

RELATIONSHIP BETWEEN HIGH-TEMPERATURE STRESS AND KEY PHYSIO-CHEMICAL, REACTIVE OXYGEN SPECIES AND ANTIOXIDANTS IN SPRING MAIZE HYBRIDS UNDER SEMI-ARID CONDITIONS

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Abstract: High-temperature stress is one of the hurdles to achieving self-sufficiency and sustainability in maize production globally. The current experimental study was executed to identify the best suitable maize hybrids for heat-prone areas based on their performance. During spring 2020 & 2021, hybrids were sown under two stress conditions (a) control sowing and (b) late sowing. Kernel yield and related characteristics varied significantly among maize hybrids across both situations ($P < 0.05$). Under High-temperature stress, correlation analysis uncovered a positive relationship between kernel yield and chlorophyll a ($r = 0.77^{**}$, 0.54^{**}), chlorophyll b ($r = 0.72^{**}$, 0.66^{**}), net photosynthetic rate ($r = 0.71^{**}$, 0.67^{**}), proline contents ($r = 0.59^*$, 0.54^{**}), hydrogen peroxide ($r = 0.54^*$, $0.17NS$), thousand kernel weight ($r = 0.71^*$, 0.38^*). Principal component and biplots analysis unveiled that the first four principal components accountable for 78.0% of the total variability among hybrids, with days to 50% silking, plant height, number of kernels per ear, kernel yield, net photosynthetic rate, hydrogen peroxide, malondialdehyde, and catalase as the primary sources of variation. Agglomerative Hierarchical Clustering (AHC) categorizes indigenous and multinational maize hybrids into three classes under stress treatments. The cluster analysis further revealed that indigenous hybrids, particularly YH-5395, YH-5482 and YH-5427 were the most heat tolerant and productive hybrids while YH-5404, P-1543 and JPL-1908 were among the most heat susceptible ones. Consequently, these hybrids are recommended for widespread cultivation, particularly in regions prone to high temperatures.

Keywords: High temperature, biplot analysis, climate change, photosynthesis, antioxidants

Introduction

Climate change is having a significant impact on crop production, affecting both the quantity and quality of crops grown around the world. Rising temperatures, changes in precipitation patterns, and extreme weather events impact agricultural systems. Several independent studies have shown that climate change has already severely affected crop yields. For every 1 °C increase in temperature, global wheat yields could fall by 6% (Lobell et al., 2011), while a second study expressed concern about the decline of maize, rice, and wheat yields in certain regions of the world as a result of a rise in temperature (Asseng et al., 2013). Moreover, extreme weather events such as

droughts, heatwaves, floods are also impacting crop yields, and these impacts are likely to become more severe in the future and will reduce crop yields by 30% (Schmidhuber and Tubiello, 2007). Therefore, efforts must be made to increase crop productivity by introducing high-yield, climate-smart crop varieties to combat climate change effects. Maize, also known as corn, is a cereal crop native to central Mexico. It is one of the world's most widely grown and important food crops, with a history of cultivation that dates back thousands of years. Maize was first domesticated by indigenous people in Mexico around 10,000 years ago. Maize is a versatile

crop that has many uses. It is most commonly used as food for both humans and animals. In terms of human consumption, it is used to make various foods, such as cornmeal, tortillas, cornstarch, and corn syrup. It is also used as feed for livestock, particularly chickens, pigs and cows. Maize is also used to produce biofuels, and its by-products manufacture a wide range of industrial and consumer products, including adhesives, plastics, and cosmetics. Additionally, maize can be used to produce ethanol, an alcohol fuel blended with gasoline to power vehicles. An area of 199.08 million hectares was planted with maize, and 1,129.44 million metric tons of produce was obtained with an average of 5.67 tons per hectare in 2020-21 globally (USDA, 2022). Pakistan cultivated maize on 1,418 million hectares in 2020-21, producing 8,465 million tonnes at an average yield of 5.97 tons per hectare (ESP, 2021). Though Pakistan's maize yield per hectare is higher than the global average (5.9 tonnes ha⁻¹), it is still quite low when compared to Turkey (11.45 tonnes ha⁻¹), the United States (10.76 tonnes ha⁻¹), Canada (9.63 tonnes ha⁻¹), Egypt (8.00 tonnes ha⁻¹), Argentina (7.94 tonnes ha⁻¹) and the European Union (7.30 tonnes ha⁻¹) (USDA, 2022). The principal causes of the low yield per hectare of maize in Pakistan are high temperature, the prevalence of drought stress, poor crop management, poor crop stand, insufficient plant population, a high incidence of disease infestation and insect-pest attack, and the use of low-quality, substandard seed for sowing (Yousaf et al., 2021).

High temperature stress is one of the major reasons for lower maize production in Pakistan. Although maize is a C4 plant and efficiently utilizes CO₂, it still is sensitive to high temperatures especially at the reproductive stage. Studies have shown that high temperatures can lead to reduced growth, delayed maturity, and lower yields in maize (Singh et al., 2013; Hall, 2002). Furthermore, high temperatures can inhibit growth and reduce the rate of photosynthesis in maize leaves (Liu et al., 2010), thus reducing the per-acre yield of maize crops. High temperatures during critical developmental stages of the crop, such as pollination and grain-filling, can lead to significant reductions in yield. Lobell et al. (2011) projected as high as 65% reduction in maize production due to High temperatures. Photosynthesis is one of the major processes that are severely affected by High temperature. The key photosynthetic pigments, including *chlorophyll a*, *chlorophyll b* and carotenoids, markedly reduced under High-temperature conditions, resulting in decreased CO₂ assimilation rate (Ashraf and Harris, 2013).

