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ENHANCING DROUGHT STRESS TOLERANCE IN MAIZE CROP THROUGH PGPR INOCULATION: ANALYSIS OF MORPHO-PHSIOLOGICAL AND BIOCHEMICAL ATTRIBUTES

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Abstract

Water scarcity is primary abiotic components brutally impede the economically important plants growth and yield world widely. Maize is the main cereal crop, ranked second after wheat cultivated for food, feed and bioenergy production purposes. The primary objective of this investigation is to access the impact of drought stress on biochemical, photosynthetic, and gas exchanging attributes of maize crop by plant growth promoting bacteria (PGPR) application. A pot-based investigation was conducted according to factorial CRD (completely randomized design) with 4 repeats. Seeds of five maize varieties were chosen and planted in loamy nutrient rich soil. Experiment was conducted using plant growth promoting rhizobacteria (PGPR) under control (D1) and drought (D2=50% field capacity). The four treatments included, D1P1 (no drought stress+ no PGPR), D1P2 (no drought stress+ PGPR), D2P1 (drought stress+ no PGPR) and D2P2 (drought stress+ PGPR). Results demonstrated that drought stress decreased all studied morpho-physiological characteristics and increased oxidative stress indicators while applying PGPR increased all the attributes such as dry weight of shoot, root, total phenolics, carotenoids, chlorophyll *a*, chlorophyll *b*, TSP (total soluble proteins), CAT, SOD and POD, ascorbic acid and gas exchange attributes and lower transpiration rate and play a pivotal role in scavenging ROS under drought stress. PGPR also lowers the lipid peroxidation and membrane degradation by reducing MDA and H_2O_2 levels in PGPR treated plants. These results suggested that PGPR treated maize varieties performed better under drought stress in comparison to non-treated plants. Among all the cultivars examined, FH-1870 performed best under control and drought stress conditions. In contrast, Sahiwal gold cultivar was more sensitive to water deficit condition but upon PGPR application performed best compared to all other cultivars. Therefore, inoculation of maize seeds with rhizobacteria is an effective strategy against drought stress. However further research is needed to explore rhizobacteria potential in different crops under various abiotic and biotic stresses.

Key words: Drought stress; Maize; PGPR inoculation; Antioxidants; Photosynthetic pigments; Gas exchange attributes

Introduction

Drought stress is a considerable devastating abiotic environmental stressor that can hinder the development of a variety of plants, specifically in a dry and semi-dry environment (Abdelaal *et al*., 2021a). Drought has an impact on agricultural production as well as crop development and growth. The sharp decline in food production is caused by the yearly increase in both the severity and frequency of droughts. Over the past 50 years, drought stress was estimated to have caused a 10% drop in the grain output. By 2050, productivity losses are expected to occur across over 50% of arable land (Akhtar *et al*., 2021). As reported by the International Food Policy Research Institute (IFPRI) most of the world's major agricultural products, including wheat, maize, and rice, have seen a severe fall. Water shortage has been linked to a 21% global decline in wheat yields and a 40% global loss in maize productivity, according to studies released between 1980 and 2015 (Siddique *et al*., 2022; Pequeno *et al*., 2021). FAO estimates that throughout 2005 to 2015, drought directly inflicted a deficit of 29 billion US dollars to agriculture in developing states (FAO, 2021). In Pakistan, there are around four droughts every ten years, and because of its dry climate, the southern area is more vulnerable to hydrological hazards (Jamro *et al*., 2019). These protracted droughts destroyed over 80% of the area's orchards, decreased food production, and slayed around 2 million faunas (Ahmed *et al*., 2019). According to reports, agricultural plants with insufficient irrigation water have stunted growth and development, stomata closure, and decreased biomass output (Ilyas *et al*., 2020; Marchin *et al*., 2020). Drought stress negatively impact the plant stages and cause numerous physiological changes including a decrease in leaf area, leaf number, chlorophyll content, stem height, and sugar output also decreased RWC (relative water contents) and cause turgor loss (AlKahtani *et al*., 2021; Abdelaal *et al*., 2020b). When a plant experiences drought, one of its initial responses is to close its stomata, which also lowers CO_2 levels and inhibits photosynthesis. In extreme cases, this can result in plant death (Siddiqui *et al*., 2019). Since reductions in water level are followed by a fall in RWC (relative water content), the decline in leaf growth during droughts also negatively impact photosynthesis (Rashwan *et al*., 2020). Furthermore, under water deficient condition, carbohydrate absorption, ion uptake, nutritional assimilation, and respiration are adversely impacted (Hafez *et al*., 2020). Consequently, significant modifications to metabolism, particularly photosynthesis, abrupt rise in the generation of ROS, or elevated radical damage occur (Sabagh *et al*., 2019; Abdelaal *et al*., 2021b). All Plants possess efficient protection pathways to scavenge ROS, these pathways which include antioxidant both enzymatic and non-enzymatic pathways, are crucial for defending mitochondria and chloroplasts from oxidative stress brought on by a range of stressors (Hafez *et al*., 2016; Abdelaal *et al*., 2020a). Like other crops, maize has a range of defense systems to impede the adverse impacts of abiotic stressors. Osmolytes accumulation, synthesis of heat shock protein, expression of stress-responsive genes, that are compatible (sugar, proline (imino acid), and the regulation of enzyme-based antioxidants like catalase (CAT), superoxide dismutase (SOD), and POD (peroxidase) are some of these processes (Sharma *et al*., 2023). Plants may eliminate hydroxyl species and singlet-state oxygen ($^1O_2$) by changing the functions of several antioxidant enzymes and non-enzymes, like phenolics, alpha-tocopherol, and vitamin c (L-ascorbic acid) (Lalarukh & Shahbaz, 2020).

PGPR are naturally existing soil microbes that reside in the rhizosphere and function as agents for biocontrol, biofertilizers, biopesticides, and bioherbicides (Xiong *et al*., 2020). By increasing systematic resistance through enhancing the production of siderophores, proteins, and lipopolysaccharides, which modifies the cell wall composition and enhances the defensive mechanism of plant (Ebrahem *et al*., 2022). Through their secretions, they help with nitrogen fixation and soil phosphorus solubilization by expanding the surface area of plant roots. PGPRs produce a variety of plant hormones, including auxins (also known as IAA), cytokinin, ACC (1-aminocyclopropane-1-carboxylic acid), and gibberellins, and they also inhibit the formation of ethylene by plants and help to attenuate the water stress and increase growth and output (Li *et al*., 2023). PGPR produces primary and secondary metabolites including organic acids, sugars, phenols, amino acid and produce antioxidant enzymes that modified the defense system of plants by reducing the damage inflicted by ROS (Jan *et al*., 2022). Overproduction of ROS altered the enzyme activity, thylakoid membrane

structure and photosynthetic pigments, PGPR elevates the stomatal conductance, photosynthetic rate (P), and efficacy of photosystem and reduces the transpiration rate (*E*) by synthesizing phytohormones that influence the vital functional and structural character of photosynthetic apparatus (Gowtham *et al*., 2022).

