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Original Article

Scrutinizing morpho-physiological parameters in drought resilient varieties of maize

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Abstract

Drought stress is one of the most relevant abiotic stress factors globally, greatly reducing the crop productivity of agricultural crops. Maize globally is an important economic cereal crop, and it is sensitive to water deficit, which is an important problem of the semi-arid areas of Pakistan. Since to test them under drought stress, the current research aimed to investigate different morphological, physiological, and biochemical traits linked to drought tolerance in maize genotypes and conducted in the laboratory of the Department of Agronomy, Faculty of Agriculture, The University of Poonch, Rawalakot, Azad Jammu and Kashmir, Pakistan. Currently two maize varieties TP-1217 and Fakhr-NARC were exposed to different PEG (5% and 10%) to study their performance. The results show that root fresh weight was higher (1.76g) in Fakhr-NARC at PEG 5% that was followed by TP-1217 with 1.72g. The shoot fresh weight of 1.63g and 1.76g observed under PEG 5%. The photosynthesis rate of 17.66% in TP-1217 and 20.32% in Fakhr-NARC under PEG 5% was observed which was significantly reduced to 11.76% and 18.33% at PEG 10%. The chlorophyll contents of 7.15 and 13.69 were observed in PEG 5% which was further reduced to 4.79 and 9.1 at PEG 10%. The relative water contents (26.74% and 43.02%) were higher in TP-1217 at both PEG concentrations. However, the sugar (38.57), proline (52.22mgg^{-1}, 694.75mgg^{-1}), and antioxidant enzymatic activities were significantly higher in Fakhr-NARC at PEG 10%. Whereas membrane integrity (18%), H_2O_2 (18.2) and MDA (37.99) contents were higher in TP1217.

Keywords: Antioxidant enzymes, Chlorophyll, Drought stress, Maize, Polyethylene glycol

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Introduction

Maize (*Zea mays* L.) is considered as "king of cereals" for its high yield potential and higher genetic range. (Kaul et al., 2011). It is grown on an area of 118 million hectares with 600 million metric tons' production annually which contributes 2.7% value added to agriculture and 0.5% to gross domestic products in Pakistan.

Maize crop is used as a feeder and food for animals and human as well as in drug and textile industries. Maize is composed on about 10% protein, 66.7% starch, 3% sugar, 8.5% fibres, 1.7% ash and 4.8% oil (Hokmalipuore, 2010). Ethanol is produced from maize which is used as a biofuel. As water has vital role in physiology of plants so its stress disturbs many developmental processes of plants that includes growth and development of plant organs and production of flowers and grains. Closuring of stomata due to water stress results in reduction of photosynthesis and water use efficiency of plants (Uhart, 1995). Harmful effects of water stress on the formation of seedlings as well as vegetative and reproductive stages of the maize plants are also reported. Maize also shows fast growth due to C4 mode of carbon fixation that increases the demand of phosphorus (Ali et al., 2015). Change in the global climate and increase in temperature due to deforestation and anthropogenic activities resulting drought conditions by plants (Characterizing drought stress and trait influence on maize yield under current and future conditions), which cause reduction in crop plants specifically in maize (25% to 30%) reduced was recorded by Ben-Ari et al., 2016. Increase in root length at seedling stage under water stress is useful for maize hybrids to adapt in water deficient conditions (Aslam et al., 2013). High demand of water for irrigation and other water usage (non-agricultural) also reduces the water resources for future use (Biswal et al., 2012).

Gradual increase in reduction of water resources would be very crucial for agricultural sector as for the growth and development of early seedlings water availability is essential and unavailability of water at this stage stops cell elongation and expansion that results in reduced seedling growth (Shao et al., 2008). In maize, cell division in meristem of primary roots is also affected by water stress that leads to decrease in the length of all the tissues of meristem. Cell growth and elongation is highly sensitive to water stress (Sacks et al., 1997). Under water stress conditions the antioxidant-enzymes such as peroxidase, superoxide-dismutase, catalase, glutathione-reductase, flavonoids, carotenoids, ascorbic acid and α-tocopherol have a vibrant role in plant growth (Abu-Ria et al., 2024; Sgherri et al., 2000).

Polyethylene glycol (PEG-6000) can be applied to uncover the tolerant and sensitive identity of crop plants. The use of PEG is one of the best methods of applying drought stress which can be used in drought prone areas while; on the other hand, it can perform well in breeding programs for the development of drought resistant varieties. PEG (6000) is more efficient for water potential which cannot be absorbed by plants (Mustamu et al., 2023; Dani and Siswoyo, 2019; Magar et al., 2019; Verslues et al., 2006).