The present experiment was planned to evaluate the performance of maize hybrids under High-temperature stress at the pre-anthesis stage. Moreover, the severity of High temperature in the pre-anthesis stage will be evaluated based on key morpho-physiological and biochemical traits. The results obtained from this study will not only advance our understanding of impacts of High temperatures on maize growth and development. Still, they will also bring a change in the livelihood of farmers by increasing their farm income.

Materials and Methods

Experimental Material and Layout

This experiment was conducted at the field area of Maize and Millets Research Institute, Yusafwala-Sahiwal (MMRI), Pakistan, over two consecutive spring seasons under field circumstances (2020 & 2021). Ten maize hybrids including six indigenous i.e., YH-5404, YH-5427, YH-5482, YH-5395, FH-1046 and YH-1898 while four multinational maize hybrids i.e., JPL-1908, NK-8441, SB-9663 and P-1543. These hybrids were sown in triplicates using a randomized complete block design with a split plot arrangement.

High-temperature stress Treatments

In both spring seasons, maize hybrids were subjected to two stress treatments: control and late planting (heat treatment). The control set was sown during the second week of February, whereas the late sown (High-temperature stress) set was planted during 3rd week of March, such that the high temperature coincided with the pre-anthesis stage of the hybrids and provided them with a heat shock. Four rows per entry, 75 cm apart and four meters in length, were sowed. The space between plants was maintained at 20 cm. Two seeds per hill were sown with the assistance of a manually-driven dibbler. Early seedlings were thinned to achieve an optimal plant population.

Recording/Masurement of Plant Parameters

Days to 50% silking were measured as the days taken by the plant to complete their 50% silking from date of sowing. Several other key morphological traits, i.e., plant height (PH), ear length (EL), ear weight (EW), number of kernels per ear (NK/E), thousand kernel weight (TKW) and kernel yield per hectare (KY) were also recorded. Net photosynthetic rate (Pn), a vital physiological parameter, was evaluated using an Infrared gas analyzer (IRGA) through CI-320, as recommended and applied by Yousef et al. (2022a). Using a Shimadzu UV-1280, the photosynthetic pigments chlorophyll a (Chl a) and chlorophyll b (Chl b) were measured. The quantity of free proline was determined using the technique described by Efeolu

et al. (2009). The quantities of reactive oxygen species, i.e., malondialdehyde (MDA) and hydrogen peroxide (H₂O₂), were determined using the procedures described by Heath and Packer (1968) and Sergiev et al. (1997), respectively. The enzymatic activity of catalase (CAT) and superoxide dismutase (SOD) was quantified following the protocol developed by Chance and Maehly (1955) with a few modifications.

Statistical Analysis

The data recorded, measured and quantified through different procedures were subjected to analysis of variance (ANOVA) and correlation coefficient analysis to compute the difference among maize hybrids on their morphological, physiological and biochemical parameters their relationship with each other (Steel et al., 1997). Principal component and

cluster analysis were applied to describe and classify maize crosses for controlled and heat-stressed plant characteristics (Sneath and Sokal, 1973). Three statistical software, namely SPSS, XLSTAT and OriginPro 2022, were utilized for data analysis and graphical display.

Metrological Data (2020 and 2021)

During the experimental study, data regarding metrological parameters, i.e., Minimum temperature/maximum temperature (°C), rainfall (mm), and relative humidity, were recorded daily. The data for the daily average maximum temperature during the stress period revealed that hybrids under High-temperature stress (40.8°C and 42.4°C) experienced 4-5 °C more temperature than control (37.4 °C and 37.5 °C) during 2020 and 2021, respectively.

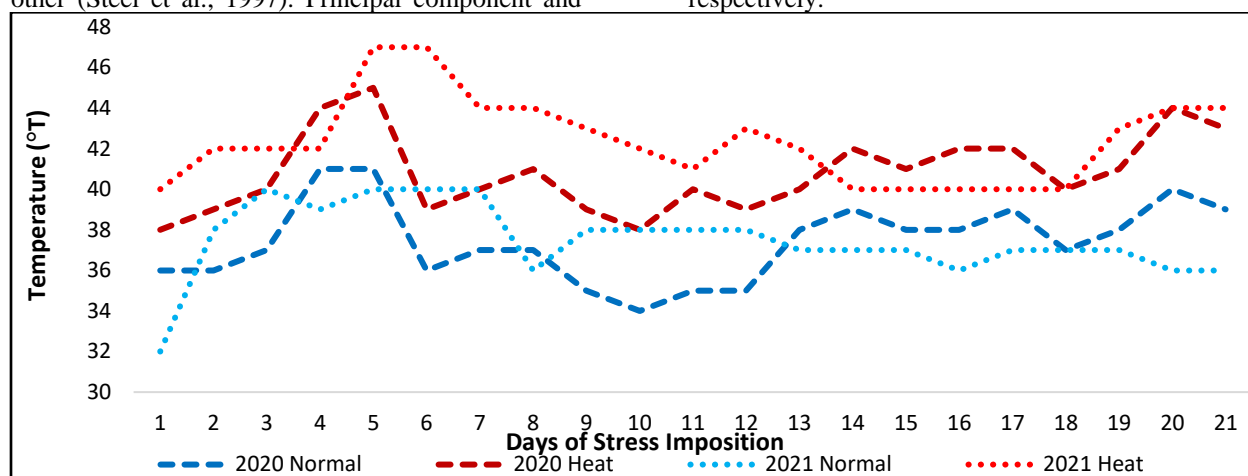


Figure 1: Variation in maximum temperature in control and High temperature stress conditions during stress period for two years (2020 & 2021) in maize hybrids

