N+control remained statistically similar to each other but were significantly different to N+PM and N+Biochar in drought stress. However, treatments S+Biochar, S+PM and S+FYM showed a significant increase in soluble proteins compared to S+control (Fig. 4B). The highest increase in protein contents and soluble proteins was noted in Neelum (N) and Sadaf (S) varieties where biochar was applied over control at 50% FC generated drought stress.

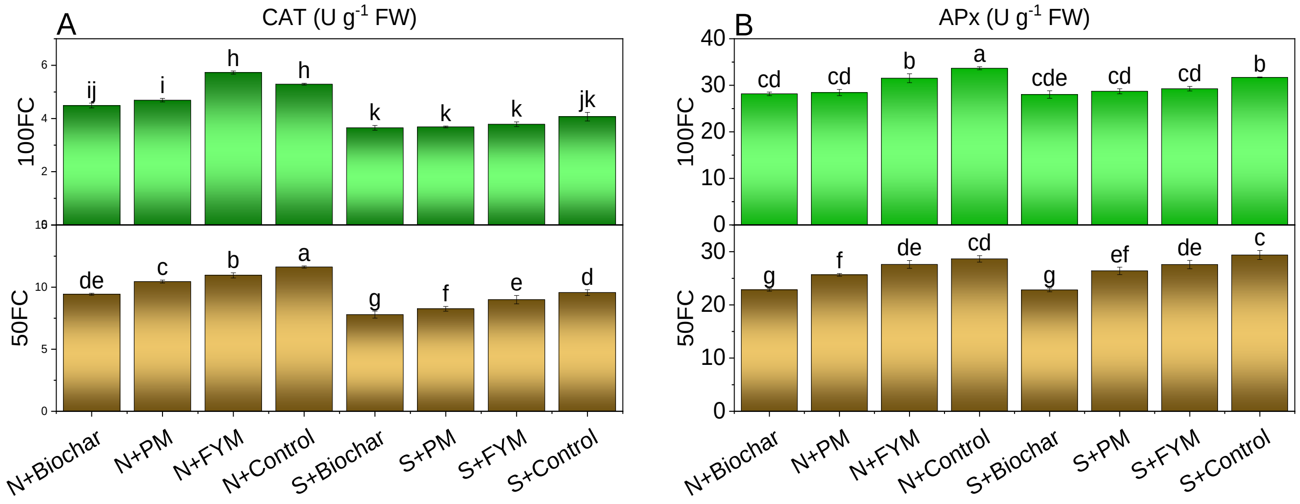

Fig. 1. Values represent the mean of 2018 and 2019. Effect of treatments on CAT (A) and APx (B) in leaf tissues of maize varieties (neelum = drought tolerant and sadaf = drought-sensitive) under drought stress. Different letters on bars showed significant differences at $p \leq 0.05$; Fisher LSD. N = Neelum; S = Sadaf; FC = Field Capacity.