The Rawalakot Azad Jammu and Kashmir being rainfed in nature needs more attention to develop and evaluate drought tolerant varieties. Under the scenario of climate change the crop may encounter drought stress at any growth stage when it is grown under rainfed conditions. Maiz which is the important crop and is used as a fodder and feeder and other food manufacturing products therefore, this study was evaluated to find out the morphological and physiological mechanism under drought stress.

Material and Methods

Plant materials and growth conditions

The experiment was conducted at the laboratory of Department of Agronomy, Faculty of Agriculture, The-University of Poonch, Rawalakot, Azad-Jammu and Kashmir. The seeds of maize genotypes viz., TP-1217 and Fakhr-NARC were collected from National Agriculture Research Center (NARC), Islamabad. The seeds were treated with hydrogen peroxide, washed with distilled water and grown in moist-filter paper. The uniform healthy seedling with two true leaves stage were transferred to 1.5-liter pot filled with basal nutrient solutions for initial growth and development. After two weeks of transplantation, stress was applied. The experiment comprised of the following treatments.

T_1 = Control (Basal Nutrient Solution + 0%PEG)

T_2 = 5% PEG (Basal nutrient solutions (BNS) + 5%PEG)

T_3 = 10% PEG (Basal nutrient solution (BNS) +10%PEG)

The experiment was carried out in completely-randomized-design (CRD) with three-replications.

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The pH of basal-nutrient-solution was adjusted to 5.8 ± 0.1 with HCl or NaOH as necessary. The basal nutrient solution in the pot was changed every five days and constantly aerated with the help of aeration pump. Data was taken after seven days of treatments application for Root/Shoot fresh and dry weight (g), Root and Shoot length (cm), Gas exchange, Relative water content (%), Membrane stability index (%).

Determination of chlorophyll contents

To determine the chlorophyll contents the described protocol of Ali et al. (2021) was followed. A fresh sample of 25mg from the leaves was diluted in 100% of 5 mL methanol. The agitated solution was then kept overnight. The supernatant was then observed at an absorbance of 470nm, 653nm and 666nm. The chlorophyll *a*, *b* and crotonids were quantified according to Hartmut and Alan, 1983. The total soluble protein was determined according to Bibi et al., 2019 protocol. The leaf water contents were determined according to the following formula as suggested by Redondo-Gomez et al., 2011.

$$WC (\%) = (FW-DW/FW)100$$

Whereas FW is fresh weight of leaf and DW is the dry weight of leaf.

Determination of membrane stability

To determined membrane stability two leaves (0.2 g) were taken before the termination of the experiment and rinsed in 20 mL distilled water. Then, both flasks were placed in a hot water bath to take two electrical conductivities readings, first at 40 °C after 30 min (C1) and second at 100 °C after 15 min (C2) (Sairam et al., 2005).

$$MSI=[1-(C1/C2)]100$$

Gas exchange

To determine gas exchange from the leaves, three fully expanded leaves from both varieties under control and treatments were selected and their photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO_2 were measured using CIRAS-3 portable open-flow gas exchange system (PP Systems, Amesbury, MA, USA) between 10 a.m. to 2 p.m.

Determination of antioxidants enzymatic activities

The antioxidant activities in term of SOD, POD, APX and CAT were determined. The activity of SOD was measured according Giannopolitis and Ries, 1977, the POD activities were measured using the describe protocol (Cakmak et al., 1993). However, Nakano and Asada (1981) designed protocol was used to determine the APX and CAT activities. The Glutathione activities were determined according to the protocol designed previously (Khan et al., 2020).

Statistical analysis

All morphological and biochemical analysis was carried out for each treatment. Statistical analyses were done using the statistical software Statistix 8.1. Means were subjected to analysis of variance (ANOVA) and compared by using least significant-difference at 5%-level of probability.