Results

Analysis of Variance (ANOVA)

The result obtained through ANOVA demonstrated the existence of substantial variation between maize hybrids, stress treatments and seasons for studied plant parameters (Table 1). All the study traits i.e., DS 50%, PH, EL, EW, NK/E, KY, TKW, Pn, Chl a, Chl b, Proline, H₂O₂, MDA, SOD and CAT showed

highly significant variations among maize hybrids based on the studied traits. However, only four plant traits, i.e., PH, TKW, MDA and SOD showed significant variations for seasons, while the variation for other traits was non-significant. Similarly, only EW and hydrogen peroxide showed significant variance for hydrogen × season interactions.

Table 1: Mean Squares (MS) of key plant traits in ten maize hybrids under High-temperature stress conditions

Traits/SOV	Replication (R)	Seasons (S)	Error (R×S)	Hybrids (H)	Interaction H×S	Error R×S×H
DS 50%	158.41	156.49 ^{NS}	184.11	20.30 ^{**}	1.71 ^{NS}	3.67
PH	5025	4526 ^{**}	2082	568 ^{**}	98 ^{NS}	73
EL	19.39	0.043 ^{NS}	0.457	1.638	0.508 ^{NS}	0.811
EW	54.46	21.24 ^{NS}	17.51	18.60	6.21 [*]	3.27
NK/E	53255	1031 ^{NS}	1374	11359 ^{**}	1767 ^{NS}	2381
KY	3.37E+07	4.4 E+6 ^{NS}	4.83+6	1.49+6 ^{**}	1.12+5 ^{NS}	1.77+5
TKW	1094	116.3 [*]	51.1	27.3 [*]	11.3 ^{NS}	9.5
Pn	485	0.9 ^{NS}	92.5	30.2 ^{**}	3.5 ^{NS}	3.1
Chl a	1.29	0.45 ^{NS}	0.31	0.18 ^{**}	0.019 ^{NS}	0.014
Chl b	0.32	0.003 ^{NS}	0.19	0.11 ^{**}	0.006 ^{NS}	0.01
Proline	151.0	3.9 ^{NS}	4.2	2.7 ^{**}	0.53 ^{NS}	0.49

[Citation: Ghani, A., Yousaf, M.I., Hussain, K., Hussain, S., Razaq, A., Akhtar, N., Ibrar, I., Kamal, N., Ali, B., Khan, A.M., Shah, S.W.H., Khanum, S., Hassan, R.M. (2023). Relationship between high-temperature stress and key physio-chemical, reactive oxygen species and antioxidants in spring maize hybrids under semi-arid conditions. *Biol. Clin. Sci. Res. J.*, 2023: 199. doi: <https://doi.org/10.54112/bcsrj.v2023i1.199>]

H ₂ O ₂	5308	5478 ^{NS}	5816	988 ^{**}	735 [*]	425
MDA	6854.0	180.2 ^{**}	64.3	60.1 ^{**}	10.1 ^{NS}	23.7
SOD	1094.82	96.27 [*]	51.12	20.3 ^{**}	11.34 ^{NS}	9.50
CAT	30.47	19.95 ^{NS}	24.35	0.54 ^{**}	0.04 ^{NS}	0.14

** = Highly significant at 1%, * = Significant at 5%, NS = Not significant

DS = days to 50% silking (days), PH = plant height (cm), EL = ear length (cm), EW = Ear width (mm), NK/E = number of grains per cob, KY = Kernel yield (kg/ha), TKW = 1000 kernel weight (g), Pn = Net photosynthetic rate (μmol m⁻² s⁻¹), Chl a = Chlorophyll a (mg g⁻¹ FW), Chl b = Chlorophyll b (mg g⁻¹ FW), Proline = Proline contents (μg g⁻¹ FW), H₂O₂ = Hydrogen peroxide (μmole g⁻¹ FW), MDA = Malondialdehyde (nmole g⁻¹ FW), SOD = Superoxide dismutase (U mg⁻¹ Pro), CAT = Catalase (U mg⁻¹ Pro).

Correlation Coefficient Analysis

Analysis of correlation coefficients found a substantial positive correlation between kernel yield and proline (r= 0.98^{**}), SOD (r= 0.93^{**}), Pn (r= 0.93^{**}), Chl a (r= 0.92^{**}), MDA (r= 0.92^{**}), Chl b (r= 0.81^{**}) and NK/E (r= 0.58^{**}) in maize hybrids under control conditions in season 2020 (Figure 2a). However, PH (r= -0.52^{*}) and CAT (r= -0.44^{*}) had an intermediately negative association with kernel yield under control conditions. On the other hand, a

significantly positive association of kernel yield with Chl a (r= 0.77^{**}), Chl b (r= 0.72^{**}), Pn (r= 0.71^{**}), proline (r= 0.59^{**}) and H₂O₂ (r= 0.54^{**}) in maize hybrids under High-temperature stress conditions during season 2020 (Figure 2b).