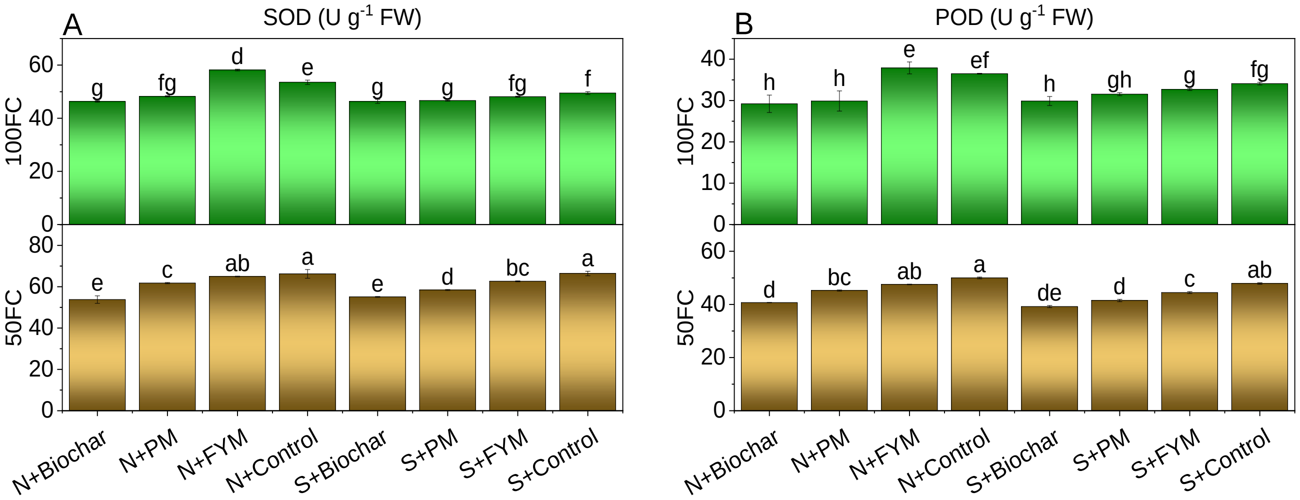

Fig. 2. Values represent the mean of 2018 and 2019. Effect of treatments on SOD (A) and POD (B) in leaf tissues of maize varieties (neelum = drought tolerant and sadaf = drought-sensitive) under drought stress. Different letters on bars showed significant differences at $p \leq 0.05$; Fisher LSD. N = Neelum; S = Sadaf; FC = Field Capacity.

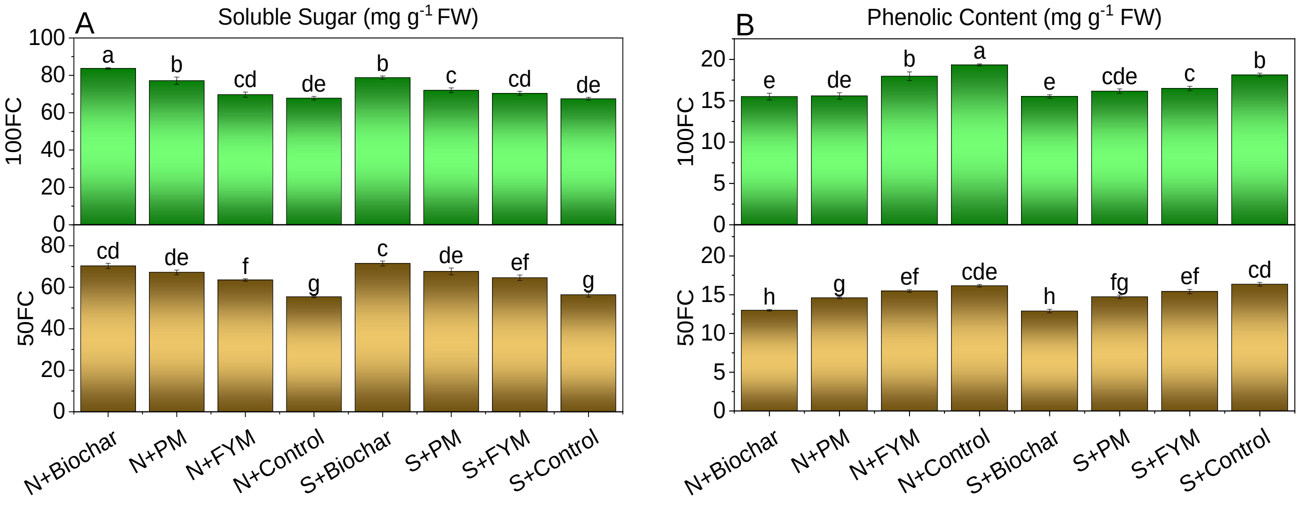

Fig. 3. Values represent the mean of 2018 and 2019. Effect of treatments on soluble sugar (A) and phenolic content (B) in leaf tissues of maize varieties (neelum = drought tolerant and sadaf = drought-sensitive) under drought stress. Different letters on bars showed significant differences at $p \leq 0.05$; Fisher LSD. N = Neelum; S = Sadaf; FC = Field Capacity.