Results

Root/Shoot fresh and dry weight

Root fresh weight increased 1.76g in Fakhr-NARC at PEG 5% while the lowest 1.52g was investigated at 10% of PEG, while the root fresh weight of 1.72g TP1217 was observed in control plants. The values of both varieties TP1217 and Fakhr-NARC were observed 1.16g and 1.76g at the treatment of PEG 5% (Figure 1A). Shoot fresh weight in control plants of TP1217 and Fakhr-NARC was 1.91g and 1.89g observed while it was decreased under PEG treatments. Under 10% PEG the reduction of 1.33g and 1.65g while it was 1.63g and 1.76g observed under PEG 5% (Figure 1B). Root dry weight 0.79g was observed in the control plants of TP1217 which was reduced to 0.23g at PEG 5% and 0.04g at PEG 10%, while in control Fakhr-NARC was 0.97g that declined to 0.65g at PEG 5% and 0.58g at PEG 10% (Figure 1C). Shoot dry weight in TP1217 and Fakhr-NARC was 0.51g and 0.56g in untreated plants respectively while it was significantly reduced to 0.23g and 0.43g at PEG 10% accordingly. However, under PEG5% a 0.39g and 0.49g root weight was observed (Figure 1D). Root length of 13.17cm in untreated TP1217 at 10% PEG and in that was reduced to 9.93cm at PEG 10% However, Fakhr-NARC the root length of 22.07cm was investigated at PEG 10% while the lowest value 19.77cm was observed in at PEG 5% (Figure 1E). Plants shoot length of 7.77cm and 8.47cm was observed in TP1217 and Fakhr-NARC respectively. The 5.83cm and 6.9cm shoot length were observed in TP1217 and Fakhr-NARC at the treatment of PEG 5% respectively (Figure 1F).

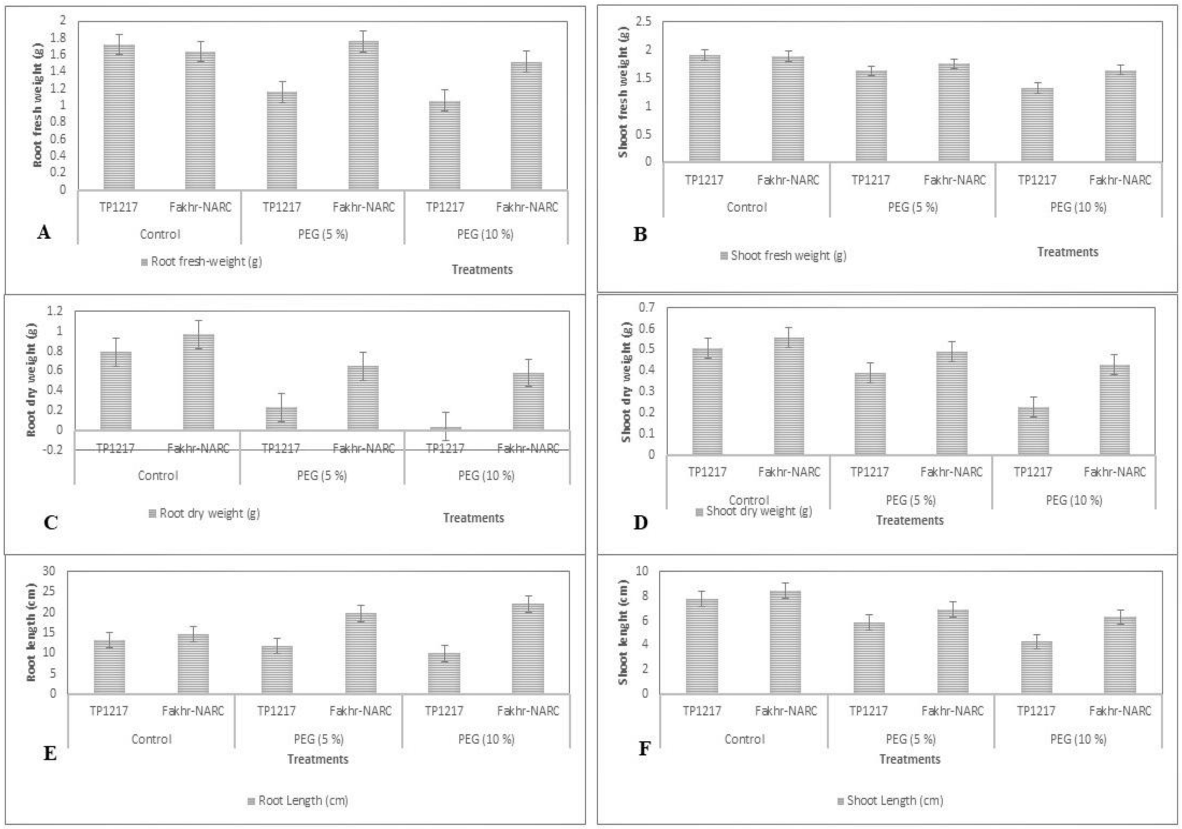

Figure-1. Effect of different concentrations of drought stress on the roots/shoots of maize cultivars. A) Root fresh weight (g), B) Shoot fresh weight (g), C) Root dry weight (g), D) Shoot Dry weight (g), E) Root length (cm), F) Shoot length (cm). Data are the mean of three replications and the level of significance were calculated with $p \leq 0.05$.