Maize (*Zea mays* L.) is the 3rd utmost significant and extensively cultivable C_4 cereal crop across the globe and occupies 197 million hectares of land and contributes to global food security (Djalovic *et al*., 2023). For almost 4.5 billion individuals in 94 developing nations, maize is considered for at least 30% of their daily intake of calories (Kálmán *et al*., 2024). Yield estimates state that 15-20% of maize's yearly grain output to be lost due to drought degradation and crop loss may increase as drought spell become more severe and frequent due to climate alteration (Adewale *et al*., 2019). In this study, we aimed to investigate the role of PGPR on some physiological and biochemical aspects, gas exchange indices that are used as indicators for drought stress under natural conditions. The purpose of this study was to investigate the potential of selected rhizospheric *Bacillus* species as Plant Growth Promoting Rhizobacteria (PGPR) for their efficiency in ameliorating drought stress during maize growth by changing various attributes and yield. The aim of this study was thus to gain a greater understanding of modulation in antioxidant enzyme activities, pigment levels, gas exchange parameters, total soluble proteins and oxidative stress indicators by applying PGPR, hence their impact on dry biomass of maize. It is tentatively hypothesized that PGPR application could ameliorate the negative impact of drought stress by altering physiological, biochemical and dry matter production of maize cultivars. Additionally, it was expected that some cultivars of maize would respond better to stress conditions because of PGPR treatments, with some cultivars showing increased tolerance to drought stress.

Material and Methods

Growing conditions and research area

A pot-based investigation was conducted during 2023 in the Botany department experimental area, Government College Women University Faisalabad, Pakistan to evaluate the drought stress effect on maize. Seeds of 5 maize Cvs. (Malka 2016, Sahiwal gold, FH-988, FH-5427, FH-1870) were collected from Maize Research Station, AARI, Faisalabad.

In spring 2023 during the maize development period, the weather specifications of the experimental site were as follows average annual rainfall= 100 millimeters (14.8 in), from February to July the average temperature ranged from 20.8 ˚C to 36 ˚C, featuring a lowest of 16.4 ˚C in February and a highest of 43.7 ˚C in May. During this time, there was an average of 21.3 mm of precipitation overall, with variations from 0 mm in July and August to 50.6 mm in June.

Experimental design and field capacity

We chose plastic pots with 10 kilograms soil capacity and filled them with nutrient-rich soil (one part compost, one part loam). Experimental design used was factorial CRD (completely randomized) with four repetitions. Two FC (field capacity) levels were chosen as treatments: the first had 50% of the FC labelled as drought stress and 100% of the FC labelled as control.

PGPR culture and seed priming

The consortium of four bacterial species (*Azotobacter*, *Azosprillium*, *Pseudomonas aeruginosa*, *Bacillus*) was applied to maize seeds before sowing in plastic pots. Healthy maize seeds of 5 varieties were sterilized with ethanol and dipped into PGPR suspension other than control for 30 minutes with continuous stirring using agitator.

Seed propagation and sample assemblage:

Healthy 10 seeds from each maize cultivar treated with PGPR were sown in pot. After sowing PGPR slurry was added into the soil. All pots were irrigated with two different water levels, D1 = control (100% field capacity), and D2 = 50% of the field capacity. Data were collected at V7 stage of maize

(38 days after seed sowing) plants for analyzing physiological, morphological and biochemical attributes.

Morphological characteristics analysis

To find dry weight of root and shoot specimens were stored in paper bags and heated in oven for 48 hours at 70°C (Al-Karaki, 2000).

Chlorophyll content analysis

Using the Arnon (1949) approach, the contents of photosynthetic pigments (carotenoids, *Chl. a*, and *Chl. b*) were assessed. For that purpose, fresh leaf specimen 0.1g were chopped into tiny pieces and soaked in 5 mL of acetone (80%) for 24 hours. The optical density was then measured by operating a UV-VIS double beam spectrophotometer at 480, 663, and 645 nm.

Biochemical attribute analysis

Enzymatic antioxidants

To determine the antioxidant enzyme activity, 0.1 gram of leaf sample was ground in 2mL of 50mM phosphate buffer (7.8 pH) at minimum temperature (4°C) using a pre-frozen pestle and mortar to generate an enzyme extract. The homogenised, ground concoction was centrifuged for 10 minutes at 13,000 rpm. The remaining suspension was kept at -20°C in a different Eppendorf container to assess the various antioxidant enzyme activity (POD, SOD and CAT). An analysis based on proteins was conducted. To measure the enzyme activity of catalase and peroxidase, the Chance & Maehly (1955) approach was applied.

Catalase

To determine Catalase activity, one milliliter of H_2O_2 was put to a cuvette first, followed by 1.9 millilitres of phosphate buffer (5.9 millimolar; pH 7.8); 0.1 millilitres of purified enzyme was infused at the last to initiate the catalase reaction. The aforementioned mixture optical density was recorded at 240 nm every 20 seconds.

Peroxidase

To determine peroxidase activity, in a cuvette combine 100µL H_2O_2 (40mM), guaiacol (20mM) 100µL, and phosphate buffer 750µL. After that, added 100µL of enzyme extract to initiate the process. For three minutes, variation in optical density at 470 nm were recorded every thirty seconds in order to determine activity of peroxidase.

Superoxide dismutase (SOD)

SOD activity level was assessed by using Giannopolitis and Ries (1977) method. To measure the superoxide dismutase activity. NBT (50µL), 50µL riboflavin, 250 microliter (µL) phosphate buffer, 100µL triton-X, 100µL methionine, and 50µL enzyme extract were all included in the reaction mixtures for SOD. For fifteen minutes, the reaction mixture-filled cuvettes were placed under a fluorescent lamp, or "white beam." The mixture's optical density was then observed at 560 nm. A single unit of SOD activity corresponded to a specific quantity of enzyme that was responsible for half of the inhibition of NBT photoreduction.

Hydrogen peroxide

Velikova *et al*. (2000) methodology was utilized to estimate hydrogen peroxide (H_2O_2). For this objective, 0.5g fresh leaf part was grinded utilizing 5mL Trichloroacetic acid (0.1% w/v) and then mixture was centrifuged for 15 minutes at 12000 rpm. Afterward in a test tube, 0.5 mL of phosphate buffer (PB) of (pH 7.8), 1 mL of KI, and 0.5 mL of supernatant were mixed, vortexed, and absorbance was determined at 390 nm.

Malondialdehyde

Carmak and Horst (1991) procedure was used in order to ascertain the malondialdehyde concentration of leaves. 10 mL (0.1% w/v) Trichloroacetic acid and 0.5 g fresh plant leaf were homogenized, and the resultant concoction was centrifuged for 10 minutes at 12000 rpm. A 0.5% solution of TBA (thiobarbituric acid) was made in 20% TCA, and 4 mL of it was poured to 1 mL of supernatant. After that, the concoction was heated at 95°C for half an hour in water bath. After chilling in ice, OD (optical density) was noted at 532 and 600 nm.

Total soluble proteins

Bradford's (1976) technique was used to measure the total soluble proteins. After homogenising 0.1g of fresh plant leaf in a pestle and mortar with 2mL of phosphate buffer (7.8 pH), the mixture was centrifuged for 10 minutes at 13000 rpm to acquire the supernatant. In addition, test tubes were filled with 0.1 milliliters of supernatant and 5 milliliters of Bradford's reagent, vortexed for a short while, and allowed to rest for 30 minutes. Using a spectrophotometer, a reading was documented at 595 nm.