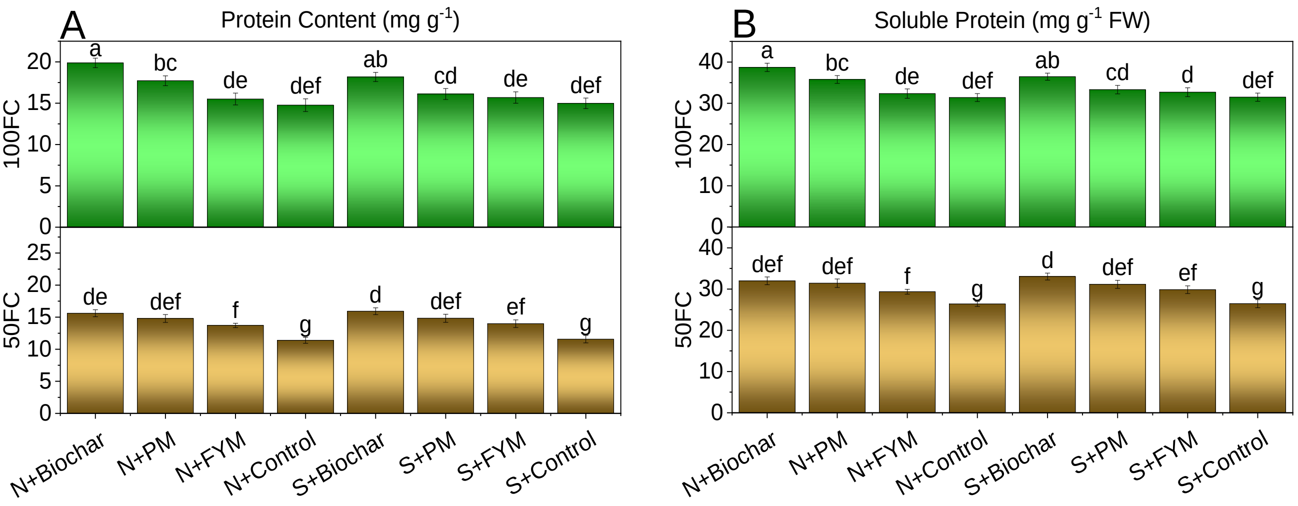

Fig. 4. Values represent the mean of 2018 and 2018. Effect of treatments on protein content (A) and soluble protein (B) in leaf tissues of maize varieties (neelum = drought tolerant and sadaf = drought-sensitive) under drought stress. Different letters on bars showed significant differences at $p \leq 0.05$; Fisher LSD. N = Neelum; S = Sadaf; FC = Field Capacity.