Similarly, in the second season (2021), it was discovered that kernel yield is positively associated with SOD (r= 0.97^{**}), MDA (r= 0.94^{**}), Chl a (r= 0.88^{**}), Pn (r= 0.85^{**}) Chl b (r= 0.82^{**}) and proline (r= 0.81^{**}) in maize hybrids under control conditions in season 2020 (Figure 3a). However, CAT (r= -0.63^{**}) and PH (r= -0.56^{**}) had a negative correlation with kernel yield under control conditions. Similar to 2020, in season 2021 too, the correlation between kernel yield and key yield-associated traits was less-stronger. The highest positive association of kernel yield was found with Pn (r= 0.67^{**}) followed by Chl b (r= 0.66^{**}), Chl a (r= 0.54^{*}), proline (r= 0.54^{*}) and DS 50% (r= 0.49^{*}) in maize hybrids under High-temperature conditions (Figure 3b). However, no significant negative correlation of found between kernel yield and any other studied plant trait in 2021.

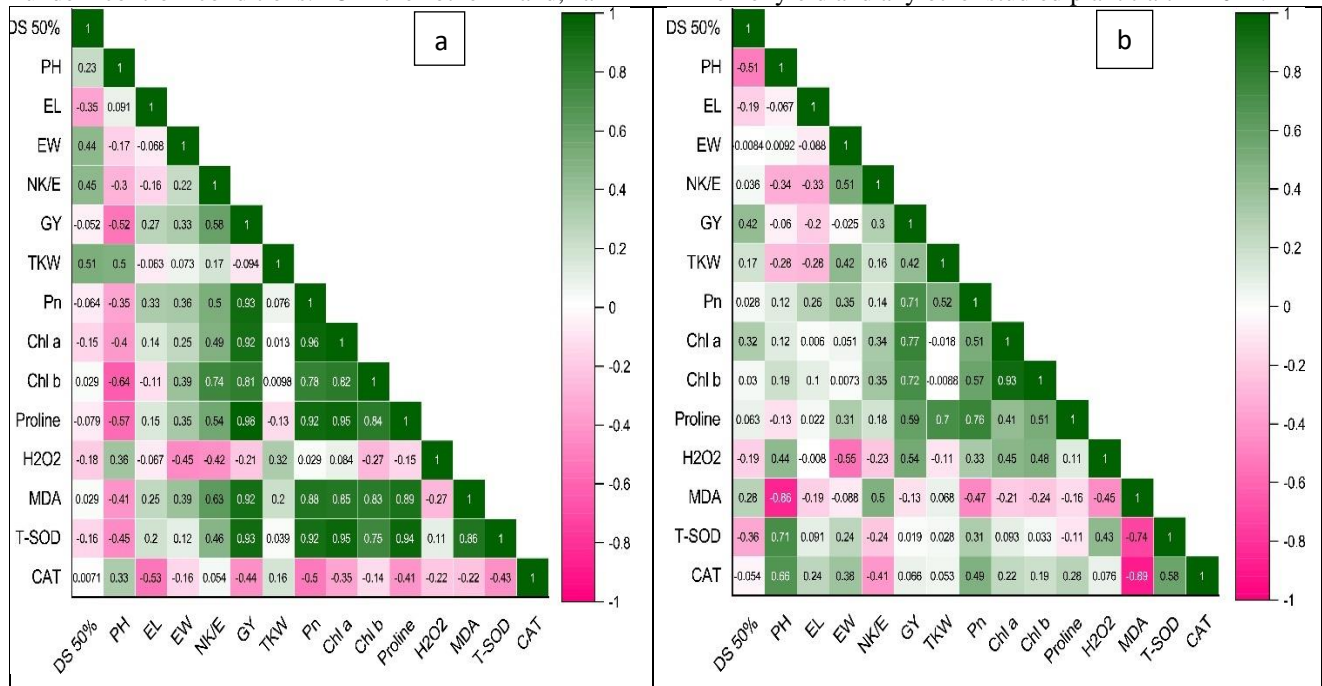


Figure 1: Correlation between key plant traits in maize under (a) Control and (b) High temperature conditions in Maize (2020)

[Citation: Ghani, A., Yousaf, M.I., Hussain, K., Hussain, S., Razaq, A., Akhtar, N., Ibrar, I., Kamal, N., Ali, B., Khan, A.M., Shah, S.W.H., Khanum, S., Hassan, R.M. (2023). Relationship between high-temperature stress and key physio-chemical, reactive oxygen species and antioxidants in spring maize hybrids under semi-arid conditions. *Biol. Clin. Sci. Res. J.*, 2023: 199. doi: <https://doi.org/10.54112/bsrj.v2023i1.199>]