The findings of this experiment are very similar to the previous observed results of Root/Shoot fresh and dry weight where the researchers obtained that drought stress affected the total fresh weight in all the cultivars of maize crop, while the drought application caused increases in the total dry weight in all the cultivars of maize. Shoot fresh and dry weight of the maize crop increased dramatically. Low concentration of dilution advanced the fresh and dry biomass due to the production of root hairs as compared to other levels. Maximum root dry weights at the higher concentration are due to the greater number of roots with the little root hairs (Naeem et al., 2017; Doğru, 2016; Kusaka, 2005; Maqbool, 2017). The results from roots fresh weight suggested that photosynthetic activity reduces due to water stress because of imbalance light reduction of intercellular carbon dioxide is due to closing of stomata that stops the supply of carbon dioxide to RUBISCO. The limitation of photosynthesis under drought stress through metabolic impairment is a more complex phenomenon than the stomatal limitation. Changes in the cellular carbon metabolism are likely to occur in the early stage of dehydration process. The rate of photosynthesis in higher plants which depends on the activity of rubisco as well as the synthesis of the RuBP (Sukiasyan, 2016; Reddy, 2004a; Lavinsky et al., 2015; Flexas et al., 2004).

Gaseous exchange

Gaseous exchange includes stomatal-conductance, transpiration-rate, photosynthesis-rate and carbon dioxide-concentration, also reduced in both PEG treatments. Photosynthetic rate in both varieties

TP1217 and Fakhr-NARC were observed with high values of 21.67% and 23% in control respectively; while under 5% it was 17.66% in TP1217 and 20.32% in Fakhr-NARC under PEG 5% which was significantly reduced to 11.76% and 18.33% at PEG 10%. (Figure 2A). Transpiration rate 4.23% and 4.53% in untreated plants was observed that was significantly reduced to 3.23% and 3.77% after PEG 5% while by increasing the concentration of PEG to 10% a significant reduction of 2.67% and 3.63% in transpiration rate was observed in both TP1217 and Fakhr-NARC respectively. Overall, these results indicate that the reduction of transpiration rat in TP1217 was more as compared to Fakhr-NARC (Figure 2B). Stomatal conductance (0.1, 0.13) and intercellular carbon dioxide (264.67, 243.67) was also significantly reduced by increasing PEG concentration dose dependently (Figure 2C, D).

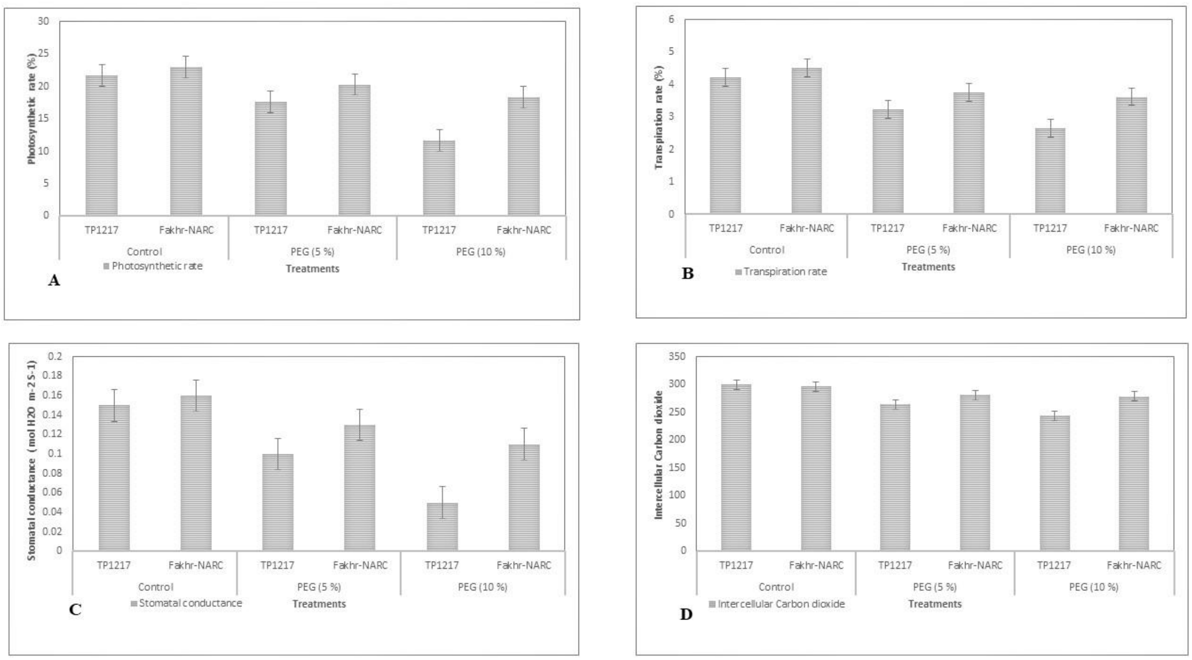

Figure-2. Effect of different concentrations of drought stress on the gaseous exchange of maize cultivars. A) Photosynthetic rate, B) Transpiration rate, C) Stomatal conductance, D) Intercellular carbon dioxide. Data are the mean of three replications and the level of significance were calculated with *p≤0.05.*

