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Biochemical Characterization of Parental Inbred Lines and Hybrids of Maize (*Zea mays* L.) under Different Irrigation Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Maize (*Zea mays* L.) is a significant crop with extensive agricultural and economic importance worldwide. With increasing concerns over water scarcity and climate change, understanding the responses of maize plants under water stress conditions is crucial to develop drought-tolerant

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cultivar (s). In present investigation, alteration in different biochemical parameters, including chlorophyll content, malondialdehyde (MDA) levels, hydrogen peroxide (H_2O_2) content, peroxidase, glutathione reductase (GR), and catalase activities, among 12 parental inbred lines along with 66 hybrids and two checks under irrigated and partial irrigated conditions were examined. Under irrigated conditions, the chlorophyll content was highest in the parental inbred line IL8, intimately followed by IL6, while IL5 exhibited the lowest content. MDA levels were significantly higher in the parental line IL8 and hybrid IL1 × IL6, whereas IL5 and IL3 × IL11 exhibited the lowest levels. H_2O_2 content was found to be highest in the parental line IL5 and hybrid IL8 × IL12, whereas IL4 and IL2 × IL5 displaying the lowest levels. Peroxidase activity was highest in IL7 and hybrid IL1 × IL7, whilst IL6 and IL4 × IL7 showed the lowest activity. Glutathione reductase activity was found highest in IL1 and IL9 × IL12, whereas IL6 and hybrid IL1 × IL3 exhibited the lowest activity. Catalase activity was highest in IL8 and IL2 × IL10, while IL4 and IL2 × IL6 displayed the lowest activity under irrigated conditions. Under partial irrigated conditions, almost similar trends were documented for the most of parameters, with slight variations in the expression levels. Notably, the drought-tolerant genotypes demonstrated higher chlorophyll content, peroxidase, glutathione reductase, and catalase activities, while drought-sensitive genotypes unveiled elevated MDA levels and H_2O_2 content. Phylogenetic analysis revealed five major clusters, indicating significant variability in different biochemical profiles among the genotypes. The heat map analysis supported the identification of distinct expression patterns of biochemical parameters, contributing to our understanding of the genotypic responses to varying irrigation conditions. These findings provide valuable insights for maize breeding programmes aimed to breed drought-tolerant cultivar (s) with enhanced antioxidant defences and stress tolerance.

Keywords: Maize; Chlorophyll; Malondialdehyde (MDA); Hydrogen Peroxide (H_2O_2); Peroxidase; Glutathione reductase (GR)' catalase activity.

1. INTRODUCTION

Maize, scientifically known as *Zea mays* and classified under the family Poaceae, was originally described by Carl Linnaeus [1]. It is a diploid plant with a genome size of 2357 MB. It is characterized as a monoecious crop, meaning it has separate male and female flowers on the same plant and relies on cross-pollination for reproduction. The origin place of maize or corn is Mexico and firstly domesticated about 10000 years ago by the indigenous people of Mexico [2,3]. It is also believed to have originated in Northern Guatemala. Maize, being a major global crop, holds significant economic importance due to its versatile applications in various sectors such as food production, industrial materials, and animal feed [4-7]. However, the growth and development of maize plants are significantly influenced by several abiotic stresses, including drought, salinity, and high temperature [8,5,6,7]. Among these stresses, maize plants exhibit heightened sensitivity and susceptibility to water stress compared to most other cereal crops [9]. The availability of water plays a crucial role in determining the productivity and nutritional quality of maize, making drought stress a primary concern [10].

Drought stands as a prominent abiotic stressor that profoundly impacts plant growth, development, and productivity [11-19]. With the anticipated consequences of global warming and population growth, future scenarios involve diminishing water resources and an expansion of arid and semiarid regions [20-21]. Consequently, investigating the mechanisms underlying plant adaptation and tolerance to drought, as well as their capacity for post-water deficit recovery, assumes paramount importance in contemporary research endeavours [22-28].

Understanding the plant's capacity to survive under drought conditions necessitates an investigation into its diverse morpho-physiological [29,5-612,14.5-6] biochemical adaptations [30-37,13,17,18,21.7] and molecular [38,15,12,19,20,6]. Additionally, exploring mechanisms related to both tolerance and recovery during rehydration is crucial [11]. Plant tolerance to water deficit entails the ability to maintain vital functions under unfavourable water conditions and swiftly restore water status and functions upon rewatering [12-20]. Recent research emphasizes the significance of the recovery phase, as it profoundly influences subsequent plant growth and development [39]. The assessment of plant tolerance to drought stress involves the identification of specific physiological characteristics that are essential for drought tolerance [40]. This indicator is

frequently employed in efforts to select drought-resistant varieties or determine tolerance levels [40].

Drought stress has been observed to decrease chlorophyll content, leading to disruptions in photosynthesis [41]. Moreover, it induces oxidative stress, characterized by the generation of reactive oxygen species (ROS), which can cause membrane lipid peroxidation, protein degradation, DNA fragmentation, and ultimately cell death [42]. These detrimental effects on cellular processes, including cell division, elongation, and differentiation, contribute to restricted plant growth and reduced yields [43]. Maize exhibits various mechanisms to respond to drought stress, including redox regulation and osmotic regulation [44] (Farooq et al., 2009). Redox regulation primarily involves the activity of enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which work to mitigate oxidative damage by clearing ROS and ensuring the normal functioning of cells under drought stress conditions [45].

Plants have developed intricate antioxidant defence mechanisms to safeguard cells against the harmful impacts of reactive oxygen species (ROS). Key enzymes involved in scavenging ROS and protecting plants from oxidative damage include catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and peroxidase (POD) [46,47]. These enzymes play critical roles in the detoxification and neutralization of ROS, contributing to the overall antioxidative capacity of plants. By efficiently removing ROS, these antioxidant enzymes help mitigate oxidative stress and maintain cellular homeostasis. Previous studies have demonstrated that the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) exhibit an increase under moderate drought conditions, while they decrease under severe drought conditions [48]. Notably, studies have reported that starch biosynthesis in maize leaves contributes to maintaining leaf growth and photosynthesis under drought stress conditions [49]. A reduction in membrane stability indicates the occurrence of lipid peroxidation caused by reactive oxygen species (ROS) [50]. The content of chlorophyll (Chl.) in plants has a positive impact on photosynthetic rate [44]. Under water stress conditions, a decrease in chlorophyll content serves as an indicative characteristic of oxidative stress and can be attributed to chlorophyll photo-oxidation and degradation [51,52]. Deficit irrigation significantly reduces chlorophyll a and b, overall chlorophyll content, and the chlorophyll a/b ratio in plants [53]. The chlorophyll stability index (CSI), which is calculated as the ratio of chlorophyll levels under stress and normal conditions, serves as an important indicator for screening genotypes with abiotic stress tolerance. Significant differences and higher CSI values indicate genotypes that exhibit tolerance to abiotic stress [54]. The objective of present investigation was to evaluate the impact of water deficit on the physiological and biochemical activities of maize inbred lines and their hybrids, focusing on their characteristics and tolerance to deficit irrigation conditions.