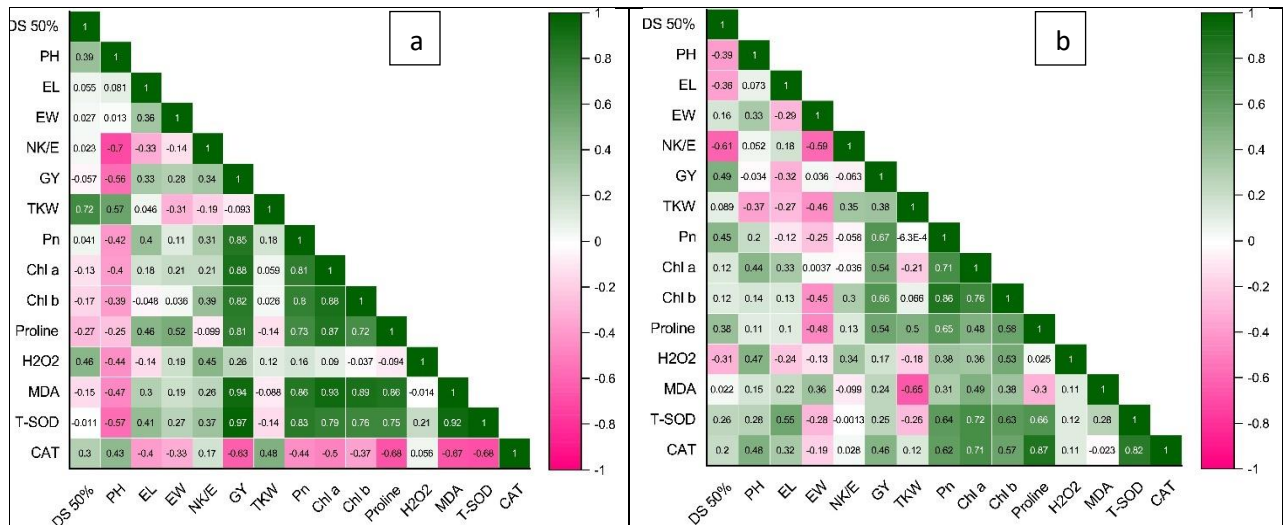


Figure 3: Correlation between key plant traits in maize under (a) Control and (b) High temperature conditions in Maize (2021)

Agglomerative Hierarchical Clustering (AHC)/Cluster Analysis

Agglomerative Hierarchical Clustering (AHC)/Cluster Analysis was applied to classify maize hybrids into groups with high homogeneity and heterogeneity between clusters. The AHC analysis categorized maize hybrids under control into three clusters/classes and High-temperature conditions during 1st season (2020) (Figure 4a). Cluster-I comprised four hybrids i.e., YH-5482, YH-5427, NK-8441 and YH-5395, all of which were top performers from optimal and high temperature conditions (Figure 4a). Cluster-II comprised six

hybrids, i.e., YH-5404, YH-1898, P-1543, FH-1046, JPL-1908 and SB-9663, the maize hybrid, which performed well under control conditions. The cluster-III, the biggest cluster, having all the maize hybrids sown under High temperature, comprised of YH-5482, YH-5427, NK-8441, YH-5395, YH-5404, YH-1898, P-1543, FH-1046, JPL-1908 and SB-9663. In the 2nd season (2021), the dendrogram was slightly changed. The cluster-II comprised nine maize hybrids, including three hybrids from High-temperature environments i.e., YH-5482, YH-5427 and YH-5395, which performed outstandingly during High temperatures (Figure 4b).

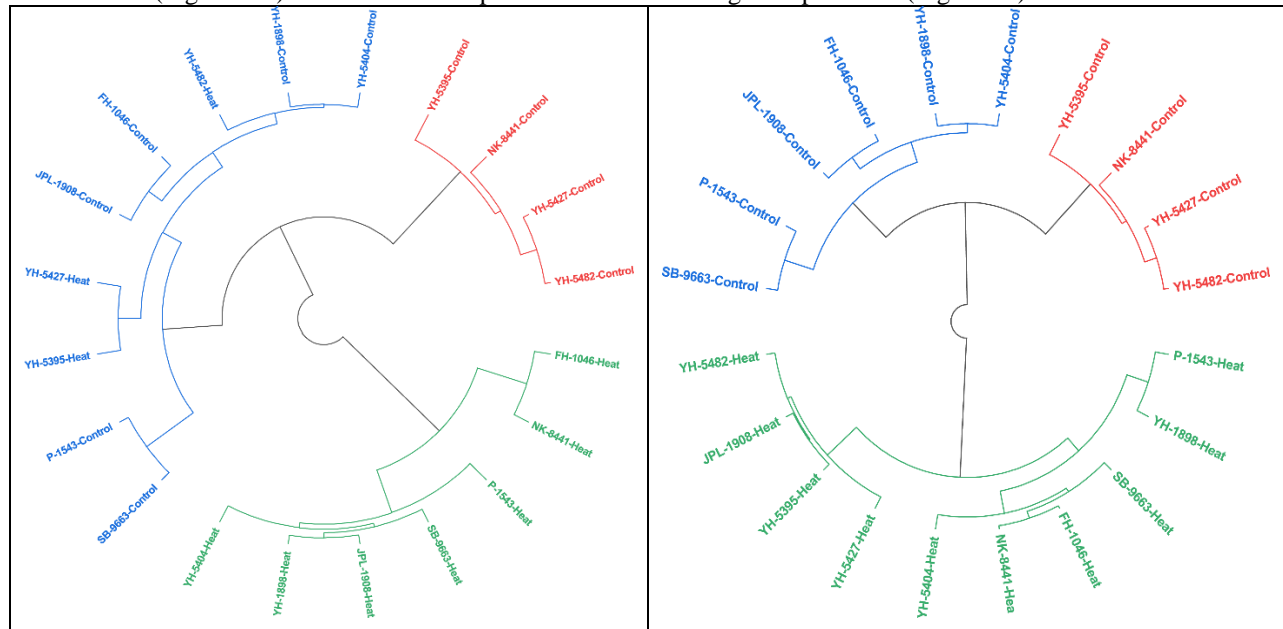


Figure 4: Cluster analysis based dendrogram for key plant traits and maize hybrids under Control and High temperature conditions in (a) (2020) and (b) 2021