Ascorbic acid

Mukherjee and Choudhri (1983) technique was used to measure ascorbic acid. Fresh plant leaves (0.25g) were grounded with a pestle and mortar in 10 milliliters of 6% TCA, and then at 4ºC centrifuged for 10 minutes at 12000 × g. Using a water bath, thiourea (10% in 70% ethanol) one drop was incorporated to an amalgamation of 4 milliliters supernatant and 2 ml of dinitrophenyl hydrazine (2%) and heated for 20 minutes. After keeping solution containing test tubes in ice, 5 ml of 80% v/v sulfuric acid was poured in at 0ºC. Then at 530 nm mixture's absorbance was estimated.

Total phenolics

To ascertain the total phenolic content Julkenen-Titto (1985) procedure was applied. Freshly picked leaves (0.1 g) blended in 2 milliliters of 80% acetone before being centrifuged for 15 minutes at 10,000 x g. The supernatant was maintained cold at -20°C. A test tube containing 100 µL of plant leaf extract (supernatant) was mixed with 0.5 ml of phenol (Folin–Cicocalteau) and 2 mL of distilled water. After adding 2.5 mL of 20% Na_2CO_3 and 5 mL of distilled water, the concoction was violently shaken for 5–10 seconds. The mixture's absorbance at 750 nm was calculated after 20 minutes.

Gas exchange attributes

Gas exchange attributes were analyzed by using IRGA (infrared gas analyzer). Transpiration rate (E), sub-stomatal (internal) CO_2 concentration, net carbon dioxide assimilation rate and stomatal conductance values of 2nd fully expanded top leaf were measured from 11:00 am to 1:00 pm.

Results

Drought stress drastically decreased ($P \leq 0.001$) the chlorophyll *a* content in maize varieties from lowest to highest percentage reduction as: FH-1870 (20.97%), Malka-2016 (24.03%), FH-988 (25.30%), YH-5427 (31.30%) and Sahiwal gold (39.16%) (Table 1). Application of PGPR considerably ($P \leq 0.001$) enhanced the Chlorophyll *a* content in maize cultivars as compared to no PGPR given plants under drought stress as: YH-5427 (9.19%), FH-1870 (10.4%), Malka-2016 (13.18%), FH-988 (21.51%) and Sahiwal gold (36.52%). Highest ($P \leq 0.001$) overall increase in chlorophyll *a* content was shown by FH-1870 in comparison to all other cultivars (Fig. 1).

Chlorophyll *b* content substantially reduced ($P \leq 0.001$) under drought stress in maize cultivars. The varieties exhibiting the highest decrease were as follows FH-1870 (16.13%), FH-988 (17.09%), YH-5427 (19.33%), Malka-2016 (26.27%), and Sahiwal gold (43.35%). PGPR application considerably ($P \leq 0.001$) improved the chlorophyll *b* in maize cultivars as compared to untreated plants under drought. The percentage increase in ascending order is as: YH-5427 (6.95%), FH-988 (10.72%), FH-1870 (10.79%), Malka-2016 (19.01%) and Sahiwal gold (40.48%). Among all Cvs. FH-1870 showed highest ($P \leq 0.001$) overall increase in chlorophyll *b* content (Fig.1; Table 1).

Table:1 Mean square values from analyses of variance for the photosynthetic pigments, dry biomass and gas exchange parameters of Maize plants grown under PGPR application and drought stress

Source of variation	df	Chl. *a*	Chl. *b*	Total chlorophyll	Carotenoids	Shoot dry weight	Root dry weight
Drought (D)	1	1.413***	24.631***	38.619***	0.0737***	2.102***	0.051***
Plant growth promoting Rhizobacteria (PGPR)	1	0.478***	9.561***	14.114***	0.025***	0.992***	0.029***
Cultivars (Cvs.)	4	0.622***	6.249***	10.144***	0.1161***	3.903***	0.045***
D× PGPR	1	0.0128ns	0.916ns	1.239*	0.0018ns	0.011ns	0.00006ns
D× Cvs.	4	0.044ns	0.382ns	0.474	0.0039***	0.142***	0.0009ns
PGPR × Cvs.	4	0.027ns	0.531ns	0.753*	0.0018ns	0.076*	0.001ns
D× PGPR ×Cvs.	4	0.0197ns	0.844ns	1.101**	0.0023*	0.047ns	0.0014ns
Error	60	0.0253	0.2560	0.2592	0.00076	0.0291	0.0006

Source of variation	df	*A*	*E*	*A*/*E*	C_i	C_i/C_a	g_s
Drought (D)	1	177.281***	12.768***	17.421***	19656.45***	0.331***	0.0045***
Plant growth promoting Rhizobacteria (PGPR)	1	52.342***	5.191***	4.931***	5951.25***	0.077**	0.00128***
Cultivars (Cvs.)	4	80.206***	8.786***	7.483***	5561.668***	0.078***	0.0047***
D× PGPR	1	0.0714ns	0.423ns	0.1987	68.45ns	0.0032ns	0.00024ns
D× Cvs.	4	11.324**	0.147ns	0.147ns	178.35ns	0.00093ns	0.00020ns
PGPR × Cvs.	4	3.922ns	0.225ns	0.2416ns	295.91ns	0.0071ns	0.000095ns
D× PGPR ×Cvs.	4	3.283ns	0.218ns	0.0791ns	359.293ns	0.0072ns	0.00014ns
Error	60	2.4843	0.2778	0.2913	373.0083	0.0104859	0.000094

* = P ≤ 0.05, ** = P ≤ 0.01, *** = P ≤ 0.001, ns = non-significant, df = degrees of freedom
Chl. *a*= Chlorophyll a, Chl. *b*= Chlorophyll b
A= Net CO_2 assimilation rate, *E*=Transpiration rate, g_s=Stomatal conductance
C_i= Sub-stomatal CO_2 concentration, C_i/C_a= Relative internal CO_2 concentration
WUE (*A*/*E*) = Water use efficiency

Drought stress imposition considerably decrease (P≤ 0.001) the total chlorophyll content in maize cultivars. Varieties exhibited the varying level of reduction percentages as FH-1870 (17.20%), FH-988 (18.16%), YH-5427 (21.64%), Malka-2016 (25.75%), and Sahiwal gold (42.79%). Application of PGPR considerably (P≤0.001) enhanced the total chlorophyll content in maize cultivars in contrast to nontreated plants under drought stress as: YH-5427 (7.15%). FH-988 (12.20%), FH-1870 (15.15%), Malka-2016 (18.18%), and Sahiwal gold (40.05%), among all cultivars highest (P≤ 0.001) overall increase was shown by FH-1870 as compared to other cultivars. Significant interaction (P≤ 0.05) exhibited between drought stress (DS) and PGPR, and between PGPR and Cvs. for total chlorophyll contents. Strongest interaction was also observed among drought, PGPR and cultivars (P≤ 0.01) (Fig.1; Table 1).

Obtruding drought stress considerably decreased (P≤ 0.001) the carotenoids content in maize cultivars. Varieties showed percentage reduction in ascending order as, FH-1870 (11.11%), FH-988 (12.5%), Malka-2016 (15.38%), YH-5427 (15.38%) and Sahiwal gold (38.63%) (Table 1). PGPR

application considerably (P≤0.001) elevated the carotenoids in maize cultivars as compared to untreated plants under water deficit condition in order: YH-5427 (4.34%) <FH-1870 (6.97%) < FH-988 (9.67%) < Malka-2016 (12%) <Sahiwal gold (30%). The highest significant (P≤ 0.001) overall increase showed by FH-1870 among all cultivars. The strongest interaction was observed between drought and cultivars. Interaction among drought, PGPR and cultivars was also significant (P≤ 0.05) for carotenoids (Fig. 1).