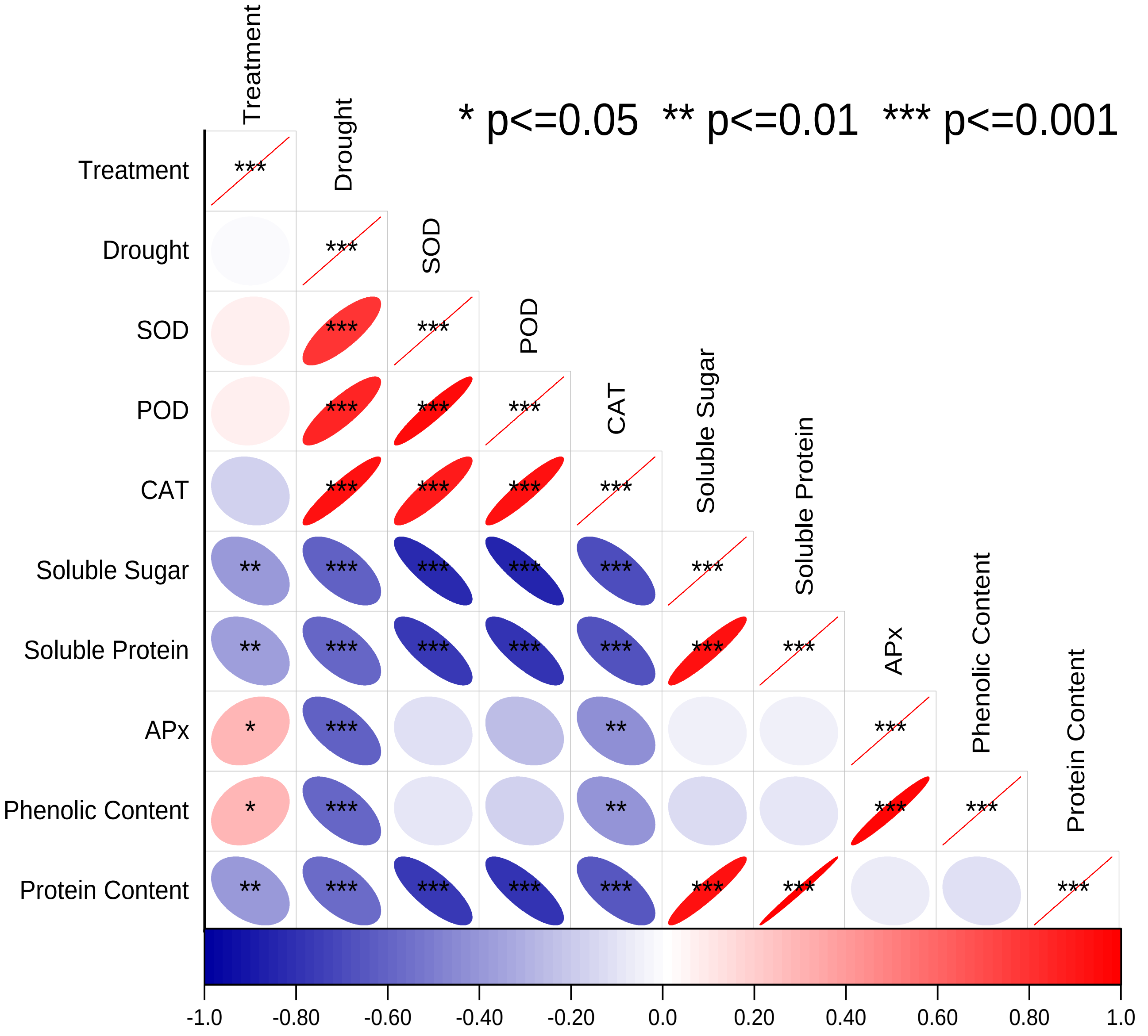

Fig. 5. Pearson correlation between treatments, drought, and maize varieties antioxidants attributes. The intensity of colour shows the strength of correlation. The red colour indicates the positive and the blue colour shows a negative correlation.

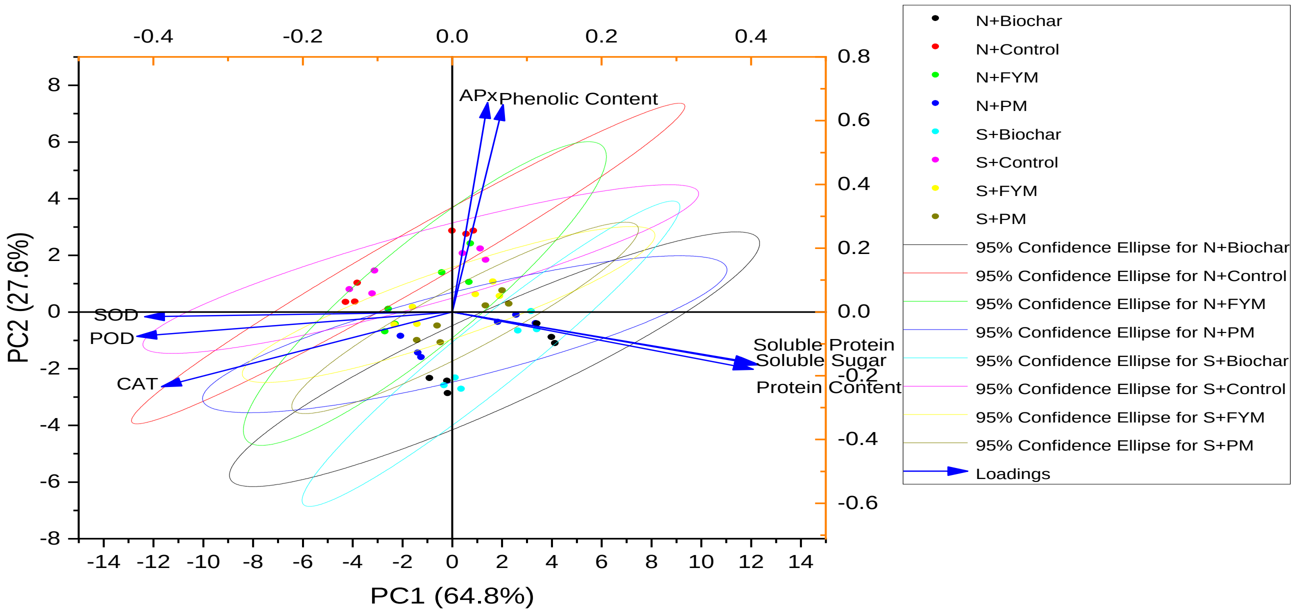

Fig. 6. Principal component analysis for studied attributes of maize varieties by using treatments as observations and drought as a group. Arrows are showing the loadings at 95% confidence. Studies 8 attributes share 92.4 principal component scores.

Pearson correlation showed that the effect of drought was significant negative in correlation with soluble sugar, soluble protein, phenolics contents and proteins contents of maize varieties (Fig. 5). However, it was significantly positive in correlation with the CAT, POD and SOD. The application of treatments was significantly positive in correlation with protein and sugar contents. Principal component analysis showed that APx and phenolics were close in contact with each other. Attributes SOD, POD and CAT were opposite in direction and far apart from soluble sugar, soluble protein and protein contents of maize varieties (Fig. 6).