Chlorophyll contents

Chlorophyll-a contents in both varieties TP1217 and Fakhr-NARC were observed with high values of 12.35 and 14.13 at the control while 7.15 and 13.69 was observed in PEG 5% which was further reduced to 4.79 and 9.1 at PEG 10% **(**Figure 3A). Under control Chlorophyll-B contents of 5.66 and 5.33 at control were observed, while 3.83 and 4.5 at PEG 5% and 2.49 and 3.83 in TP1217 and Fakhr-NARC of both varieties at PEG 10% (Figure 3B). Carotenoids contents in both varieties were observed with high values of 7.41 and 8.31 at control while lowest values 4.54 and 7.54 were observed at PEG 5% and 2.7 and 6.03 at PEG 10% (Figure 3C).

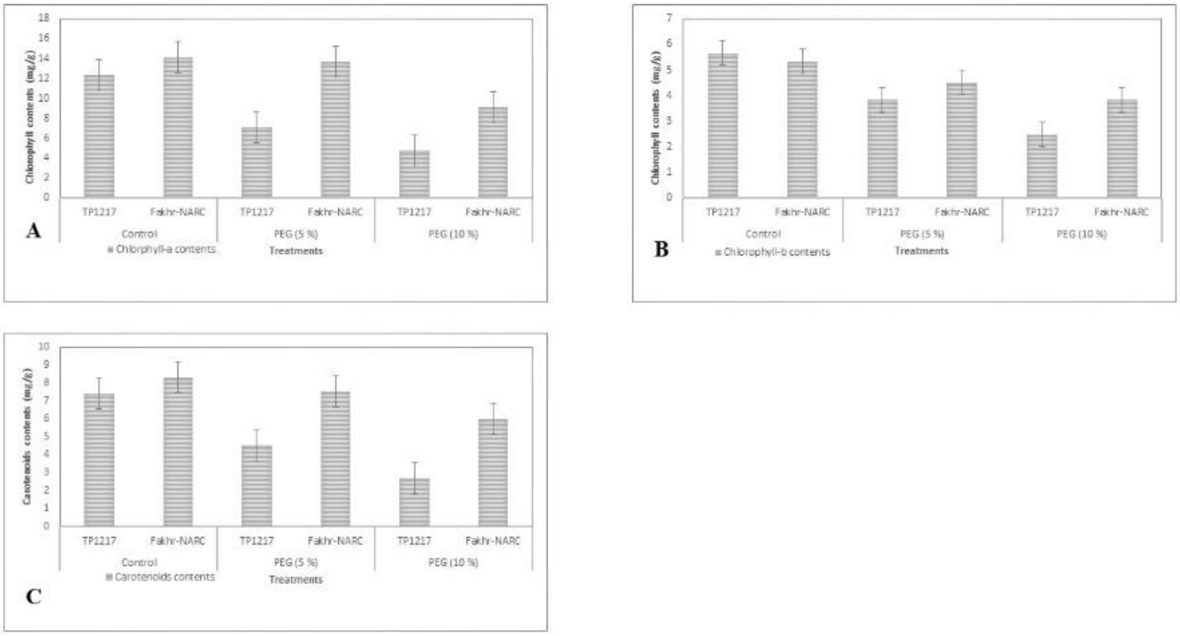

Figure-3. Effect of different concentration of drought stress on the chlorophyll contents of maize cultivars. A) Chlorophyll-a contents, B) Chlorophyll-b contents, C) Carotenoid contents. Data are the mean of three replications and the level of significance were calculated with *p≤0.05*.