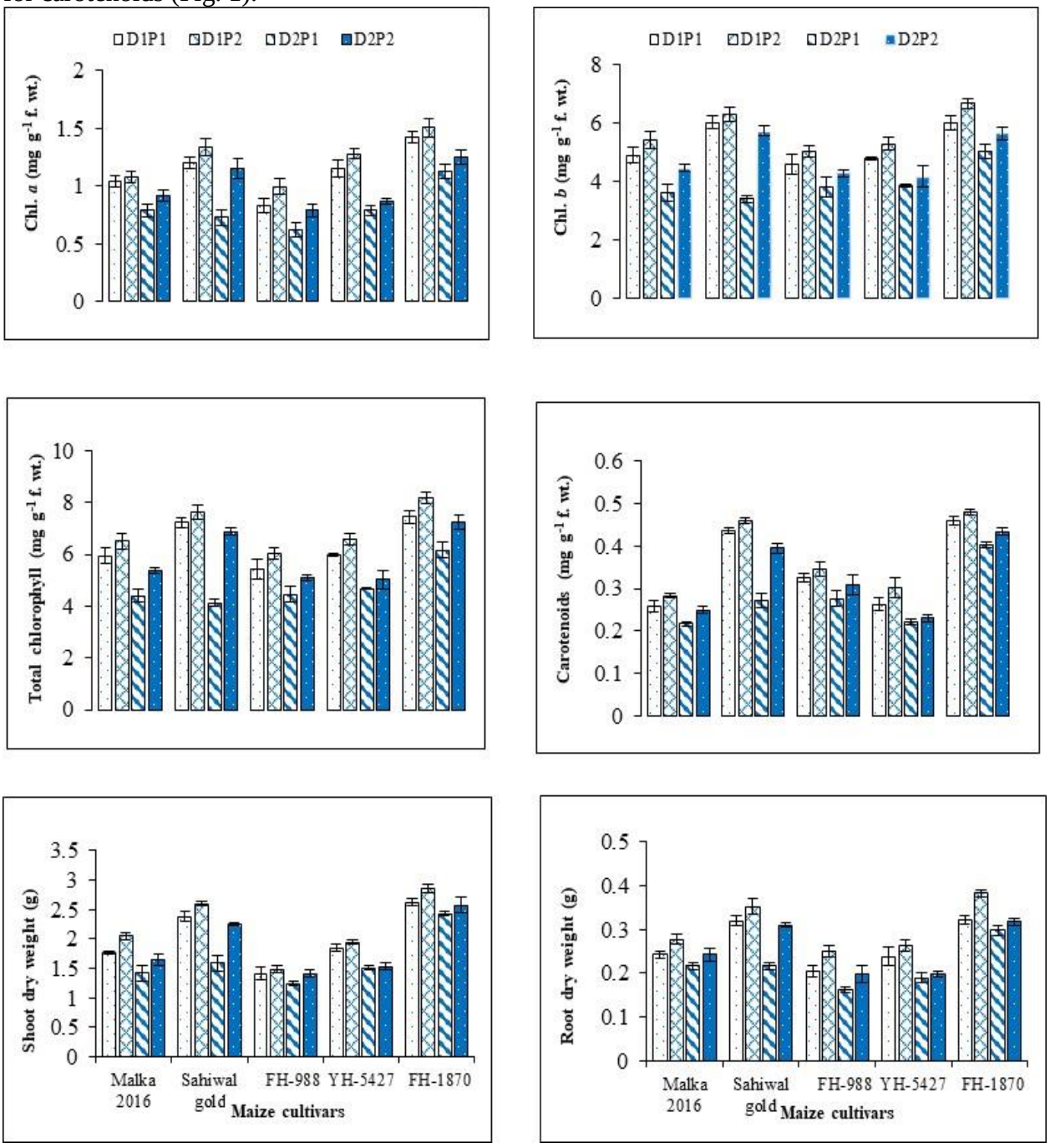

Fig. 1. Photosynthetic pigment and dry biomass of Maize (*Zea mays* L.) plants raised from PGPR treatment under drought and no-drought stress (D1P1= No drought + No PGPR, D1P2= No drought + PGPR, D2P1= Drought + No PGPR, D2P2= Drought +PGPR).

Drought stress considerably decreased (P≤0.001) shoot dry weight in maize cultivars. Varieties showed decrease in percentages as: FH-1870 (7.25%), FH-988 (12.05%), YH-5427 (18.37%), Malka-2016 (20.22%), and Sahiwal gold (32.77%). Application of PGPR considerably (P≤0.001) enhanced the SDW (shoot dry weight) in maize Cvs. as compared to nontreated plants under drought stress as: YH-5427 (1.9%), FH-1870 (5.8%), FH-988 (12.04%), Malka-2016 (13.93%), and Sahiwal gold (29.88%). FH-1870 exhibited highest (P≤ 0.001) overall increase in SDW in comparison to all other

cultivars. Significant interaction (P≤0.01) between drought stress and cultivars, and between PGPR and cultivars (P≤0.05) respectively were observed (Fig.1; Table 1)

Root dry weight substantially reduced (P≤ 0.001) under drought stress in maize varieties. The results showed distinct variation in percentages as follows: FH-1870 (6.25%), Malka-2016 (8.33%), YH-5427 (20.83%), FH-988 (21.95%), and Sahiwal gold (31.25%) (Table 1). Applying PGPR considerably (P≤0.001) enhanced the root dry weight in maize varieties in comparison to no PGPR given plants under drought condition as: YH-5427 (5%), FH-1870 (6.66%), Malka-2016 (8.33%), FH-988 (20%) and Sahiwal gold (29.03%). Among all cultivars FH-1870 showed highest (P≤ 0.001) overall increase in root dry weight compared to other cultivars (Fig.1).

Drought stress imposition considerably decreased (P≤ 0.001) the net CO_2 assimilation rate (*A*) in maize cultivars. The percentage variations in increasing order for *A* under drought stress were as follows: FH-1870 (5.84%), YH-5427 (12.72%), FH-988 (28.47%), Malka-2016 (28.50%), and Sahiwal gold (38.40%). PGPR application considerably (P≤0.001) enhanced the *A* in maize cultivars as compared to control in ascending order as: YH-5427 (1.62%), Malka-2016 (7.7%), FH-1870 (7.9%), FH-988 (12.54%), and Sahiwal gold (30.61%). Highest (P≤ 0.001) overall increase was shown by Sahiwal gold compared to all other cultivars. Strong interaction (P≤0.01) was observed between drought and cultivars (Fig. 2; Table 1).

