

MORPHOMETRIC, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTRIZATION OF LOCAL AND EXOTIC MAIZE HYBRID UNDER HEAT AND WATER-STRESS STRESS CONDITIONS AT POST-ANTHESIS STAGE

HUSSAIN S¹, GHANI A², KHALID M³, NAYAB SF⁴, HASSAN IU⁵, SATTAR A⁶, KAUSAR S⁷, SALEEM M⁸, ASIF M⁹, AKRAM F¹⁰, IMRAN M¹¹, ULLAH R¹², SARWAR MI¹³, KAMAL N¹⁴, KHAN MA¹⁵, SAJJAD MH¹⁶, YOUSAF MI*^{1, 2}

¹Cotton Research Station (CRS), 63100, Bahawalpur, Pakistan ²Maize and Millets Research Institute (MMRI), Yusafwala, Sahiwal, 57000, Pakistan ³Regional Agricultural Research Institute, Bahawalpur, 63100, Pakistan ⁴Sorghum Research Sub-Station, Dera Ghazi Khan, 32200, Pakistan ⁵Plant Virology Section, Plant Pathology Research Institute, 38000, Faisalabad. ⁶Soil and Water Testing Laboratory, Lodhran, 59320, Pakistan ⁷Pesticide Quality Control Laboratory, Bahawalpur, 63100, Pakistan ⁸Soil and Water Testing Laboratory, Pakpattan, 57400, Pakistan ⁹Soil and Water Testing Laboratory, Bahawalnagar, 62300, Pakistan ¹⁰Soil and Water Testing Laboratory, Nankana Sahib, 39100, Pakistan ¹¹Soil and Water Testing Laboratory, Rahim Yar Khan, 64200, Pakistan ¹²Agricultural Research Station, Bahawalpur, 63100, Pakistan ¹³Soil and Water Testing Laboratory, Ayub Agricultural Research Institute, 38000, Faisalabad ¹⁴Wheat Research Sub-Station, Murree, 47150, Pakistan ¹⁵Department of Plant Breeding and Genetics, The Islamia University of Bahawalpur, 63100, Pakistan ¹⁶Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, 38000, Pakistan *Correspondence author email address: irfanpbg.uaf@gmail.com

(Received, 05th August 2023, Revised 29th October 2023, Published 26th November 2023)

Abstract: The research project was carried out at the Maize and Millets Research Institute in Yusafwala-Sahiwal during the crop season of 2022) using a split-plot design. The present investigation explored the effects of individual and combined effects of water stress and heat stresses on morpho-phenological, physiological and biochemical traits in eight local (YH-5482, YH-5427, YH-5407, FH-1046, YH-5399, JPL-1908, SB-9663 and YH-1898) and two exotic maize hybrids (NK-8441 and P-1543). Significant variations were observed among maize hybrids under discrete and shared effects of water stress and heat stresses for key morphometric and other agronomically important, grain-related traits. Correlation analysis exposed strong positive correlation of grain yield with SPAD value ($r= 0.88^{**}$), Proline ($r= 0.79^{**}$), transpiration rate ($r= 0.78^{**}$), superoxide dismutase ($r= 0.73^{**}$), stomatal conductance ($r= 0.72^{**}$) while negatively correlated with hydrogen peroxide ($r= -0.87^{**}$). Cluster analysis grouped local and exotic maize hybrids into three groups under stress conditions and exposed that local maize genotypes/hybrids, predominantly YH-5427, YH-5482 and YH-5395 were the maximum stress tolerance under individual as well as combined heat + water-stress conditions.

Keywords: Photosynthesis, Grain Quality, Water Stress, Antioxidant Activity, Maize, Stress Physiology

Introduction

Maize, also known as corn, holds significant importance and serves a multitude of purposes in various aspects of human life. This versatile crop is a staple food source for millions around the world. Its significance lies in its role as a dietary staple, providing essential nutrients and energy (Murdia et al., 2016). Maize is a rich source of carbohydrates, fibre, and various vitamins and minerals, making it a crucial component of many diets. Beyond its role as a food source, maize has extensive industrial applications (Kumar et al., 2021). One of the most notable uses is in the production of ethanol, a biofuel that contributes to reducing the world's dependence on fossil fuels. Maize is also used to manufacture several products for instance corn oil, maizebased liquid syrup, and starch which have diverse applications in the food industry. Additionally, maize plays a key role in animal husbandry, serving as a primary ingredient in livestock feed. Livestock and poultry rely on maize-based feeds for their growth and sustenance (Erenstein et al., 2022). The crop's adaptability to different climates and growing conditions further enhances its importance as a global agricultural commodity, contributing to food security and economic stability. Maize is not only essential for human and animal consumption but also has cultural and ornamental value. It has been an integral part of local cultures for centuries, serving as a symbol of heritage