Principal Component Analysis (PCA)

Principal component and biplot analysis were applied to investigate and classify maize hybrids

[Citation: Ghani, A., Yousaf, M.I., Hussain, K., Hussain, S., Razaq, A., Akhtar, N., Ibrar, I., Kamal, N., Ali, B., Khan, A.M., Shah, S.W.H., Khanum, S., Hassan, R.M. (2023). Relationship between high-temperature stress and key physio-chemical, reactive oxygen species and antioxidants in spring maize hybrids under semi-arid conditions. *Biol. Clin. Sci. Res. J.*, 2023: 199. doi: <https://doi.org/10.54112/bcsrj.v2023i1.199>]

under contrasting environments. In the current study, PCA extracted 15 principal components (PCs) based on morphological, physiological and biochemical traits under control and stress conditions (Table 2). Among these 15 PCs, only four PCs had an eigenvalue greater than 1 under both control and High-temperature conditions (Table 2). These 4 PCs contributed 78.7% to the variation in the data under control conditions while 82.0% to the total variability under High-temperature conditions (Table 2).

The PC1 accounted for 43.8% of total variability under control conditions and 45.8% under High temperature (Table 2). In PC1, DS 50%, PH, NK/E, KY, Pn, H₂O₂, MDA and CAT were the key contributing traits under both control and High-temperature conditions (Table 3). In PC2, which accounted for 17.9% variability in data under control while 18.1% variability under High-temperature conditions., Chl a & b were the major variability contributing traits in maize hybrids under control and High-temperature conditions (Table 3). In principal component 3, which accounted for 9.5% of the variation in control, EL is the only trait that

significantly contributes towards variations. On the other hand, PC3 contributed 11.3% to variation under high-temperature conditions, and EW, NK/E and TKW were the major contributing traits. The last PC, PC4, contributed 7.6% to variation under control while 6.9% under High-temperature conditions having thousand kernel weight and ear length as the major contributors in variations.

Principal component analysis-based PC1/PC2 biplots were generated to observe the pattern of association between maize hybrids and studied traits under both treatments. The PC1/PC2 biplot of control treatment showed proline contents, plant height, catalase and chlorophyll a & b as the most distinctive traits under stress. Chlorophyll a & b, superoxide dismutase, proline contents, net photosynthetic rate and kernel yield were among the major discriminating traits. Similarly, under High-temperature conditions, five maize hybrids, i.e., YH-5427, YH-5482, FH-1046, SB-9663 and YH-5395 were the best performers. The angle between corresponding lines showed that kernel yield significantly associated with chlorophyll a, chlorophyll b, net photosynthetic rate and ear length.