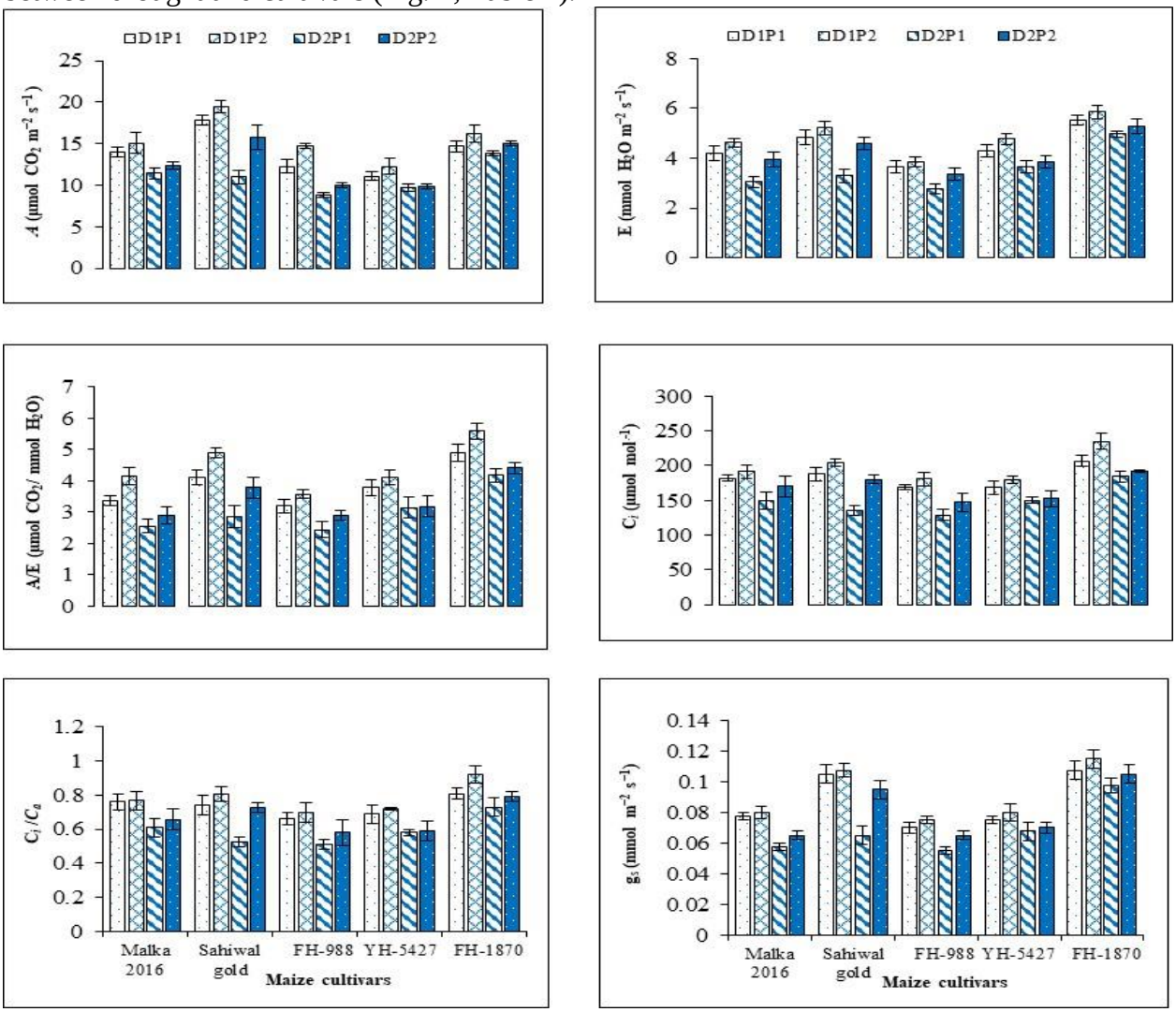

Fig. 2. Gas exchange attributes of Maize plants raised from PGPR treatment under drought and no-drought stress (D1P1= No drought + No PGPR, D1P2= No drought + PGPR, D2P1= Drought + No PGPR, D2P2= Drought +PGPR).

Drought stress considerably decreased (P≤ 0.001) transpiration rate (*E*) in maize cultivars. The percentage changes for *E* were as follows: FH-1870 (10.12%), YH-5427 (14.45%), FH-988 (24.31%), Malka-2016 (27.45%) and Sahiwal gold (31.60%) (Table 1). PGPR application considerably

(P≤0.001) increased the *E* in maize cultivars as compared to untreated plants as: YH-5427 (5.35%), FH-1870 (5.87%), FH-988 (17.26%), Malka-2016 (22.72%) and Sahiwal gold (28.26%). FH-1870 exhibited highest (P≤ 0.001) overall increase in *E* as compared to other cultivars (Fig. 2).

Drought stress imposition considerably reduced (P≤0.001) the water use efficiency (WUE) (*A*/*E*) in maize cultivars. The *A*/*E* values from lowest to highest percentage observed were as: FH-1870 (14.54%), YH-5427 (16.93%), FH-988 (23.51%), Malka-2016 (24.03%), and Sahiwal gold (30.41%) (Table 1; Fig. 2). Application of PGPR considerably (P≤0.001) enhanced the *A*/*E* in maize cultivars as compared to no PGPR given plants under drought stress. The percentage increase in WUE was in following order: YH-5427 (1.56%), FH-1870 (5.21%), Malka-2016 (11.72%), FH-988 (15.27%), and Sahiwal gold (24.33%). Highest (P≤ 0.001) overall increase in WUE was shown by FH-1870 as compared to other cultivars.

Sub-stomatal CO_2 concentration drastically reduced (P≤ 0.001) under DS in maize Cvs. The varieties exhibiting values in ascending order were as: FH-1870 (10.65%), YH-5427 (10.86%), Malka-2016 (17.90%), FH-988 (23.85%), and Sahiwal gold (28.19%). PGPR application considerably (P≤0.001) enhanced the Sub stomatal CO_2 concentration in maize cultivars as compared to nontreated plants under water deficit condition as: YH-5427 (1.64%), FH-1870 (4.1%), Malka-2016 (12.48%), FH-988 (12.58%), and Sahiwal gold (25.10%). Among all cultivars highest (P≤ 0.001) overall increase showed by FH-1870 (Table 1; Fig. 2).

Imposition of drought stress substantially reduced (P≤ 0.001) the relative internal CO_2 concentration (C_i/C_a) in maize cultivars (Table 1). The percentage changes for (C_i/C_a) under drought stress were as follows FH-1870 (10.86%), Malka-2016 (19.73%), YH-5427 (15.94%), FH-988 (22.72%), and Sahiwal gold (29.72%). Application of PGPR considerably (P≤0.001) increased the (C_i/C_a) in maize cultivars as compared to no PGPR given plants under drought stress. The percentage increase was in following sequence: YH-5427 (1.88%), Malka-2016 (7.57%), FH-1870 (7.59%), FH-988 (13.72%), and Sahiwal gold (28.76%). FH-1870 exhibited highest (P≤ 0.001) overall increase in comparison to other cultivars (Fig. 2).

Stomatal conductance (g_s) reduced considerably (P≤ 0.001) under drought stress in maize Cvs. The percentage changes for g_s under drought stress were as follows: FH-1870 (9.34%), YH-5427 (10.66%), FH-988 (21.42%), Malka-2016 (26.92%), and Sahiwal gold (38.09%). PGPR application drastically (P≤0.001) increased the stomatal conductance in maize cultivars as compared to nontreated plants under drought stress showed varied percentage responses in following sequence: FH-1870 (6.66%), Malka-2016 (10.76%), YH-5427 (14.28%), FH-988 (15.38%), and Sahiwal gold (31.57%). However, FH-1870 showed highest (P≤ 0.001) increase in stomatal conductance under water deficit and control environment (no drought stress) (Fig. 2; Table 1).