Relative water contents (%)

Reduction in relative water contents (%) occurs more in drought susceptible than the resistant cultivars. In the current study, Fakhr-NARC showed 4.29% relative water contents under PEG was 5% while 13.1% loss at PEG10%, while 26.74% and 43.02% loss in relative water contents was observed at PEG 5% and 10% respectively in variety TP1217 (Figure 4A). Membrane stability index (%) was also reduced in PEG treatments in both the cultivars but variety Fakhr-NARC showed 18% (Figure 4B). The high amount of proline (61%) contents in Fakhr-NARC was observed under PEG treatments while the lowest values 0.53% and 0.57% at control (Figure 4C). Hydrogen peroxide contents (nmolg^{-1}) increased with the increasing the concentration of PEG from 5% (8.8 and 5.6) while at PEG 10% it increased to 18.2 and 9.9 in TP1217 and Fakhr-NARC respectively. (Figure 4D). Sugar contents in both TP1217 and Fakhr-NARC were observed higher (27.9 and 38.57) at PEG 10% whereas it was 24.23 and 30.57 at PEG 5% (Figure 4E). Protein contents (mgg^{-1}) also increased with enhancing the PEG concentration from 5% to 10%. The highest protein contents of 52.33 mgg^{-1} and 62.33 mgg^{-1} were observed at PEG 10% while 42.33mgg^{-1} and 50mgg^{-1} at PEG 5% (Figure 4F).

Malondialdehyde (MDA) contents in both varieties were observed with more contents of (37.99 and 26.68) under PEG 10% in TP1217 and Fakhr-NARC respectively; whereas it was 31.56 and 21.83 at PEG 5% however, in control plants MDA contents were reduced to10.46 and 8.9 in both varieties respectively (Figure 4G).

Enzymatic activity

Catalase activities (CAT) at both concentration of PEG was observed in both varieties TP1217 and Fakhr-NARC. The highest CAT activities of 1.87 and 3.11 at PEG 10% were observed that was followed by 1.17 and 2.31 at PEG 5% (Figure 5A). Like CAT the activities of POD (0.44,1.12) and SOD (438.75, 694.75) were also higher at PEG 10% in TP1217 and Fakhr-NARC respectively. However, at PEG 5% POD was 0.42 and 0.44, and SOD was 152.18 and 170.67. (Figure 5B and C). Glutathione reductase activity in both varieties TP1217 and Fakhr-NARC were observed with high values of 1.57 and at PEG 10% and 1.16 and 1.53 were observed at PEG 5% (Figure 5D). Guaiacol peroxidase activity (7.94) in TP1217 was found PEG 10% and the guaiacol peroxidase activity of variety Fakhr-NARC was found with high values 4.28 at PEG 5% treatment (Figure 5E). Ascorbate peroxidase (APX) in both varieties TP1217 and Fakhr-NARC were 16.27 and 26 At the treatment PEG 10% and 15.31, 21.41 were found at the treatment PEG 5% (Figure 5F).