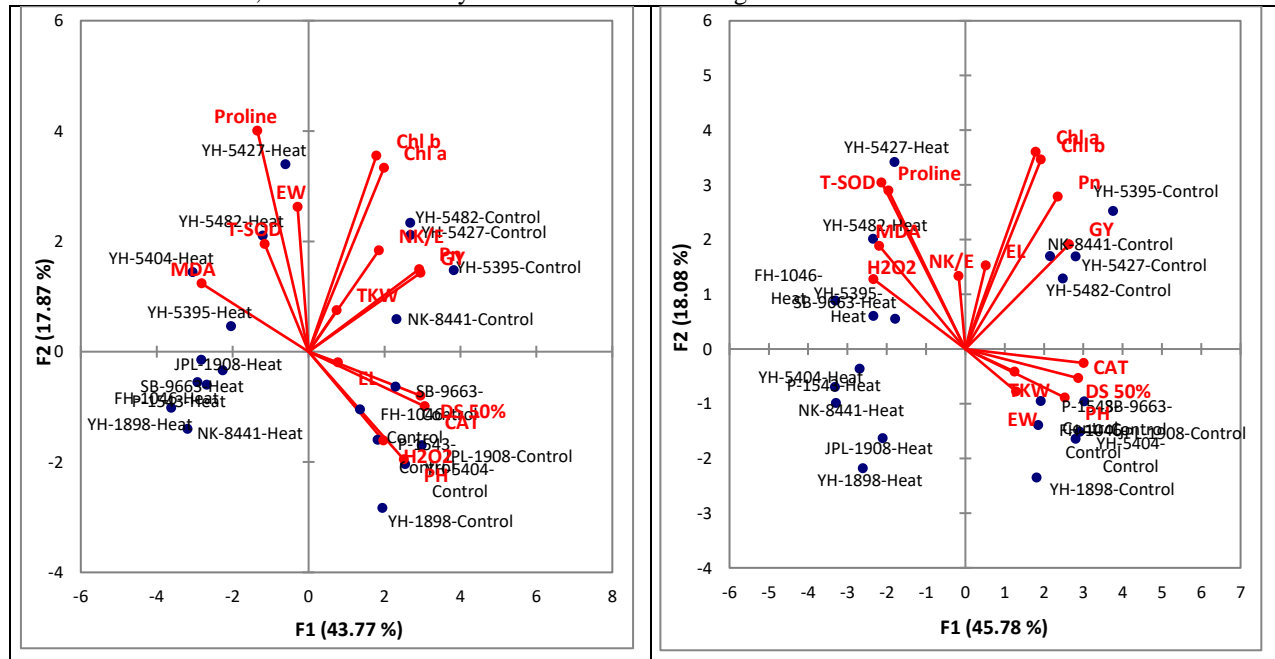


Figure 4: PC1/PC2 biplots for key plant traits and maize hybrids under Control and High temperature conditions in (a) (2020) and (b) 2021 **Table 2:** Principal Components (PCs) for key plant morphological, physiological and biochemical traits in maize hybrids under control and High temperature conditions (2020 & 2021)

	Control Conditions														
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Eigenvalue	6.57	2.68	1.43	1.14	0.97	0.81	0.45	0.34	0.26	0.16	0.11	0.04	0.03	0.02	0.00
Variability (%)	43.8	17.9	9.5	7.6	6.5	5.4	3.0	2.3	1.7	1.1	0.7	0.3	0.2	0.2	0.0
Cumulative %	43.8	61.6	71.1	78.7	85.2	90.6	93.6	95.8	97.5	98.6	99.3	99.6	99.8	100.0	100.0
	High temperature Conditions														

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Eigenvalue	6.87	2.71	1.69	1.03	0.85	0.47	0.42	0.32	0.25	0.16	0.11	0.07	0.03	0.02	0.01
Variability (%)	45.8	18.1	11.3	6.9	5.7	3.1	2.8	2.1	1.7	1.0	0.7	0.4	0.2	0.1	0.0
Cumulative %	45.8	63.9	75.1	82.0	87.7	90.8	93.6	95.7	97.4	98.5	99.2	99.6	99.8	100.0	100.0

Table 3: Principal Components (PCs) correlation with key plant morphological, physiological and biochemical traits in maize hybrids under control and High temperature conditions (2020 & 2021 combined)

	Control				High temperature			
	PC1	PC 2	PC 3	PC 4	PC 1	PC 2	PC 3	PC 4
DS 50%	0.9133	-0.1577	-0.2193	-0.0683	0.9320	-0.1085	-0.0134	0.0141
PH	0.7773	-0.3868	0.0455	0.2626	0.8215	-0.1811	0.1047	0.0740
EL	0.2394	-0.0386	0.6515	0.0745	0.1684	0.3114	0.2749	0.8124
EW	-0.0900	0.5205	-0.4036	0.2187	0.4194	-0.1588	0.7330	-0.2526
NK/E	0.5727	0.3631	-0.4452	-0.3468	-0.0546	0.2716	-0.7027	-0.1334
GY	0.9152	0.2835	0.0348	-0.0726	0.8546	0.3901	0.0201	-0.1497
TKW	0.2295	0.1491	-0.5582	0.6317	0.4073	-0.0845	-0.6219	0.2549
Pn	0.9035	0.2964	0.1115	0.1097	0.7632	0.5681	-0.0045	-0.0506
Chl a	0.6161	0.6603	0.2407	-0.0959	0.5823	0.7350	0.1672	-0.0776
Chl b	0.5543	0.7041	0.1552	-0.2104	0.6237	0.7066	-0.1497	-0.1934
Proline	-0.4181	0.7933	0.0632	0.1653	-0.6337	0.5898	0.0186	0.1576
H2O2	0.6107	-0.3194	0.0359	0.2742	-0.7574	0.2602	-0.1341	-0.2770
MDA	-0.8739	0.2450	-0.1577	-0.1581	-0.7100	0.3841	0.3279	-0.1513
T-SOD	-0.3586	0.3863	0.3913	0.5417	-0.6902	0.6199	0.0986	0.1583
CAT	0.9488	-0.1964	-0.0045	0.0153	0.9767	-0.0521	-0.0195	0.0087

PC = Principal Components

Discussion

High temperature significantly impacts the growth and development of maize (*Zea mays* L.). High temperatures during the vegetative and reproductive stages can cause a reduction in growth and yield. High temperatures during the vegetative stage can cause stunted growth and delay the transition to the reproductive stage. High temperatures during the reproductive stage can cause reduced pollination, and kernel set and ultimately lead to lower yields. Although maize is one of the most adaptive cereal crop of the world, yet it is quite sensitive to high temperatures at reproductive stages, especially early flowering.

The current study was designed to evaluate local and multinational commercial hybrids for their heat tolerance and to elucidate the role of physiological and biochemical traits, especially ROS and antioxidants, in determining heat tolerance. The results from ANOVA showed the presence of highly significant variations among maize hybrids, which depicts the differences in their performance and genetic potential under optimal and High-temperature conditions. Similar results were reported by Yousaf et al. (2017) and Yousaf et al. (2022b), who unveiled the presence of significant variation in maize hybrids under High-temperature conditions.