2. MATERIALS AND METHODS

2.1 Plant Material and Growth Conditions

The experimental material consist of 12 maize inbred lines were obtained from Sam Higgonbottom Agriculture Science and Technology University, Prayagraj, U.P., India (Table 1). A half diallel analysis method as proposed by Jinks and Hayman [55], was employed to development of 66 F_1 hybrids. A drought-tolerant HKI1105 and drought-susceptible HKI1128, varieties were used as a check. A total of 80 genotypes, consisting of 12 parents, 66 F_1 hybrids, along with two checks, were grown in a randomized complete block design (RCBD) with two replications during Kharif 2020-21. Each genotype was planted in two rows measuring 4 meters in length, with a spacing of 60 cm between rows and 20 cm between plants. Drought stress was imposed by withholding irrigation starting from 10 days prior to flowering, and irrigation was resumed once the soil moisture reached the temporary wilting point. At the reproductive stage after planting, leaf samples were collected from the second leaf from the top to analyze chlorophyll, malondialdehyde, H_2O_2, peroxidase, glutathione reductase, and catalase contents.

Table 1. List of inbred lines with their parentage used in study

S. No.	Lines	Parentage	Source
1	IL-1	CM-13	SHUATS, Allahabad
2	IL-2	CML-193	SHUATS, Allahabad
3	IL-3	CML-439	SHUATS, Allahabad
4	IL-4	NBPGR-36417	SHUATS, Allahabad
5	IL-5	NBPGR-36417 X NBPGR-33000	SHUATS, Allahabad
6	IL-6	(103) NBPGR-36548 × (97) NBPGR-36407	SHUATS, Allahabad
7	IL-7	DMR-N 21 × NBPGR-32809	SHUATS, Allahabad
8	IL-8	LM- 13 × NBPGR-31899	SHUATS, Allahabad
9	IL-9	CML-224-1 × NBPGR-32809	SHUATS, Allahabad
10	IL-10	NBPGR-36550 × NBPGR-36407	SHUATS, Allahabad
11	IL-11	KL- 153237 × VL- 1016536	SHUATS, Allahabad
12	IL-12	CML- 161 × VL- 1056	SHUATS, Allahabad

2.2 Biochemical Estimation of Maize Genotypes

2.2.1 Chlorophyll content (mgcm^{-2})

Total chlorophyll was calculated as per method suggested by Arnon et al. [56]. Total chlorophyll content was estimated on 60 days after sowing. A fresh 100mg random leaf sample (each row) from field after 60 days sowing was collected. Then leaf sample was crushed finely in 10 ml (80% Acetone) and transferred into falcon tube. Then centrifuged for 15 minutes at 10000 rpm and the green supernatant was transferred into fresh 15ml falcon tube. Readings were taken in a spectrophotometer at 645 nm, 663 nm and 470nm using a Spectrophotometer (UV–visible-160A, Shimadzu). Chlorophyll a and b concentrations was calculated by using the method of Arnon [56]. Total chlorophyll was the summation of chlorophyll a and b concentrations, and chlorophyll a/b ratio was the ratio between the concentration of chlorophyll a divided by the concentration of chlorophyll b. The chlorophyll stability index (CSI) was determined as described by Koleyoreas [57] using the equation:

CSI % = [Total chlorophyll content (stress) / Total chlorophyll content (control)] $*$ 100.

2.2.2 Malondialdehyde (MDA) test (Lipid peroxidation Assay)

The method proposed by Heath and Packer [58] was employed for measuring the lipid peroxidation in terms of malondialdehyde (MDA) by thiobarbituric acid (TBA) content. Around 25 mg leaf sample was taken crushed in fine powder. Then 500 microliters of 0.1% trichloro-acetic acid were added and vortexed and centrifuged at 10,000 rpm for 10 min. Around 100 microliters of supernatant were taken in an Eppendorf tube and 200 microliters 0.5% thiobarbituric acid was added. Then reaction mixture was heated for 95°C for 30 minutes and quickly kept at -80°C for 2 minutes to stop the reaction. After 2 minutes contents come at room temperature and centrifuged at 10000 rpm for 10 min and supernatant was taken to take absorption at 532 nm in UV Spectrophotometer. The MDA content was expressed as µmol g^{-1} FW.

2.2.3 Estimation of hydrogen peroxide (H_2O_2, mmol g^{-1} FW)

Hydrogen peroxide was determined by using the protocol proposed by Alexieva et al. [59]. Leaf sample (25.0 mg) was taken and crushed into a fine powder. Approximately 500 microliters of 0.1% trichloro-acetic acid were added and vortexed and centrifuged at 10000 rpm for 10 min. Hundred microliters of supernatant were taken in an Eppendorf tube and 200 microliters of 0.5% TBA was added. The reaction mixture was heated at 95°C for 30 minutes and quickly kept at - 80°C for 2 minutes to stop the reaction. After 2 minutes allowed to come at room temperature and centrifuged it at 10000 rpm for 10 min and supernatant was taken to take reading at 532 nm absorption.

2.2.4 Peroxidase activity (units/mg protein)

Peroxidase activity assay was estimated as per the protocol given by Kar and Mishra [60]. Enzyme extraction was done by homogenizing 100 mg leaf sample of normal and water-logged plants in 5.0 ml, 0.1M phosphate buffer (pH 6.4). The crude extract was centrifuged at 10,000 rpm for 20 minutes at 4ºC. Supernatant was stored at 4ºC till the enzymatic activity was performed.

Reaction mixture was prepared by adding 4.6 ml 0.1M phosphate buffer (pH 6.4), 0.2 ml pyrogallol (50 µM) and 0.1 ml 50 µM H_2O_2 and 0.1 ml enzyme extract. Mixture was incubated at 25ºC for 5 minutes. Then 0.5 ml 5.0 per cent H_2SO_4 was added to terminate the reaction. Absorbance was measured at 420 nm with the help of spectrophotometer.

2.2.5 Glutathione reductase activity (GR)

Glutathione reductase activity was estimated by the method of Smith et al. [61]. The reagents used were 50 mM potassium phosphate buffer (pH 7.6) containing 1 mM EDTA, 5 mM NADPH, 6 mM 5,5'-dithio-bis (2-nitrobenzoic acid) [DTNB] and 0.2 mM oxidized glutathione (GSSG). To prepare the reaction mixture to determine GR activity, diluted enzyme extract (25 p1), DTNB (250 p1), 1mM EDTA (20 pl) and 0.2 mM GSSG (100 pl) were added in 175 pl of 50 mM potassium phosphate buffer (pH 7.6). To initiate GR activity 50 pl of 5 mM NADPH added to the reaction mixture. Absorbance was taken using spectrophotometer at 412 nm was followed at 15 s interval up to 2 min.

2.2.6 Catalase activity (units/mg protein)

Catalase activity was determined according to the protocol given by Aebi [62]. The leaf sample (100 mg) was collected from normal and water-logged plants and samples were homogenized in 5.0 ml of 0.1M phosphate buffer (pH 6.4). The crude extract was centrifuged at 10,000 rpm for 20 minutes at 4ºC. The enzyme extract was stored at low temperature until completion of enzyme assay. The enzymatic activity was assayed by taking 2.6 ml, 0.1M phosphate buffer (pH 6.4), 0.1 ml enzyme extract and 0.1 ml and 1.0 percent H_2O_2. The reaction mixture was mixed rapidly at room temperature. A blank was prepared similarly in which 0.1M phosphate buffer (pH 6.4) was added in reaction mixture instead of enzyme extract. The absorbance of the reaction mixture was read immediately at 2300 nm with the UV Spectrophotometer at an interval of 15 second were noted for 2 minutes.

2.3 Statistical Analysis of Biochemical Traits of Maize Genotypes

A comprehensive statistical analysis was conducted to analyze biochemical parameters using the NTSYS pc software (version 2.02) [63]. The dendrogram and heat map were generated to visualize the relationships between the different biochemical parameters. To construct the dendrogram, the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm was employed [64].