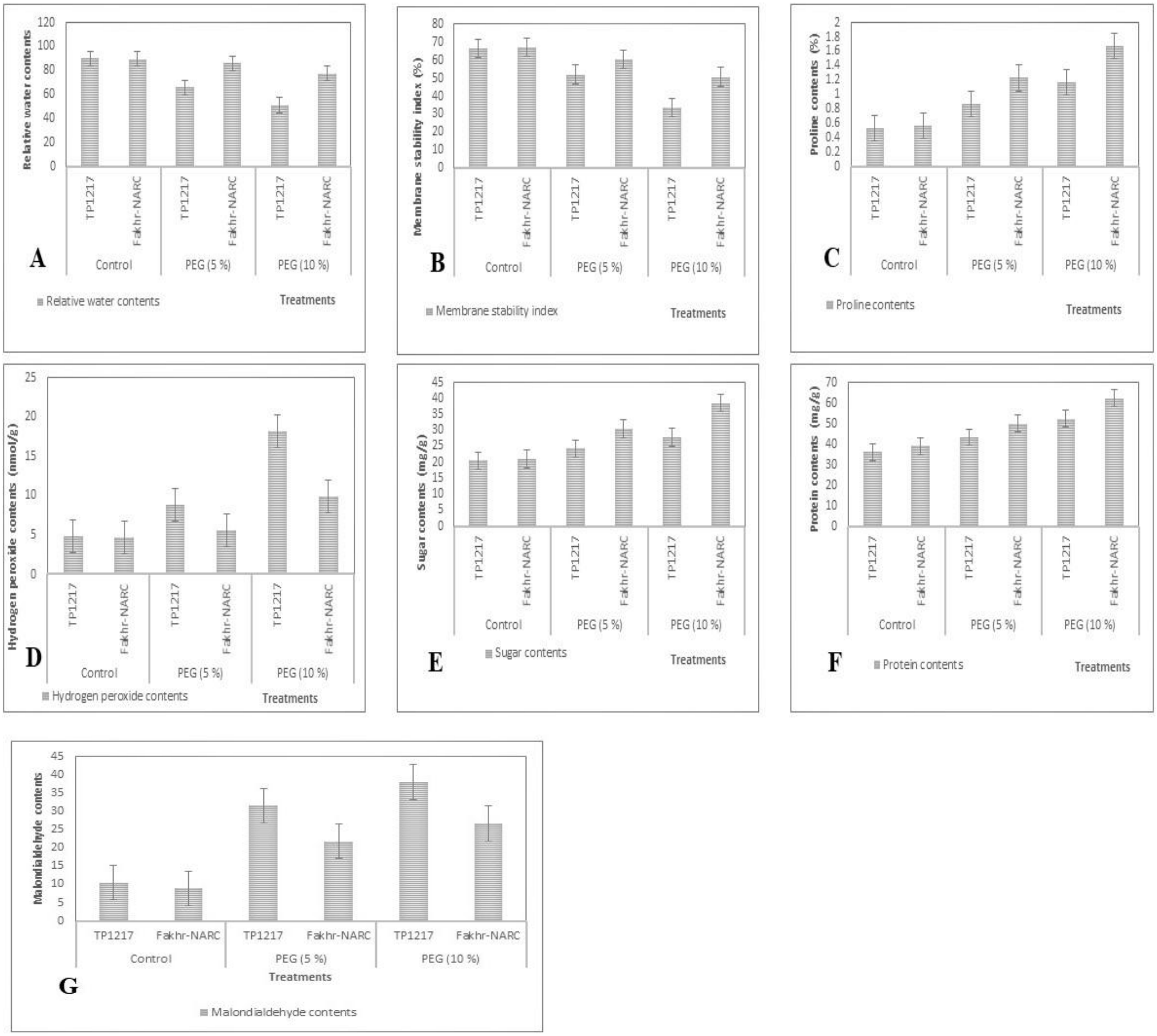

Figure-4. Effect of different concentration of drought stress on the relative water contents of maize cultivars. A) Relative water contents B) Membrane stability, C) Proline contents, D) Hydrogen peroxide, E) Sugar contents, F) Protein contents, G) Malondialdehyde contents. Data are the mean of three replications and the level of significance were calculated with *p≤0.05.*

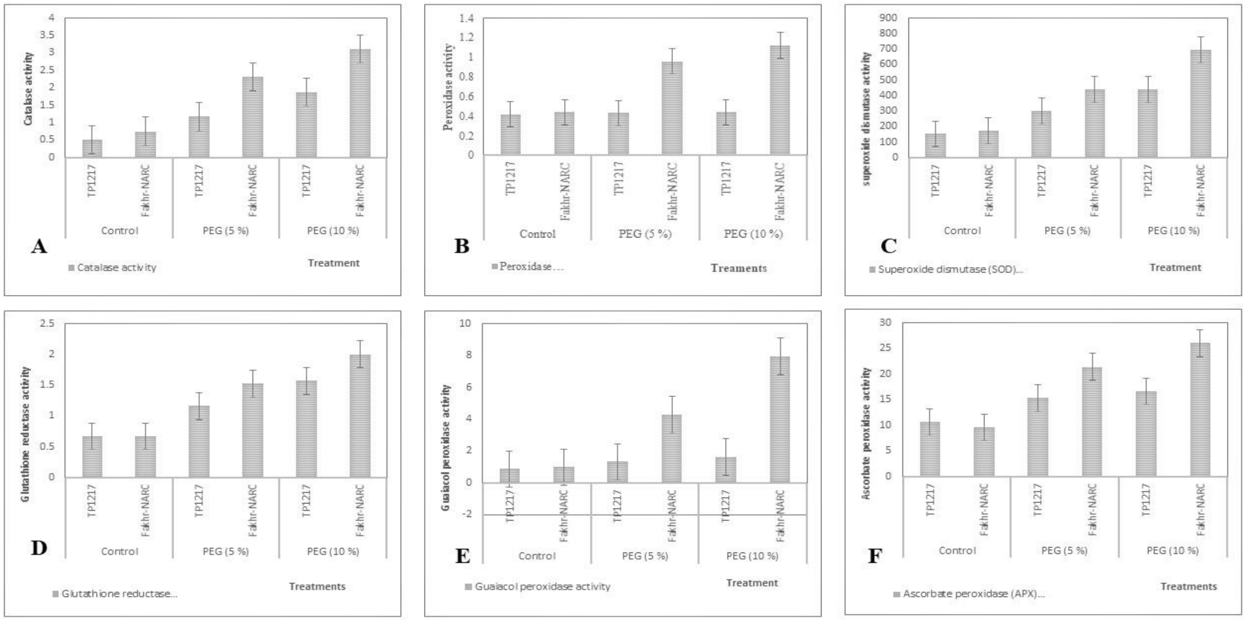

Figure-5. Effect of different concentrations of drought stress on the antioxidant enzymatic activities of maize cultivars. A) Catalase activity, B) Peroxidase activity, C) Superoxide dismutase activity, D) Glutathione reductase activity, E) Guaiacol peroxidase activity, F) Ascorbate peroxidase activity. Data are the mean of three replications and the level of significance were calculated with *p≤0.05*.