Discussion

Agricultural land degradation and water resource depletion bring worldwide attention towards using BC, FYM and PM for conserving the soil and water in arid and dry regions. A reduction in water availability severely affects the functioning of plants by disturbing the plant's physiological activities (Abideen *et al*., 2020). Water deficit increases the ROS stress in plants by increasing the CAT, SOD, APx, POD and phenolic compounds production (Sharma *et al*., 2012; Abideen *et al*., 2020). In the study, it was observed that antioxidants production (CAT, APx and SOD) was increased significantly in both droughts tolerant and sensitive varieties. A similar response was also observed by Cia *et al*., (2012) that drought-tolerant varieties exhibited greater CAT and APx contents than drought-sensitive varieties (Cia *et al*., 2012). Plants having greater drought tolerance level can accumulate greater antioxidants in them, allowing greater tolerance against stresses, whilst reduced production of antioxidants in plants indicate the plant sensitivity towards (drought) stress (Anjum *et al*., 2016). These ROS produce hydrogen peroxide (H_2O_2), which interferes with plant physiological activities upon further production of OH. Uncharged OH further reacts with membranes and leads to the formation of malondialdehyde compounds. Phenolic compounds are well known for their antioxidant activity and distinctly increased phenolic contents under drought stress proved its role in the prevention of drought stress ROS damage (Bourgou *et al*., 2010; Caliskan *et al*., 2017) and a similar result was also observed in the present study.

Under drought conditions, elevation in the phenolic compounds leads to an increase in the accumulation of soluble sugars in the maize tissues, as also reported by Gharibi *et al*., (2016). Previous research showed that phenolic acid regulates the accumulation and translocation of carbohydrates from source to sink. For instance, an increase in carbohydrate contents in the source of the sink, under elevated CO_2, leads to elevation of phenolic contents in the plants (Herrmann & Weaver, 1999; Gharibi *et al*., 2016). The result was also in line with Mohammadkhani and Heidari (Mohammadkhani & Heidari, 2008a), who observed that soluble sugar contents were increased 1.18-1.90 times in two cultivars of maize. Studies showed that drought stress induces the conversion of carbohydrates (starch and sucrose) and hexose into alcohols and proline. Sugars protect the cell from drought damage either by substituting its hydroxyl group for water or it forms a biological glass in the dehydrated plant cell (Leopold *et al*., 1994).

Protein and soluble protein contents in the maize tissues were increased under drought stress. More protein and soluble protein contents were observed at 100% FC than 50% FC which confirmed that drought stress reduced the total proteins and soluble protein contents in the maize tissues, and results were consistent with Mohammadkhani and Heidari (Mohammadkhani & Heidari, 2008b). Alharby & Fahad (2020) reported that drought stress increased the metabolism of carbohydrates and proteins. Reduction in plant proteins can occur due to three reasons (1) reduced assimilation of N (Bradford & Hsiao, 1982), (2) increased protein degradation due to oxidative stress (3) reduced production of proteins under drought stress.

In the present study, drought stress increases the activity of antioxidants and phenolic compounds accumulation under control conditions, whereas the application of biochar, FYM, and poultry manure reduced the phenolic contents in the maize tissues. However, study results showed that the application of biochar, FYM and PM reduced the drought stress on plants, which was reflected by reduced antioxidant activity. Similar results were reported by Paryan *et al*., (2012), that application of FYM improved the growth of maize under water scarcity. The application of biochar significantly reduced the CAT, APx and SOD activity in the maize by increasing the leaf solutes accumulation in the leaves due to less water potential under water stress. This may prevent dehydration of plants and thus regulate the uptake of water and water consumption by plants, as reported by Akhtar *et al*., (2014) in maize. Biochar has a hydrophilic surface area and residual pores, which improve the water holding capacity of soil and reduce water evaporation (Nadeem *et al*., 2017). In the present study, the application of amendments like FYM, biochar, and PM were increased the proteins and soluble protein contents in the maize tissues. Similar results were observed by Alharby & Fahad (2020) that biochar application under drought stress improves the proteins and total proteins contents in the maize, which alleviates the harmful effect of stress, thereby maintaining the turgor pressure of the cell (Alharby & Fahad, 2020). Parian *et al*., (2012) reported that FYM application under drought stress improves plant growth, reduced electrolyte leakage and proteins contents in the maize tissues and grains. Biochar, FYM and PM are also enriched with mineral nutrients, which promote root hair production and increase the root exploration area of plants for uptake of water (Farhad *et al*., 2011; Paryan *et al*., 2012; Abideen *et al*., 2020). Biochar application provided significantly improved results than FYM and PM, which could be due to its more ability to retain nutrients than others. Moreover, biochar also improves soil hydro-physical properties (Agbna *et al*., 2017; Singh *et al*., 2019).

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