3. RESULTS AND DISCUSSION

3.1 Biochemical Analysis of the Inbred Lines and Their Hybrids

To discover drought-tolerant genes by association analysis, physiological and biochemical traits were tested in maize genotypes. The mean values of drought tolerance indexes *viz*., chlorophyll, malondialdehyde (MDA), and H_2O_2 content along with peroxidase, glutathione reductase and catalase activities were estimated in elite eighty maize genotypes subjected to drought treatment at the reproductive stage. Extensive variation was documented for all biochemical parameters during drought stress. The descriptive statistics for the phenotypes related to drought stress are presented in the Table 2. Wide range of variation was observed among the accessions in terms of the drought tolerance indexes for biochemical traits.

3.2 Chlorophyll Content

Chlorophylls have been rightly designated as “pigments of life” because of their central role in living systems responsible for harvesting sunlight and transforming its energy into biochemical energy essential for life on earth. Chlorophyll is one of the major chloroplast components in photosynthesis and had a positive correlation with the photosynthetic rate. Reductions in chlorophyll contents under deficit irrigation conditions can be considered a typical symptom of oxidative stress that causes pigment photo-oxidation and chlorophyll degradation. The chlorophyll contents *i.e*., Chl. a, Chl. b, and total Chl. are very viable parameters that indicate water stress conditions [44]. Deficit irrigation leads to the inhibition of photosynthesis by damaging the photosynthetic apparatus [65], which leads to a decrease in photosynthetic pigments and a reduction in the consumption of energy and carbon required for chlorophyll synthesis inside the plants [66].

The parental inbred line IL10 demonstrated the highest chlorophyll content (0.89 ± 0.02) closely followed by IL6 (0.88 ± 0.02), IL9 (0.84 ± 0.02), and IL8 (0.81 ± 0.02). Conversely, IL5 displayed the lowest chlorophyll content (0.69 ± 0.02).

However, genotypes *viz*., IL1 (0.74 ± 0.02), IL2 (0.75 ± 0.02), and IL4 (0.75 ± 0.02) exhibited intermediate chlorophyll levels under irrigated conditions. Similarly, under partial irrigated conditions, inbreed IL10 maintained the highest chlorophyll content (0.80 ± 0.02), tracked by IL6 (0.80 ± 0.02) and IL9 (0.79 ± 0.02). In contrast, IL5 exhibited the lowest chlorophyll content (0.62 ± 0.02), while IL4 (0.69 ± 0.02), IL2, IL3, IL8 (0.70 ± 0.02) showed slightly higher but comparable levels.

Concerning hybrids under irrigated conditions, the IL2 × IL10 hybrid displayed a notably high chlorophyll content (0.89 ± 0.02), along with IL7 × IL10 (0.89 ± 0.02), IL2 × IL6 (0.88 ± 0.02), and IL4 × IL6 (0.88 ± 0.02). Whilst, the hybrid IL4 × IL5 exhibited the lowest chlorophyll content (0.69 ± 0.02), and similarly, IL6 × IL10 (0.69 ± 0.02), IL3 × IL6 (0.71 ± 0.02), and IL8 × IL11 (0.71 ± 0.02) displayed relatively lower chlorophyll levels. Likewise, under partial irrigated conditions, the hybrid IL4 × IL12 and IL2 × IL10 showed a comparatively high chlorophyll content (0.82 ± 0.02), tracked by IL4 × IL6 (0.81 ± 0.02) and IL1 × IL11 (0.81 ± 0.02). Whereas hybrid IL2 × IL5 displayed a comparatively low chlorophyll content (0.60 ± 0.02) tracked by IL4 × IL5, IL6 × IL10 (0.62 ± 0.02) (Table 2). These findings provide scientific evidence regarding the variation in chlorophyll content among different parental inbred lines and their hybrids under different irrigation conditions in maize. In this investigation, water deficiency stress caused a significant decrease in the chlorophyll contents in the maize inbred lines and their hybrids (Fig. 1 and Fig. 2). For chlorophyll content, Kumari et al. [67] and Manasa et al. [68] reported that under drought conditions, there is decrease in chlorophyll content. Similarly, Anjum et al. [69] and Naghizadeh et al. [51] reported that deficit irrigation caused a significant reduction in the chlorophyll contents in maize hybrids. The drought-tolerant varieties have higher Chl. a, Chl. b, and total Chl., compared to drought-sensitive maize hybrids.

3.3 Malondialdehyde Content (MDA TEST)

The MDA content is an indicator of oxidative injury and acts as a marker for membrane lipid peroxidation owing to exposing plants to stress [66]. Drought-induced overproduction of ROS increases MDA, which results in a disrupted cell bilayer structure and leads to the porosity of the cell membrane, with a decrease in membrane stability reflecting the extent of lipid peroxidation caused by ROS [50].

Under irrigated conditions, among the parental inbred lines, IL8 exhibited the highest MDA content (16.4 ± 0.2 nmol/g FW), followed by IL6 (15.9 ± 0.2 nmol/g FW), and IL4 (15.8 ± 0.2 nmol/g FW). Conversely, IL5 displayed the lowest MDA content (11.6 ± 0.2 nmol/g FW), while IL1 and IL10 exhibited slightly higher but comparable levels (12.5 ± 0.2 nmol/g FW). Under partial irrigated conditions, IL9 demonstrated the highest MDA content (10.4 ± 0.2 nmol/g FW), followed by IL7 (10.3 ± 0.2 nmol/g FW), and IL4 (10.2 ± 0.2 nmol/g FW). However, the lowest MDA content was observed in IL5 (8.5 ± 0.2 nmol/g FW).

Regarding hybrids under irrigated conditions, the hybrids IL1 × IL6 and IL9 × IL10 exhibited substantially higher MDA content (17.4 ± 0.2 nmol/g FW), followed by IL1 × IL9, IL4 × IL11 (16.6 ± 0.2 nmol/g FW), and IL5 × IL7 (15.9 ± 0.2 nmol/g FW). On the other hand, the hybrid IL3 × IL11, IL8 × IL10 displayed the lowest MDA content (10.3 ± 0.2 nmol/g FW), followed by IL3 × IL12, IL8 × IL11, (10.5 ± 0.2 nmol/g FW), and IL2 × IL7 (10.8 ± 0.2 nmol/g FW). Under partial irrigated conditions, the hybrids *viz*., IL1 × IL9, IL10 × IL11, IL4 × IL11 exhibited notably high MDA content (14.6 ± 0.2 nmol/g FW), along with IL1 × IL8, IL9 × IL12, IL4 × IL10 (14.3 ± 0.2 nmol/g FW). Similarly, the hybrids IL4 × IL7, IL6 × IL12 exhibited the lowest MDA content (7.2 ± 0.2 nmol/g FW), tracked by IL3 × IL6, IL3 × IL8, IL3 × IL11, and IL8 × IL10 (7.3 ± 0.2 nmol/g FW) under partial irrigated conditions (Table 2, Fig. 1 and Fig. 2).

The content of MDA is often employed as an indicator of lipid peroxidation in plant tissues, resulting from oxidative stress induced by various abiotic stresses. Recently, an increase in MDA content under drought stress has been reported in leaves of drought-sensitive genotype of maize, whereas no change is observed in tolerant genotypes [70,71].