Drought stress considerably triggered (P≤0.001) the MDA production in maize cultivars. The varieties exhibiting increase from lowest to highest percentage were as follows: FH-1870 (11.89%), YH-5427 (14.09%), Malka-2016 (15.63%), FH-988 (16.65%), and Sahiwal gold (19.11%). Application of PGPR dramatically (P≤0.001) reduced the MDA production in maize cultivars as compared to no PGPR given plants under drought stress; The reduction percentages in descending order were Sahiwal gold (17.39%), Malka-2016 (16.42%), FH-1870 (8.10%), FH-988 (7.67%), and YH-5427 (4.55%). The highest (P≤ 0.001) overall decrease was shown by FH-1870 as compared to other cultivars (Fig. 3; Table 2).

Obtruding drought stress substantially increased (P≤0.001) the H_2O_2 production in maize cultivars. The varieties exhibiting lowest to highest percentage were FH-1870 (8.70%), YH-5427 (12.73%), Malka-2016 (22.48%), FH-988 (24.51%), and Sahiwal gold (36.95%). PGPR application substantially (P≤0.001) decreased the H_2O_2 production in maize cultivars as compared to nontreated plants as: Sahiwal gold (17.21%), FH-1870 (16.32%), Malka-2016 (16.56%), FH-988 (12.90%), and YH-5427 (6.40%). Highest (P≤ 0.001) overall decrease was exhibited by FH-1870 in comparison to other cultivars. Interaction between drought and cultivars exhibited that FH-1870 showed significant tolerance (P≤ 0.001) under DS than other cultivars (Fig. 3; Table 2).

Table:2 Mean square values from analyses of variance for the gas exchange and biochemical attributes of Maize plants grown under PGPR application and drought stress

Source of variation	df	MDA	H_2O_2	CAT	POD
Drought (D)	1	177453.48***	3453.67***	12.188***	195.161***
Plant growth promoting Rhizobacteria (PGPR)	1	91048.86***	1314.09***	3.615***	52.852***
Cultivars (Cvs.)	4	161123.1***	611.32***	19.096***	80.994***
D× PGPR	1	54.03ns	4.246ns	0.0981ns	4.225 ns
D× Cvs.	4	2116.29ns	217.12 ***	0.150ns	3.859ns
PGPR × Cvs.	4	845.61ns	17.41ns	0.082ns	0.846ns
D× PGPR ×Cvs.	4	5080.80ns	35.36ns	0.0197ns	0.391ns
Error	60	3809.72	28.435	0.291	2.029

Source of variation	df	SOD	Total Soluble proteins	Total phenolics	AsA
Drought (D)	1	8654.964***	0.00008***	0.103***	0.199***
Plant growth promoting Rhizobacteria (PGPR)	1	2342.009***	0.00005***	0.025***	0.044***
Cultivars (Cvs.)	4	3613.075***	0.00006***	0.018***	0.188***
D× PGPR	1	5.735ns	0.0000002ns	0.000000041ns	0.0023ns
D× Cvs.	4	105.874ns	0.000003*	0.00046ns	0.0027ns
PGPR × Cvs.	4	40.664ns	0.000006**	0.00027ns	0.00088ns
D× PGPR ×Cvs.	4	43.144ns	0.000003*	0.00023ns	0.00033ns
Error	60	76.397	0.000001	0.00052	0.00177

* = P ≤ 0.05, ** = P ≤ 0.01, *** = P ≤ 0.001, ns = non-significant, df = degrees of freedom
POD = Peroxidase; SOD = Superoxide dismutase; CAT = Catalase; AsA= Ascorbic acid
MDA= Malondialdehyde, H_2O_2= Hydrogen peroxide

Drought stress considerably enhanced (P≤ 0.001) the catalase activity in maize cultivars. The varieties exhibited increase in following sequence, Sahiwal gold (13.57%), YH-5427 (15.52%), FH-988 (15.75%), Malka 2016 (16.07%), and FH-1870 (16.74%). PGPR application considerably (P≤0.001) improved the catalase activity in maize cultivars as compared to nontreated plants under drought stress as: YH-5427 (8.43%), Malka-2016 (9.31%), FH-988 (9.34%), FH-1870 (9.50%), and Sahiwal gold (11.65%). Highest (P≤ 0.001) overall increase was observed in FH-1870 compared to other cultivars (Fig. 3; Table 2).

Drought stress (D) stress substantially increased (P≤0.001) superoxide dismutase (SOD) in all maize cultivars. Varieties illustrated the increase percentages as: Sahiwal gold (14.18%), FH-988 (18.76%), Malka 2016 (19.02%). YH-5427 (22.89%) and FH-1870 (23.75%). Applying plant growth promoting rhizobacteria (PGPR) considerably enhanced (P ≤0.001) SOD as compared to nontreated plants under drought stress. Varieties reporting the significant spike in SOD activity as followed: YH-5427 (7.51%), Malka 2016 (9.19%), FH-988 (9.35%), FH-1870 (10.39%), and Sahiwal gold (12.99%). All cultivars exhibited disparity in which highest (P≤ 0.001) overall increase was shown by FH-1870 as compared to other cultivars (Fig. 3; Table 2).