Correlation analysis was applied to foresee the association of kernel yield with different plant variables under different growing conditions. The results from both seasons (2020 & 2021) exposed a significantly positive association of kernel yield with chlorophyll a, chlorophyll b, net photosynthetic rate, proline contents, days to 50% silking and hydrogen peroxide under High-temperature conditions. Higher contents of chlorophyll a and b are necessary for proper photosynthetic activity in maize under High-temperature conditions which will assure the translocation of sufficient food reserves to sink, resulting in higher production (Khajeh-Hosseini et al., 2016). However, the correlation of ear width was negative with kernel yield under stress. This might be because the increase in ear width is due to the increase of the woody part of the ear called, which take most of the food reserve under stress, thus impacting kernel yield (Wang et al., 2018). The higher the days taken to silking, the more will be biomass that will strengthen the sink, and eventually, high yield will be achieved (Yousaf et al., 2017; Shehzad et al., 2019). Therefore, it's pivotal to select hybridizing parents or successive generations based on these kernel yield-associated traits under High-temperature conditions, especially net photosynthetic rate, chlorophyll a, chlorophyll b, proline contents and days to silking to develop heat tolerant maize hybrids.

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Many researchers used cluster analysis to classify genotypes from various crop species under various ecological conditions, such as drought and High temperature and discovered that this method was helpful for parent selection in hybrid breeding programs to develop heat-resistant genotypes (Saeed et al., 2018; Bhatti et al., 2020; Khalid et al., 2020). Al-Naggar et al. (2020) used cluster analysis to evaluate and categorize 19 maize hybrids and showed that this method successfully characterized maize hybrids based on their genetic diversity. In this experiment, agglomerative hierarchical cluster analysis classified maize hybrids according to their productivity under heat-stress conditions. The cluster analysis grouped the maize hybrids into three subgroups under both seasons, i.e., 2020 and 2021. The categorization was based on the performance of the maize hybrids under control and High-temperature conditions. The results revealed that cluster-I hybrid, i.e., YH-5482, YH-5427, NK-8441 and YH-5395, out-yield other hybrids included in the study under control conditions and under High-temperature conditions. This study re-verified the heat tolerance ability of these hybrids as claimed in several heat-related field studies (Ghani et al., 2020, Yousaf et al., 2021; Yousaf et al., 2022b). However, the performance of a few hybrids included in cluster-III, i.e., YH-5404, P-1543, JPL-1908 and SB-9663, was excellent under control but under High temperature, their performance was below average, indicating their High-temperature sensitivity. The differential response of maize hybrids to High-temperature tolerance was due to their higher net photosynthetic rate, higher quantities of chlorophyll a & b, the higher number of kernels per ear and significant accumulation of enzymatic antioxidants, especially catalase and superoxide dismutase accumulation under stress conditions as reported in several studies (Hussain et al., 2019; Tiwari and Yadav, 2019; Sabagh et al., 2020; Waqas et al., 2021, Riaz et al., 2021).

Biplot and principal component analyses were also used to group maize hybrids into various categories. Using PCA and biplot analysis, it was determined that YH-5482, YH-5427, NK-8441, and YH-5395 were the most productive and heat-tolerant maize hybrids under High-temperature conditions, demonstrating a significant positive association with net photosynthetic rate, plant height, days to 50% silking, chlorophyll a & b, and proline contents. This increase in grain productivity may be linked to increased plant height-based biomass, a higher number of days taken to achieve 50% silking, increased photosynthetic efficiency and greater accumulation of chlorophyll a & b due to their strong association with kernel yield in maize under High temperature as suggested by Lambert et al. (2014).

Most of the imported maize hybrids were primarily heat-susceptible, as revealed by biplot analysis. Ben-Asher et al. (2008) and Yousaf et al. (2022a) discovered that kernel yield was improved with the increase in net photosynthetic rate under high temperature. The principal component analysis further showed that the most distinctive characteristics under High-temperature conditions included chlorophyll a & b, days to 50% silking, plant height, superoxide dismutase, proline contents, catalase and kernel yield. Hence, these characters could be used in selecting parents to develop maize hybrids for the areas experiencing high temperatures. Similar results were observed by Yousaf et al., (2018) and Yousaf et al. (2022b), who found that the indigenous hybrids were more heat tolerant than exotic ones and classified them using multivariate approaches under High-temperature conditions. Ghani et al. (2020) also described that the locally produced yellow maize hybrid YH-5427 had high grain yields under High-temperature conditions.

Conclusion

The findings of the current field study revealed the existence of highly substantial differences in kernel yield and associated morpho-physiological and biochemical parameters across maize hybrids grown under control and heat-stress conditions. Correlation analysis suggested that net photosynthetic rate, days to 50% silking, proline contents, catalase, plant height, chlorophyll a & b and superoxide dismutase were the most significant traits for the choice of parents under High-temperature conditions, as these traits showed significant relationships with kernel yield. The findings from PCA, cluster analysis, and biplot graphs were highly comparable. They demonstrated that locally bred maize hybrids, particularly YH-5482, YH-5427, NK-8441 and YH-5395 were far more heat tolerant than other exotic hybrids like NK-8441, P-1543, SB-9663 and JPL-1908.

Conflict of interest

The authors declared the absence of a conflict of interest.

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