3.4 H_2O_2 Content

Hydrogen peroxide is a reactive oxygen species (ROS) that can play a role in various physiological processes in plants, including responses to stress and defence mechanisms. However, its levels can vary depending on environmental factors, plant health, and other conditions. The studies investigated the H_2O_2

(hydrogen peroxide) content in different maize parental inbred lines and hybrids under varying irrigation conditions, revealing distinctive patterns of H_2O_2 accumulation. Under irrigated conditions, the parental inbred line IL5 exhibited the highest H_2O_2 content (68.2 ± 0.2 nmol/g FW), followed by IL1 (64.4 ± 0.2 nmol/g FW) and IL3 (64.3 ± 0.2 nmol/g FW). Contrariwise, the parental inbred line IL4 (38.2 ± 0.2 nmol/g FW) displayed the lowest H_2O_2 content, along with IL9 (45.4 ± 0.2 nmol/g FW) and IL7 (50.7 ± 0.2 nmol/g FW) showing higher levels of H_2O_2 under the same irrigation conditions. Additionally, IL3 (47.6 ± 0.2 nmol/g FW) demonstrated the highest H_2O_2 content, followed by IL8 (46.5 ± 0.2 nmol/g FW) and IL10 (45.5 ± 0.2 nmol/g FW) under partial irrigated condition. Whilst, IL4 (33.9 ± 0.2 nmol/g FW) revealed the lowest H_2O_2 content, trailed by IL2 (38.8 ± 0.2 nmol/g FW) and IL9 (38.6 ± 0.2 nmol/g FW) under partial irrigated condition (Table 2).

Among the hybrids, under irrigated conditions, the hybrid IL8 × IL12 demonstrated the highest H_2O_2 content (76.9 ± 0.2 nmol/g FW), followed by IL9 × IL10 (73.1 ± 0.2 nmol/g FW), IL7 × IL12 (70.8 ± 0.2 nmol/g FW), and IL8 × IL11 (70.1 ± 0.2 nmol/g FW). Whereas, the hybrid IL2 × IL5 showed the lowest H_2O_2 content (42.1 ± 0.2 nmol/g FW), tracked by IL9 × IL11, IL10 × IL12, IL11 × IL12 (all three 43.2 ± 0.2 nmol/g FW) and IL3 × IL5 (46.9 ± 0.2 nmol/g FW), Whilst under partial irrigated condition, the hybrid IL1 × IL8 demonstrated relatively low H_2O_2 content (53.5 ± 0.2 nmol/g FW), trailed by IL4 × IL10 (51.8 ± 0.2 nmol/g FW), IL5 × IL8 (50.5 ± 0.2 nmol/g FW), and IL3 × IL6 (50.2 ± 0.2 nmol/g FW). In contrast, the hybrid IL4 × IL5 exhibited the lowest H_2O_2 content (34.9 ± 0.2 nmol/g FW), tracked by IL3 × IL11 (35.7 ± 0.2 nmol/g FW), IL1 × IL6 (36.7 ± 0.2 nmol/g FW), and IL3 × IL12 (36.3 ± 0.2 nmol/g FW) (Table 2).

These findings shed light on the diverse responses of maize genotypes to H_2O_2 accumulation under different irrigation conditions, providing valuable insights into their oxidative stress tolerance mechanisms. The results contribute to the understanding of plant stress responses and may have implications for the development of stress-resistant maize varieties in agricultural practices. Tolerant genotypes did not accumulate more H_2O_2 under drought stress, indicating less severe oxidative damage. Accumulation of higher H_2O_2 content in leaf tissue of sensitive genotype of maize, as compared to tolerant ones under drought stress induced by polyethylene glycol has been reported [72, 71]. Higher H_2O_2 content in the leaves of drought sensitive maize under drought stress as compared to drought tolerant maize has been also observed [70,71].

3.5 Peroxidase Activity

Peroxidase is most important enzyme in the decomposition of H_2O_2 into water and oxygen in the cytosol and chloroplast. Changes in peroxidase activity have been frequently correlated to the response of tolerance or susceptibility of plants to stresses [73]. The data clearly shows that activity of peroxidase increased with increase in stress in roots and shoots of all the genotypes.

Peroxidase activity, an essential enzyme involved in plant defence against oxidative stress, was investigated in various maize parental inbred lines and hybrids under different irrigation conditions. The investigation revealed distinct patterns of peroxidase activity across the genotypes. Under irrigated conditions, the parental inbred lines IL7 and IL11 exhibited the highest peroxidase activity (31.9 ± 0.2 units/mg protein), closely followed by IL2 (29.8 ± 0.2 units/mg protein) and IL9 (29.4 ± 0.2 units/mg protein). On the other hand, IL6 (19.1 ± 0.2 units/mg protein) and IL5 (22.7 ± 0.2 units/mg protein) displayed the lowest peroxidase activity, with IL10 (24.2 ± 0.2 units/mg protein) showing an intermediate level. Moreover, under the same partial irrigated conditions, IL2 (19.3 ± 0.2 units/mg protein) displayed the highest peroxidase activity, tracked by IL6 (17.1 ± 0.2 units/mg protein) and IL3 (16.3 ± 0.2 units/mg protein). Similarly, IL5 (14.4 ± 0.2 units/mg protein) demonstrated the lowest peroxidase activity, followed by IL1 (14.1 ± 0.2 units/mg protein) and IL12 (13.9 ± 0.2 units/mg protein).

Additionally, under irrigated conditions, the hybrid IL1 × IL7 (31.8 ± 0.2 units/mg protein) exhibited the highest peroxidase activity, intimately followed by IL2 × IL10, (31.4 ± 0.2 units/mg protein) and IL3 × IL9 (30.9 ± 0.2 units/mg protein). In contrast, the hybrid IL4 × IL7 (18.4 ± 0.2 units/mg protein) displayed relatively lower peroxidase activity, along with IL7 × IL10 (19.1 ± 0.2 units/mg protein) and IL6 × IL10 (19.9 ± 0.2 units/mg protein) under the same conditions. Furthermore, under partial irrigated conditions, the hybrid IL1 × IL4 (23.1 ± 0.2 units/mg protein) demonstrated the highest peroxidase activity, followed by IL5 × IL12 (20.1 ± 0.2 units/mg

protein) and IL6 × IL11 (19.9 ± 0.2 units/mg protein), whereas the hybrid IL6 × IL11 (12.5 ± 0.2 units/mg protein) confirmed the lowest peroxidase activity, followed by IL11× IL12 (13.4 ± 0.2 units/mg protein) and IL4 × IL6 (14.1 ± 0.2 units/mg protein).

In agreement with our results, an increase in peroxidase activity in drought tolerant and sensitive genotypes of maize under water stress has also been reported by Kolarovic *et al.*[74]. Such commonalities were found in other crop species including cotton, rice and wheat [75-80] Abedi and Pakniyat [81] documented that under water stress conditions, the peroxidase activity increased in the oilseed rape plants. Similar results were also obtained by Chugh et al. [71, Moharramnejad et al.[82] and Xie et al.[83] in maize.

3.6 Glutathione Reductase Activity

Glutathione reductase (GR) is a critical enzyme that helps to reduced glutathione (GSH) from its oxidized form (GSSG). Glutathione is a tripeptide composed of three amino acids *viz*., glutamate, cysteine, and glycine [18]. It plays a crucial role in protecting plant cells from oxidative stress by scavenging harmful reactive oxygen species (ROS) and protecting cellular components from damage [17]. In plants, including maize, glutathione reductase activity is closely linked to the antioxidant defence system, as it helps to maintain an adequate pool of reduced glutathione. The balance between GSH and GSSG is essential for cellular redox signalling and stress responses [23].