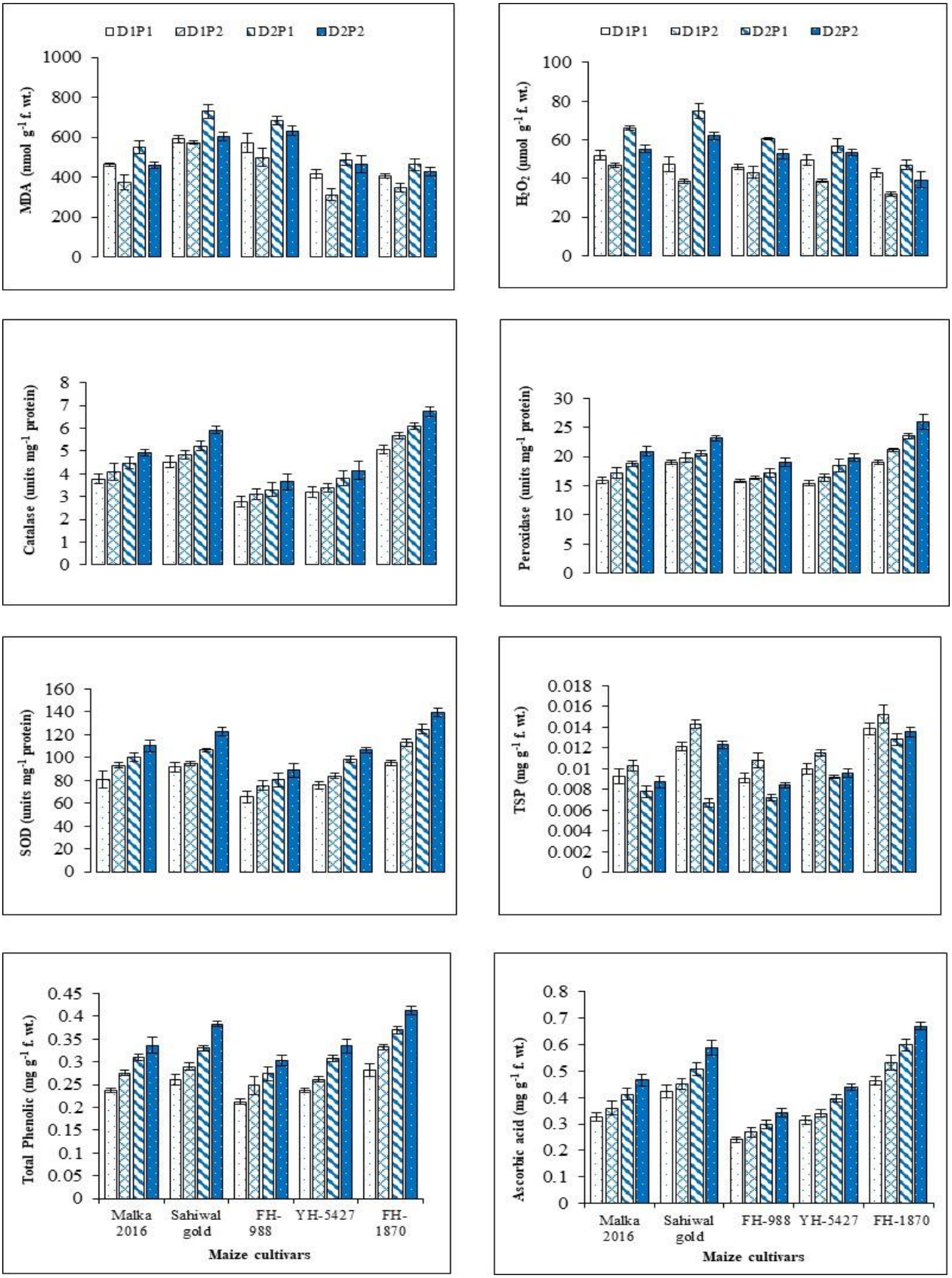

Fig. 3. ROS (MDA, H_2O_2), Antioxidants activities and total soluble proteins of Maize plants raised from PGPR treated seeds under control and water deficit condition (D1P1= No drought + No PGPR, D1P2= No drought + PGPR, D2P1= Drought + No PGPR, D2P2= Drought + PGPR).

Imposition of drought stress on maize cultivars caused a significant increase (P≤0.001) in peroxidase activity. Varieties demonstrating the increase from lowest to highest percentage values were Sahiwal gold (7.69%), YH-5427 (16.16%), FH-988 (8.26%), Malka-2016 (14.98%), and FH-1870 (19.25%) (Table 2). PGPR application considerably (P≤0.001) enhanced the peroxidase activity in maize cultivars as compared to no PGPR given plants under water deficit condition as: YH-5427 (7.06%), FH-1870 (9.30%), FH-988 (9.81%), Malka-2016 (10.37%), and Sahiwal gold (11.23%). The highest (P≤ 0.001) overall increase was observed in FH-1870 as compared to other cultivars (Fig. 3).

Imposition of drought stress on maize cultivars caused significant reduction (P≤0.001) in total soluble proteins (TSP). The percentage changes for TSP from lowest to highest were FH-1870 (7.14%), YH-5427 (8.08%), Malka-2016 (15.55%), FH-988 (22.22%), Sahiwal gold (45%). Application of PGPR remarkably (P≤0.001) enhanced the TSP in maize cultivars as compared to nontreated plants under drought stress. PGPR showed increase in TSP as: YH-5427 (4.16%), FH-1870 (7.14%), Malka-2016 (10.34%), FH-988 (15.47%), and Sahiwal gold (44.16%). Among all cultivars highest (P≤ 0.001) overall increase was shown by FH-1870 as compared to other cultivars. Significant interaction (P≤ 0.05) between drought stress (DS) and cultivars, PGPR and cultivars (P≤ 0.01) and among all three major factors (drought, PGPR and cultivars) (P≤ 0.05) were observed (Fig. 3; Table 2).

Drought stress obtruding on maize cultivars caused significantly enhanced (P≤0.001) total phenolic content. The percentage changes were as follows: Sahiwal gold (21.21%), FH-988 (22.22%), Malka-2016 (22.58%), YH-5427 (22.58%), and FH-1870 (24.32%). PGPR application significantly (P≤0.001) enhanced the total phenolics in maize cultivars as compared to no PGPR given plants under water deficit condition in following sequence, YH-5427 (6.06%), Malka-2016 (8.82%), FH-1870 (9.75%), FH-988 (10%), and Sahiwal gold (13.15%). Among all cultivars FH-1870 showed the highest significant (P≤ 0.001) overall increase in comparison to other cultivars (Fig. 3; Table 2).

Leaf ascorbic acid (AsA) showed considerable increase (P≤0.001) under DS in maize cultivars. Varieties exhibiting the highest increase in AsA under drought stress were Sahiwal gold (17.64%), Malka-2016 (19.51%), FH-988 (20%), YH-5427 (20%), and FH-1870 (23.33%). PGPR application remarkably (P≤0.001) enhanced the leaf AsA in maize cultivars as compared to nontreated plants under DS condition. The increase in ascending order was as follows: YH-5427 (9.09%), FH-1870 (10.44%), FH-988 (11.76%), Malka-2016 (12.76%), and Sahiwal gold (13.55%). FH-1870 exhibited highly significant (P≤ 0.001) overall increase among other cultivars (Fig. 3; Table 2).

Discussion

Drought is a primary abiotic stressor that impairs plant growth and development, restricts agricultural output, raises the overall expense of crop production considerably, and eventually affects the availability of food as well as feed. In response to drought stress, plants employ a range of interconnected physiological, biochemical, nutritional, molecular, metabolic, and cellular processes in response to drought stress (Chieb & Gachomo *et al*., 2023).

Photosynthetic pigments are essential for light absorption and the generation of reducing energy, including NADPH and ATP (adenosine triphosphate), as well as for photosynthesis and carbon fixation in plants, carotenoids, however, serve other purposes and aid plants in overcoming the challenges posed by water constraint (Farooq *et al*. 2019). Our research revealed that drought-stressed maize cultivars displayed a significant reduction in photosynthetic pigments, possibly as a result of their stomatal conductance decreasing to prevent water loss, lowers water availability cause stomatal closure slows transpiration, which in turn reduce the rate of photosynthetic by lowering CO_2 and nutrient intake. Like our findings, Asrar & Elhindi (2020) and Kiani *et al*. (2020) reported decreased chlorophyll level under water stressed condition sunflower, *Vaccinium myrtillus*, and marigolds plants significantly. In our trial PGPR inoculation enhances antioxidant enzyme activity which defend chlorophyll and other photosynthetic pigments from ROS damage triggered by drought stress. Our results coincide with the findings of Gashash *et al*. (2022) that PGPR application on tomato plants enhanced the chlorophyll content in contrast to non-treated cultivars.

Drought stress decreased photosynthesis and water availability leading to reduced carbohydrate production which ultimately reduced cell growth and decreased plant root and shoot dry weight in our research plants. Similar to our findings Faisal *et al.* (2019) described that wheat and rice plant subjected to water deficit condition showed highest reduction in shoot and root dry weight. Contradictory PGPR treatment enhances water uptake and retention improve nutrient availability in maize cultivars exhibited significant increase in plant shoot, and root dry weight. Contrariwise, wheat and rice plants inoculated with PGPR enhance root growth and dry weight (Kumar *et al*., 2019) and PGPR treated wheat, soybean and maize enhance shoot dry weight (Zhang & Tang, 2020).

In our trial DS reduced the gas exchanging characteristics in maize varieties. Reduction might be due to the stomatal closure which limits the CO_2 uptake and damage of photosynthetic apparatus. Similarly, research on wheat plants exhibited that water scarcity limits stomatal conductance and photosynthetic rate cause decline in plant growth and development (Khan *et al*., 2020b). PGPR induced increase in antioxidant activity protected photosynthetic apparatus from damage hence, increased chlorophyll content and improved stomatal conductance and photosynthetic rate in maize plants under drought stress. Likewise, Khan *et al*. (2020a) reported wheat plants inoculated with PGPR increased net photosynthetic rate (*P*), transpiration rate (*E*) and improved *WUE*. Zhang *et al*. (2020) also reported an increase in stomatal conductance, intercellular CO_2 concentration and photosynthetic activity in maize plants under drought stress condition in comparison to non-treated plants.