Discussion

Drought stress is one of the most serious problems which is created due to the scare water supply to the root zone that affect the morphological and physiological attributes of plants (Haghpanah et al., 2024). The most important consequences which are passed by plants is the osmotic imbalance, dehydration, impaired photosynthesis and oxidative stress. the results from the current study revealed that the fresh and dry weight of root and shoot are reduced in both tolerant and sensitive varieties, but the reduction is more significant in the sensitive as compared to the tolerant these results are in the line with the finding of (Ward et al., 1999). It was stated that the Chlorophyll contents (µg g^{-1}) were reduced in the sensitive varieties as compared to tolerant because it may be due to the high production of ROS (O_2 and H_2O_2) which may induce the high production of lipid peroxidation and finally results disturbance in chlorophyll contents (Luo et al., 2023). On the other hand these results are also same with the findings of Younis (2000) and Rahman et al. (2004), who stated that water stress decreases the chlorophyll a, chlorophyll b, total chlorophyll and carotenoids.

Proline contents (nmol g^{-1}) in maize cultivars tend to increase under PEG treatments and its concentration is higher in drought tolerant cultivars, our these results are in line with the finding of (Reddy, 2004b; Cetinkaya, 2014; Lama, 2016). Proline, soluble sugar and soluble proteins reduced water potential of plant cells in drought conditions that prevents dehydration of cells and prevent normal plant growth (Ozturk et al., 2021). The contents of hydrogen peroxide in the treated plants in the sensitive plants were induced by increasing the concentration from PEG 5% to 10%. These results are in line with the findings of Chung (2011), who stated that in drought sensitive crops produce more contents of hydrogen peroxide than in tolerant crop due to inability of scavenging enzymes peroxidase and catalase to breakdown in the susceptible cultivars. The activities of CAT, POD, SOD, APX, GRA and GPA were increased in tolerant variety as compared to the sensitive however, these activities were significantly increased by increasing the PEG from 5% to PEG 10%. The increase in the activity of antioxidant enzymes detoxify the excess amount of ROS (reactive oxygen species) which maintain the balance between the formation and removal of ROS under stress conditions (Singh et al., 2021; Hasan et al., 2018). MDA contents in the current experiment were significantly higher in the sensitive variety as compared to the tolerant one. The MDA contents in the sensitive variety were significantly increased by increasing the contents from PEG 5% to PEG 10% significantly these findings are in agreement with the results of Castillo et al. (2008), who stated that the heigh amount of MDA is an oxidative feature which break the membrane damage under drought stress.

Glutathione reductase activity was also increase in the resistant variety as compared to the sensitive. These findings are same with results of Lama (2016) and Cetinkaya (2014), who observed that glutathione reductase activity increased under drought stress conditions but significantly increase was noticed in the drought resistant cultivar.

Conclusion

The results revealed that under drought stress the variety Fakhr-NARC showed a bitter growth in terms of fresh and dry weight under PEG 10%. However, the chlorophyll contents, water contents, membrane stability were also increased under PEG 5%, whole MDA contents were higher in TP1217 at 5% PEG. The hydrogen peroxide contents were also higher in TP1217 at PEG10%. However, there was no significant changes observed in stomatal conductance and intracellular spaces in Fakhr-NARC at both concentrations of PEG. The sugar and proline contents were higher in Fakhr-NARC; however, the contents of hydrogen peroxide and antioxidant activities were higher in Fakhr-NARC at PEG10%. These results indicate that under drought conditions Fakhr-NARC perform well even at higher drought. The variety Fakhr-NARC showed the better results in all the studied parameters. From the current study it is concluded that further study is needed to uncover the molecular mechanisms in drought tolerant variety.

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Contribution of Authors

Zakaria M: Conceived idea, designed and performed the experiments.
Hussain S & Ali E: Supervised study and wrote the initial draft of the article.
Shah JM & Raza SH: Data analysis and interpretation.
Wazir M, Ali A & Shehzad M: Reviewed literature, critiqued and edited the final draft of the manuscript.

All authors read and approved the final draft of the manuscript.

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