Under irrigated conditions, the parental inbred line *namely* IL1 displayed the highest GR activity (0.37 ± 0.02), followed by IL9 (0.34 ± 0.02), and IL2 and IL7 (0.32 ± 0.02). Conversely, the lowest GR activity was observed in IL6 (0.22 ± 0.02) followed by IL8 (0.25 ± 0.02) and IL3 (0.28 ± 0.02) correspondingly, under partial irrigated conditions, IL1 demonstrated the highest GR activity (0.36 ± 0.02), trailed by IL9, IL7 (both 0.29 ± 0.02), However, the lowest GR activity was observed in IL6 (0.19 ± 0.02), followed by IL8 (0.20 ± 0.02) and IL3, IL11 (both 0.24 ± 0.02).

In respect to hybrids, under irrigated conditions, the hybrid IL9 × IL12 exhibited the highest GR activity (0.40 ± 0.02), tracked by IL6 × IL12, IL10 × IL11 (0.37 ± 0.02), and IL2 × IL5 (0.36 ± 0.02). On the other hand, the hybrid IL1 × IL3 showed the lowest GR activity (0.21 ± 0.02), tracked by IL3 × IL8, IL3 × IL9, IL4 × IL5, IL3 × IL12 and IL5 × IL6 (0.23 ± 0.02 each). Whereas, under partial irrigated conditions, the hybrids IL2 × IL5 demonstrated the highest GR activity (0.36 ± 0.02), followed by IL9 × IL12, IL2 × IL3, IL5 × IL8 (0.35 ± 0.02 each), Conversely, the hybrids IL1 × IL3, IL5 × IL6 (0.18 ± 0.02) tracked by IL3 × IL7 (0.19± 0.02) showed the lowest GR activity. The results indicate significant variations in GR activity among different maize inbred lines and their hybrids under varying irrigation conditions. These findings contribute to our understanding of the antioxidative defence mechanisms in maize and may have implications for breeding programmes aimed to develop drought-tolerant maize varieties.Glutathione reductase (GR) activity was increased under drought in plant species such as maize [84], wheat [85], rice [86-87]. GR activity was increased with short-term drought treatment in leaves in wheat [88]. Total GR activity was increased in the drought-tolerant sugarcane genotype under severe water stress but not mild stress, but was increased even under mild stress in non–drought-tolerant cultivars [89]. GR activity was increased in a drought-resistant wheat cultivar subjected to 100% oxygen and water stress [90]. However, under continuous drought, dual-targeted GR transcripts were upregulated in the drought-sensitive cultivar but downregulated in the resistant cultivar [91].

3.7 Catalase Activity

Catalase (CAT) decomposes H_2O_2 into water and oxygen at different cellular locations [46]. A decline in CAT activity is considered as a common response to many stresses [92]. During stress conditions, CAT activity is supposedly decreased due to the inhibition of enzyme synthesis or change in the assembly of enzyme subunits. It may also be associated with degradation caused by induced peroxisomal proteases or due to the photo-inactivation of the enzyme [81].

Catalase activity, a key enzyme involved in the detoxification of hydrogen peroxide, was assessed in various maize parental inbred lines and hybrids under different irrigation conditions. The study revealed distinct patterns of catalase activity across the genotypes. Under irrigated conditions, the parental inbred line IL8 exhibited the highest catalase activity (11.9 ± 0.2 units/mg protein), followed by IL10 (11.2 ± 0.2 units/mg protein) and IL11 (10.8 ± 0.2 units/mg protein).

Contrariwise, IL7 and IL12 displayed the lowest catalase activity (9.6 ± 0.2 units/mg protein. Additionally, under the partial irrigated conditions, IL5 and IL11 demonstrated the highest catalase activity (7.8 ± 0.2 units/mg protein), followed by IL7 (7.6 ± 0.2 units/mg protein) and IL6 and IL12 (7.3 ± 0.2 units/mg protein). Furthermore, under partial irrigated conditions, the parental inbred line IL4 and IL10 exhibited the lowest catalase activity (6.9 ± 0.2 units/mg protein).

Regarding hybrids under irrigated conditions, the hybrid IL2 × IL10 and IL4 × IL12 demonstrated the highest catalase activity (11.9 ± 0.2 units/mg protein), tracked by IL1 × IL11, IL3 × IL11, and IL6 × IL9 (11.7 ± 0.2 units/mg protein). In contrast, the hybrid IL7 × IL9 and IL2 × IL6, IL4 × IL8 displayed relatively lower catalase activity (8.8 ± 0.2 units/mg protein), along with IL4 × IL9 (9.3 ± 0.2 units/mg protein) under the same conditions. Conversely, under partial irrigated conditions, the hybrids *viz*., IL1 × IL8, IL3 × IL5, IL4 × IL11, IL6 × IL12, and IL10 × IL12 showed the highest catalase activity (8.3 ± 0.2 units/mg protein), tracked by IL2 × IL9 (8.2 ± 0.2 units/mg protein). The hybrids *namely* IL1 × IL3, IL1 × IL7, IL10 × IL11, IL3 × IL4 and IL4 × IL10, and demonstrated the lowest catalase activity (6.3 ± 0.2 units/mg protein), followed by IL8 × IL9, IL5 × IL10, IL2 × IL11, and IL2 × IL14 (6.5 ± 0.2 units/mg protein).

In the absence of natural scavengers such as CAT and POD, high level of H_2O_2 accumulates in tissues. Catalase is heterogeneous in nature under drought stress. It might be increased and remain unchanged or decreased on exposure to water stress [93]. Like our results by Anjum *et al.* [94] also observed that catalase activity decreased due to water deficiency as compared with well water conditions in maize hybrids. Xie *et al.* [83] demonstrated a decline in the catalase enzyme activity in maize hybrids under drought stress conditions.

3.8 Diversity and Expression Analysis among Biochemical Parameters

A heat map was conducted on a set of 12 inbred lines along with 66 hybrids, and two checks to examine their biochemical profiles. The analysis resulted in the formation of distinct clusters based on a map (Fig. 3). These clusters encompassed a total of 80 genotypes, which were primarily classified into five major clusters. Cluster I, the largest group, consisted of 39 genotypes. Cluster II contained four genotypes, while Cluster III comprised seven genotypes. Cluster IV encompassed 23 genotypes, and Cluster V confined five genotypes. Furthermore, additional subdivisions were observed within these clusters. It is noteworthy that this clustering pattern indicates significant variations in the biochemical, and antioxidant profiles among the genotypes under study.

Heat maps are commonly employed in expression analysis studies to visually represent data and facilitate quality control. In this investigation, heat map analysis was utilized to examine 80 genotypes based on their expression levels of six biochemical parameters *viz*., chlorophyll, MDA and H_2O_2 contents along with peroxidase, glutathione reductase, and catalase activities. The analysis was conducted under two different irrigation conditions: irrigated and partially irrigated. The heat map visualization employed a colour key, where the colour blue was utilized to represent a range of values from 0.69 to 76.9. By utilizing this approach, the heat map facilitated the identification of patterns and variations in the expression levels of the different biochemical parameters among the genotypes under different irrigation (irrigated and partially irrigated) conditions.