The overproduction of H_2O_2 and MDA is the indicator of oxidative stress under water stress. MDA is produced through lipid peroxidation and H_2O_2 is a harmful radical that damage plant protein and components cause lipid peroxidation of plant membrane ultimately leads to death of plant (Tayyab *et al*., 2020). MDA and H_2O_2 overproduction react with protein, lipids and DNA and cause oxidative imbalance (Gill & Tuteja, 2019). Our results demonstrated that maize plants subjected to drought stress remarkably enhanced the MDA and H_2O_2 content by impairing antioxidant defence system that enhance ROS production and cause lipid peroxidation which produce H_2O_2 and MDA as an indicator of oxidative stress. Aligning with our results, a recent study showed that water stress remarkably enhanced the MDA and H_2O_2 content in rice plants (Urmi *et al*., 2023). Furthermore, maize plants treated with PGPR exhibited significant decrease in MDA and H_2O_2 content. The PGPR modulates the activity of antioxidant enzymes to stop radical impairment imposed by drought stress. Al-Turki *et al*. (2023) reported that PGPR treatment upregulated antioxidant gene expression, boosting the activity of antioxidant enzymes ultimately decreased superoxide and protected the chloroplast from the effects of ROS. Gowtham *et al*. (2020) stated that tomato plants inoculated with the PGPR produced from ACC deaminase significantly decreased MDA and H_2O_2 content by increasing APX and SOD activity as compared to non-treated plants.

Antioxidant enzymes scavenge ROS and serve as markers of plant defense against stress. Enzymatic antioxidants including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) can defend plants from oxidative damage (Azeem *et al*., 2022). According to our study antioxidant activities increased in maize plants because reactive oxygen species increased under drought stress and damage cellular components such as photosynthetic apparatus and to overcome this damage plants regulates the ABA activity which induces antioxidant gene expression and increase antioxidant enzyme activity. Similar results were demonstrated by Kumar *et al*. (2019) in wheat plants under drought stress. In present research, maize plants treated with PGPR increased antioxidants (SOD, CAT, POD) activity under drought stress in comparison to non-treated cultivars. SOD is an essential enzyme that scavenges reactive oxygen species (ROS) and functions as the first line of defense. SOD uses several substrates to donate electrons in order to convert superoxide anion (O_2^-) to H_2O_2 (hydrogen peroxide). Furthermore, H_2O_2 is broken down by APX, CAT, and, POD into molecular oxygen and water and also lessen lipid peroxidation and membrane damage by converting lipid hydroperoxide to alcohol (Rajput *et al*., 2021). Likewise, Nautiyal *et al*. (2019) demonstrated that the PGPR treated spinach, carrot and lettuce plants boosted antioxidant activity and increased the growth of plant than non-treated plants. Furthermore, according to research by He *et al*. (2021), ryegrass (*Lolium perenne* L.)

plants treated with PGPR (*Pseudomonas* sp. M30-35 and *Bacillus* sp. WM13-24) under water scarcity environment showed greater activities of ROS detoxifying enzymes, particularly catalase, peroxidase and superoxide dismutase in contrast to non-inoculated plants.

The decrease in total soluble protein in our drought-stressed maize cultivars might be caused by decreased water availability, which lowers nutrient absorption and the availability of amino acids needed for protein synthesis. Our results agreed with those obtained by Khan *et al*. (2020b) who described that drought stress highly reduced the protein content in *Brassica napus* plants and Choukri *et al*. (2020) observed reduction in lentil plants. In this research, our experimental findings exhibited that plants treated with the PGPR increases total soluble protein as PGPR produces exopolysaccharides which help to hold water content and induce expression of protein coding gene and increase total soluble proteins as compared to non-treated plant. Akin to our research Qaseem *et al*. (2019) stated increase in protein content in maize plants inoculated with PGPR in contrast to non-inoculated plants.

Total phenolics are the largest and most important class of secondary metabolites, are known to contribute significantly to increase resistance to drought stress (Salam *et al*., 2023). Phenolics are potent antioxidants that can detoxify reactive oxygen species (ROS) and defends the plant from negative consequences by the modulation of the phenylpropanoids pathway (Hatami, 2019). Applying of PGPR can alleviated the drastic effects of drought stress on phenolic contents (Bouremani *et al*., 2023). Our results demonstrated that, plants exposed to water stress showed increased phenolic production due to enhanced antioxidant activity which protect phenolic compounds from oxidative damage and increased total phenolics. Similar results were observed by Gharibi *et al*. (2019) in *Achillea* species under drought stress. Our plants when treated with PGPR significantly enhanced the total phenolic contents of leaf under drought stress by enhancing the antioxidant enzyme and modulating phenylpropanoids pathway. Similar to our study, wheat and maize plants inoculated with PGPR showed increase in phenolic contents a resistant mechanism to water scarcity (Islam *et al*., 2019).

Ascorbic acid (AsA) is the prevalent antioxidant component required for a variety of biological processes in plants (Chaturvedi *et al*., 2022). Ascorbic acid functions primarily as a redox buffer, through the APX reaction, it can convert H_2O_2 to H_2O and immediately scavenge $O_2^{\bullet-}$, OH^-, and $^1O_2$ under abiotic stress (Liang *et al*., 2019). In our study maize plants increased ascorbic acid under drought stress as a protective mechanism against oxidative damage. Likewise, Zhang *et al*. (2020) stated that soybean and maize plants showed an increase in ascorbic acid under drought stress. Our research demonstrated that ascorbic acid significantly enhanced by applying PGPR under drought stress by improving water uptake and enhancing antioxidant activity in comparison to non-treated cultivars. Similar to our findings ascorbic acid improves the common bean's antioxidant activity and secondary metabolites when it comes to water stress (Gaafar *et al*., 2020). Related to our research Asghari *et al*. (2020), inspected that PGPR innoculation (*Azotobacter chroococcum* and *Azospirillum brasilense*) on pennyroyal (*Mentha pulegium* L.) against water stress remarkably decreased the negative effects on low growth characteristics and synthesis of secondary metabolites. Tomato plants treated with PGPR mixture of two strains significantly increased ascorbic acid accumulation as compared to non-inoculants (Gashash *et al*., 2022).

Conclusion

Maize seed inoculation with PGPR can alleviate deleterious effects of drought stress by efficiently improving growth (dry biomass production), gas exchange attributes and photosynthetic pigment contents under drought stress. PGPR application played a promising role in suppressing oxidative stress indicators like hydrogen peroxide and MDA limiting them to a level for signal transduction to trigger the antioxidants defense mechanism of maize. Among the varieties examined, FH-1870 showed a stronger inherent tolerance to drought, while Sahiwal gold benefited most from PGPR application but was less effective under control conditions. In contrast, YH-5427 showed limited response to both drought and PGPR treatments, indicating varietal sensitivity. Overall, this research emphasizes the potential of PGPR as an effective biotechnological tool to increase drought tolerance in maize, with the specific responses of different varieties highlighting the importance of tailored

approaches for crop improvement under drought conditions. However, there is further need for more studies in field conditions with various crops under variable ecological constraints to validate these findings.

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