Based on the heat map analysis conducted under irrigated conditions, certain observations can be made regarding the expression levels of different biochemical parameters in the genotypes studied. The expression of chlorophyll content was found to be highest in the parent IL10 and the hybrid IL2 × IL10, while it was lowest in IL5 and IL6 × IL10. In terms of the MDA test, the expression was highest in IL9 and IL1 × IL9 for the parent and hybrid, respectively, whereas it was lowest in IL2 and IL6 × IL12 Regarding H_2O_2 content, IL3 and IL1 × IL8 exhibited the highest expression, while IL4 and IL4 × IL5 showed the lowest expression. For peroxidase activity, IL2 and IL1 × IL4 displayed the highest expression, whereas IL7 and IL6 × IL11 exhibited the lowest expression. Glutathione reductase activity was found to be highest in IL1 and IL9 × IL12, while it was lowest in IL6 and HKI 1128 for the parent and a check variety. Lastly, catalase activity showed maximum expression in IL5 and IL1 × IL8, and minimum expression was recorded in genotypes IL9 and IL10 × IL11 for the parent and hybrid.

Under partial irrigated conditions, the expression of chlorophyll content was highest in the genotypes IL10 and IL2 × IL10, while it was

Table 2. Biochemical profile of the inbred lines, their hybrids and check cultivars under irrigated and partial irrigated conditions

Genotype	Chlorophyll Content		Malondialdehyde Content		H_2O_2 Content		Peroxidase		Glutathione		Catalase	
	I	PI	I	PI	I	PI	I	PI	I	PI	I	PI
IL1	0.74	0.71	12.5	9.7	64.4	42.4	25.8	14.1	0.37	0.36	10.4	7.20
IL2	0.75	0.70	13.5	8.4	57.5	38.8	29.8	19.3	0.32	0.30	9.80	7.10
IL3	0.77	0.70	12.7	9.7	64.3	47.6	28.4	16.3	0.28	0.24	9.80	6.50
IL4	0.75	0.69	15.8	10.2	38.2	33.9	29.2	14.8	0.30	0.27	8.80	6.90
IL5	0.69	0.62	11.6	8.5	68.2	45.4	22.7	14.4	0.33	0.27	9.30	7.80
IL6	0.88	0.80	15.9	9.4	56.5	44.3	19.1	17.1	0.22	0.19	9.60	7.30
IL7	0.76	0.71	15.5	10.3	50.7	43.5	31.9	13.2	0.32	0.28	10.70	7.60
IL8	0.81	0.70	16.4	9.8	62.3	46.5	26.7	15.5	0.25	0.20	11.90	7.10
IL9	0.84	0.79	14.5	10.4	45.4	38.6	29.4	14.7	0.34	0.29	10.70	6.50
IL10	0.89	0.80	12.5	9.7	58.7	45.5	24.2	15.1	0.31	0.28	11.20	6.90
IL11	0.76	0.75	13.5	8.4	54.3	39.6	31.9	15.9	0.29	0.24	10.80	7.80
IL12	0.77	0.71	12.7	9.7	56.4	40.5	25.5	13.9	0.33	0.29	9.60	7.30
IL1 × IL2	0.80	0.76	12.9	10.3	42.1	38.5	21.3	18.9	0.30	0.26	10.70	7.80
IL1 × IL3	0.82	0.79	15.2	9.5	53.3	37.9	28.9	15.7	0.21	0.18	11.20	6.30
IL1 × IL4	0.87	0.80	13.5	10.7	56.2	49.3	29.6	20.1	0.26	0.22	10.80	7.80
IL1 × IL5	0.78	0.71	11.7	9.2	63.6	38.6	30.9	15.9	0.26	0.21	9.60	6.50
IL1 × IL6	0.76	0.73	17.4	10.3	56.2	36.7	28.9	16.4	0.29	0.22	10.90	7.90
IL1 × IL7	0.77	0.72	15.1	10.4	59.4	41.1	31.8	18.7	0.32	0.28	10.20	6.30
IL1 × IL8	0.73	0.69	15.8	14.3	55.3	53.5	30.4	19.6	0.30	0.27	10.20	8.30
IL1 × IL9	0.78	0.71	16.6	14.6	55.7	46.9	27.6	17.2	0.28	0.21	10.80	7.80
IL1 × IL10	0.76	0.71	15.7	10.3	69.6	45.3	29.4	17.3	0.30	0.26	10.30	7.10
IL1 × IL11	0.74	0.69	12.8	9.6	66.4	44.4	27.8	19.1	0.27	0.21	11.7	7.70
IL1 × IL12	0.75	0.69	15.9	10.9	55.7	47.7	31.4	16.7	0.33	0.28	10.30	7.20
IL2 × IL3	0.77	0.70	14.6	10.2	55.7	49.4	27.3	17.2	0.34	0.29	10.40	7.10
IL2 × IL4	0.75	0.72	15.3	10.2	60.7	47.3	30.6	19.4	0.28	0.24	9.80	6.50
IL2 × IL5	0.69	0.60	12.7	9.6	53.7	45.6	21.8	16.6	0.36	0.31	9.80	6.90
IL2 × IL6	0.88	0.80	10.9	7.8	48.9	46.8	25.8	17.3	0.29	0.22	8.80	7.80
IL2 × IL7	0.76	0.75	10.8	9.9	53.9	47.1	24.2	16.5	0.25	0.20	9.3	7.3
IL2 × IL8	0.81	0.75	11.2	8.5	51.6	46.9	28.3	17.7	0.28	0.22	9.6	7.6

Genotype	Chlorophyll Content		Malondialdehyde Content		H_2O_2 Content		Peroxidase		Glutathione		Catalase	
	I	PI	I	PI	I	PI	I	PI	I	PI	I	PI
IL2 × IL9	0.84	0.79	12.9	8.2	52.5	47.9	26.9	19.7	0.32	0.29	10.9	8.2
IL2 × IL10	0.89	0.82	12.2	9.4	59.5	42.1	31.4	16.9	0.29	0.22	11.9	7.8
IL2 × IL11	0.76	0.71	13.3	7.9	48.3	40.7	26.5	16.4	0.31	0.28	10.7	6.5
IL2 × IL12	0.77	0.7	12.5	8.4	49.8	46.2	24.8	15.3	0.28	0.22	11.2	7.9
IL3 × IL4	0.76	0.71	13.7	7.2	66.4	40.1	26.4	14.4	0.30	0.27	10.8	6.3
IL3 × IL5	0.77	0.7	13.2	9.9	46.9	39.8	27.1	17.8	0.30	0.26	9.6	8.3
IL3 × IL6	0.71	0.69	12.8	7.4	55.7	50.2	20.5	17.5	0.26	0.22	10.9	7.8
IL3 × IL7	0.78	0.72	12.7	8.3	50.5	42.7	25.1	17.1	0.24	0.19	10.2	7.1
IL3 × IL8	0.76	0.7	13.8	7.3	65.5	47.3	20.3	18.5	0.23	0.19	10.2	7.7
IL3 × IL9	0.74	0.69	12.6	8.9	48.1	42.4	23.1	19.6	0.23	0.21	10.8	7.2
IL3 × IL10	0.75	0.7	10.9	7.5	64.8	39.1	30.9	16.3	0.28	0.23	10.3	7.1
IL3 × IL11	0.77	0.72	10.3	7.3	54.6	35.7	26.6	16.7	0.26	0.22	11.7	6.5
IL3 × IL12	0.75	0.73	10.5	8.2	57.8	36.3	23.7	16.6	0.24	0.20	10.3	6.9
IL4 × IL5	0.69	0.62	12.2	9.1	54.3	34.9	23.3	16.2	0.23	0.19	10.4	7.8
IL4 × IL6	0.88	0.81	13.6	8.4	53.1	38.1	24.9	14.1	0.29	0.21	9.8	7.3
IL4 × IL7	0.76	0.72	12.7	7.2	57.9	42.2	18.4	15.9	0.32	0.27	9.8	7.6
IL4 × IL8	0.81	0.78	17.4	10.3	52.5	43.8	25.7	17.2	0.27	0.22	8.8	6.5
IL4 × IL9	0.84	0.8	15.1	10.4	54.2	45.5	24.2	18.6	0.27	0.21	9.3	7.9
IL4 × IL10	0.80	0.76	15.8	14.3	68.5	51.8	24.2	16.9	0.25	0.21	9.6	6.3
IL4 × IL11	0.82	0.79	16.6	14.6	61.4	39.8	21.6	16.6	0.30	0.25	10.9	8.3
IL4 × IL12	0.87	0.82	15.7	10.3	52.9	46.6	29.2	16.9	0.24	0.20	11.9	7.8
IL5 × IL6	0.78	0.74	12.8	9.6	55.4	48.1	23.7	16.6	0.23	0.18	10.7	7.1
IL5 × IL7	0.76	0.71	15.9	10.9	51.9	42.1	23.9	17.5	0.26	0.21	11.2	7.7
IL5 × IL8	0.77	0.72	14.6	10.2	57.1	50.5	21.5	16.3	0.34	0.30	10.8	7.2
IL5 × IL9	0.73	0.69	15.3	10.2	52.4	40.8	23.2	17.5	0.26	0.21	9.6	7.1
IL5 × IL10	0.78	0.71	12.7	9.6	50.4	42.9	23.8	17.1	0.29	0.22	10.9	6.5
IL5 × IL11	0.76	0.74	10.9	7.8	60.8	48.1	24.4	16.6	0.32	0.28	10.2	6.9
IL5 × IL12	0.74	0.69	10.8	9.9	63.4	44.6	20.1	17.3	0.28	0.22	10.2	7.8
IL6 × IL7	0.75	0.71	11.2	8.5	59.7	36.8	20.6	15.3	0.34	0.30	10.8	7.3
IL6 × IL8	0.77	0.72	12.9	8.2	61.3	45.3	23.1	15.5	0.30	0.27	10.3	7.6
IL6 × IL9	0.75	0.71	12.2	9.4	68.4	49.3	24.9	16.9	0.30	0.26	11.7	6.5
IL6 × IL10	0.69	0.62	13.3	7.9	51.9	44.7	22.1	17.3	0.26	0.22	10.3	7.9

Genotype	Chlorophyll Content		Malondialdehyde Content		H_2O_2 Content		Peroxidase		Glutathione		Catalase	
	I	PI	I	PI	I	PI	I	PI	I	PI	I	PI
IL6 × IL11	0.87	0.8	12.5	8.4	60.7	42.2	19.9	12.5	0.28	0.21	10.4	6.3
IL6 × IL12	0.76	0.72	13.7	7.2	67.7	45.9	22.3	15.4	0.37	0.32	9.8	8.3
IL7 × IL8	0.81	0.76	13.2	9.9	59.6	45.5	24.5	16.6	0.35	0.30	9.8	7.8
IL7 × IL9	0.84	0.8	12.8	7.4	61.4	38.1	22.4	15.7	0.31	0.25	8.8	7.1
IL7 × IL10	0.89	0.81	12.7	8.3	66.2	38.7	19.1	17.1	0.31	0.26	9.3	7.7
IL7 × IL11	0.76	0.71	13.8	7.3	60.7	47.8	24.9	16.4	0.26	0.21	9.6	7.2
IL7 × IL12	0.77	0.7	12.6	8.9	70.8	42.9	23.8	16.2	0.24	0.20	10.9	7.1
IL8 × IL9	0.76	0.72	10.9	7.5	59.4	46.5	25.3	15.6	0.26	0.21	11.9	6.5
IL8 × IL10	0.77	0.73	10.3	7.3	62.6	45.3	22.5	16.4	0.30	0.28	10.7	6.9
IL8 × IL11	0.71	0.68	10.5	8.2	70.1	41.1	22.5	15.7	0.29	0.24	11.2	7.8
IL8 × IL12	0.80	0.76	12.2	9.1	76.9	47.7	30.5	16.1	0.28	0.22	10.8	7.3
IL9 × IL10	0.82	0.75	17.4	10.3	73.1	46.1	22.2	16.7	0.27	0.21	9.6	7.6
IL9 × IL11	0.87	0.81	15.1	10.4	44.2	38.3	20.1	14.2	0.26	0.22	10.9	6.5
IL9 × IL12	0.78	0.74	15.8	14.3	58.3	41.7	22.2	16.4	0.40	0.35	10.2	7.9
IL10 × IL11	0.76	0.72	16.6	14.6	50.5	44.3	23.1	15.8	0.37	0.31	10.2	6.3
IL10 × IL12	0.77	0.71	15.7	10.3	60.8	43.3	28.2	15.8	0.34	0.29	10.8	8.3
IL11 × IL12	0.73	0.69	15.5	10.3	57.8	43.4	23.5	13.4	0.34	0.28	10.3	7.8
HKI 1105	0.78	0.70	16.4	9.8	55.7	45.4	28.5	14.5	0.35	0.30	11.7	7.1
HKI 1128	0.76	0.71	14.5	10.4	58.7	46.5	38.7	15.7	0.26	0.24	10.3	7.7
SEm	**1.023**	**1.659**	**1.081**	**1.236**	**1.728**	**1.965**	**2.68**	**2.541**	**0.196**	**1.235**	**1.641**	**1.234**
CV	**6.02**	**5.23**	**7.5**	**8.56**	**6.32**	**3.45**	**5.36**	**4.53**	**3.36**	**5.23**	**4.23**	**6.32**

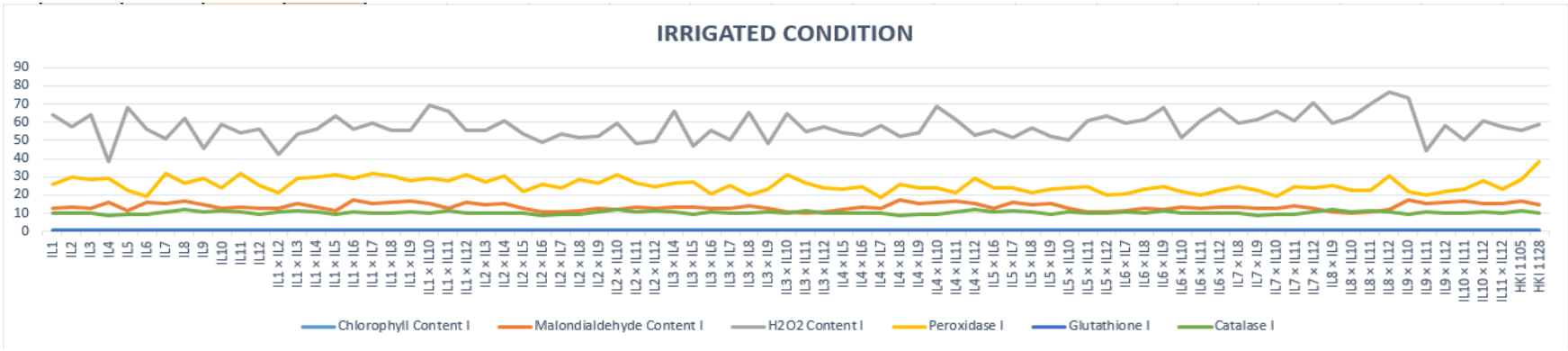

Fig. 1. Line diagram of six important biochemical traits recorded in 80 maize genotypes under irrigated condition

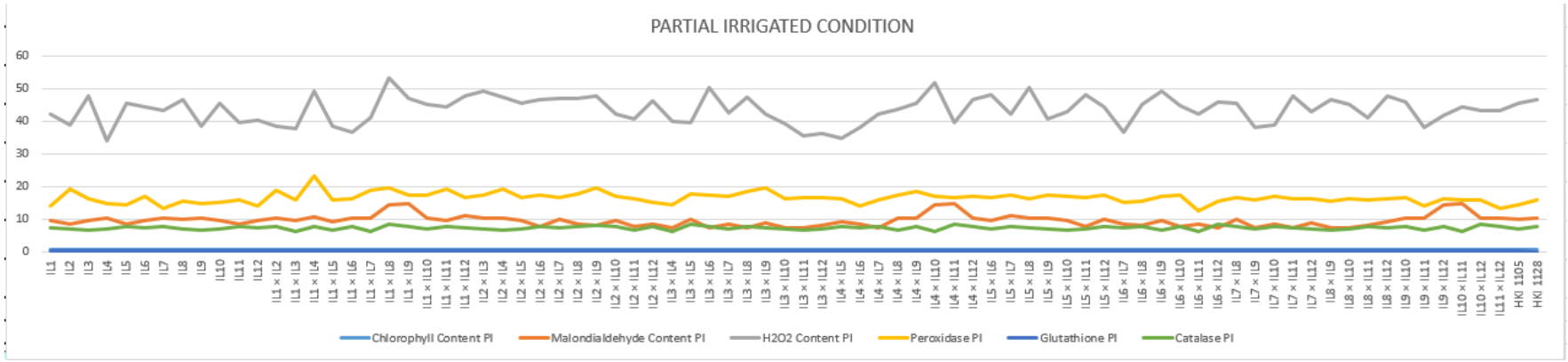

Fig. 2 Line diagram of six important biochemical traits recorded in 80 maize genotypes under partial irrigated condition

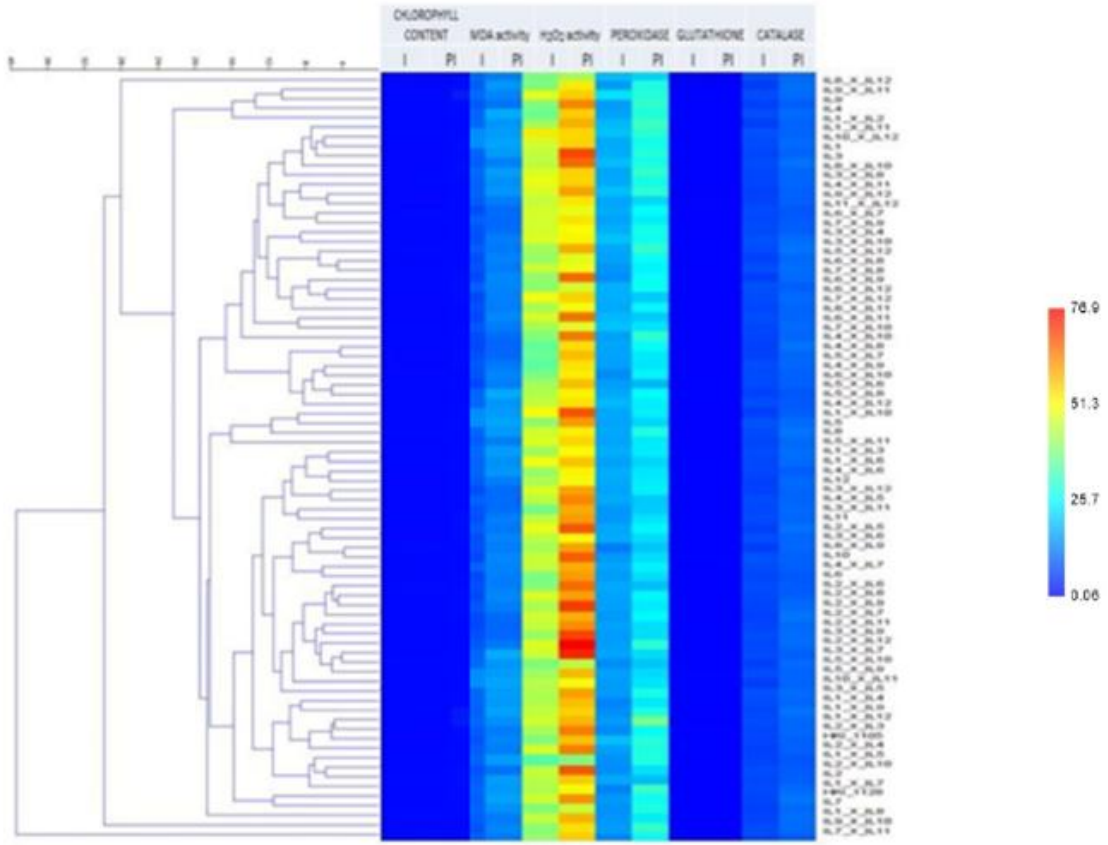

Fig. 3. Dendrogram and heat map analysis of various biochemical parameter in inbred lines, their hybrids and check cultivars under irrigated (I) and partial irrigated condition (PI)

lowest in IL5 and IL6 × IL10. In terms of the MDA test, expression was highest in the genotypes IL8 and IL1 × IL6, while it was lowest in IL5 and IL8 × IL10. Regarding H_2O_2 content, IL5 and IL8 × IL12 showed the highest expression level, whereas IL4 and IL1 × IL2 exhibited the lowest expression. For peroxidase activity, IL7 and HKI 1128 displayed the highest expression for the parent and check variety, respectively, whereas IL6 and IL4 × IL7 exhibited the lowest expression. Glutathione reductase activity was found to be highest in IL1 and IL9 × IL12, while it was lowest in IL6 and HKI 1128 for the parent and check cultivar. Lastly, catalase activity showed maximum expression level in genotypes IL8 and IL2 × IL10, and minimum expression was recorded for the genotypes IL4 and IL7 × IL9.

According Santos et al. [95], genotypes from the same group are genetically similar and their combinations may cause inferior variability when compared with the other groups. The inbred lines in distant groups, is indicative of being genetic divergence and can be considered promising in artificial crosses. Moreover, the divergence and genetic relations studies regarding physiological quality and biochemical composition support the selection strategies, aiming at the quality of seeds.

4. CONCLUSION

This study investigated the variation in different biochemical parameters related to antioxidant defence mechanisms in maize parental inbred lines and their hybrids under different irrigation conditions. The results revealed significant differences in chlorophyll, MDA, and H_2O_2 contents, along with peroxidase, glutathione reductase, and catalase activities among the genotypes studied. Overall, the phylogenetic analysis and heat map visualization highlighted the diversity in the antioxidant defence mechanisms among the maize genotypes, suggesting potential variations in stress response and antioxidant activity. These findings can have important implications for maize breeding programmes aimed to develop drought-tolerant varieties with improved antioxidant capacity and stress resilience.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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