[Citation: Hussain, S., Ghani, A., Khalid, M., Nayab, S.F., Hassan, I.U., Sattar, A., Kausar, S., Saleem, M., Asif, M., Akram, F., Imran, M., Ullah, R., Sarwar, M.I., Kamal, N., Khan, M.A., Sajjad, M.H., Yousaf, M.I. (2023). Morphometric, physiological and biochemical charactrization of local and exotic maize hybrid under heat and water-stress stress conditions at post-anthesis stage. *Biol. Clin. Sci. Res. J.*, **2023**: *548*. doi: <u>https://doi.org/10.54112/bcsrj.v2023i1.548</u>]

1

and tradition. Moreover, maize is used in various arts and crafts applications, including decorations, and ornaments, and even as an ingredient in paints and dyes.

Maize is cultivated as both an autumn and spring crop in Pakistan. The area under maize crop in Pakistan is increasing day by day, especially under spring crop due to the problem faced by the farming community for two other key alternative crops i.e., Cotton and Sugarcane. However, it was observed that maize production in Pakistan (5.83 tons ha-1) is comparably lower than in Turkey (12.83 tons ha-1), USA (10.88 tons ha-1), Canada (10.07 tons ha-1), Egypt (8.0 tons ha-1), Ukraine (6.67 tons ha-1), China (6.44 tons ha-1) and Russia (6.00 tons ha-1) (USDA, 2023). The key reasons for lower grain yield in maize in Pakistan are primarily heat and water-stress stresses, lower availability of standard, high-quality seed, high disease infestation rate, insect-pest attack (shoot fly, fall armyworm, corn borer), higher rates of inputs and poor crop management.

Heat and water stress individually and especially when combined can have profound and detrimental effects on maize, which is a critical staple crop worldwide (Waqas et al., 2021). High temperatures, often associated with climate change, can disrupt maize growth and development in several ways. Elevated temperatures can accelerate the rate of evaporation from the soil and increase transpiration from the plant, leading to water stress and reduced water availability for the crop (Luan and Vico, 2021). This not only inhibits germination and early growth but can also result in smaller grains and lower overall yields. Extreme heat can also interfere with pollination, causing poor grain formation and further yield reduction. Water stress, on the other hand, directly impacts maize by limiting water availability, which is crucial for its growth. Prolonged water scarcity during critical growth stages can stunt the plant's development, decrease the number of grains per ear, and lead to smaller, less productive plants. Water-stress stress can also increase susceptibility to pests and diseases, as the plant's defences are compromised when it's under water stress (Ozturk et al., 2021).

Heat and water stress are two critical environmental factors that have a significant impact on the global production of primary crops. These stresses have been linked to substantial losses, ranging from 31% to a staggering 81% in grain yield of important crops across the world (Farooq et al., 2017). Rising temperatures and prolonged periods of high heat can disrupt various physiological processes in plants, affecting their growth, flowering, and grain formation (Nelimor et al., 2020). Water stress, including both drought and excess moisture in some cases, further compounds the issue by limiting the availability of water for plant uptake and disrupting nutrient absorption. This double whammy of heat and water stress not only reduces crop yield but also threatens food security and the livelihoods of countless farmers globally (Lipiec et al., 2013). Adapting agricultural practices, developing stress-tolerant crop varieties, and implementing water management strategies are critical steps in mitigating these losses and ensuring sustainable food production in the face of changing climatic conditions (Riaz et al., 2021). However, the damage of these stresses is further intensified and determined by several factors including magnitude, period, genus, and phase of plant stress contact (Fahad et al., 2017).

When heat and water stress occur simultaneously, their combined impact can be particularly devastating. The stressors often amplify each other's negative effects, resulting in even greater reductions in maize yields. The combination of high temperatures and limited water availability exacerbates water stress. impairs photosynthesis, and can lead to irreversible damage to the plant's tissues. This can ultimately result in significant crop losses, posing a significant threat to food security in regions where maize is a dietary staple. Like other crops, maize has a number of defence mechanisms to fend off the damaging effects of abiotic stresses. These mechanisms include the production of heat shock protein and activation of stressresponsive genes, the build-up of compatible osmolytes, the activation of enzymatic antioxidants and the accumulation of compatible osmolytes (Hussain et al., 2019).

The effects of individual heat and water-stress stresses have been quantified in a number of studies using their morphophysiological parameters; yet, the cumulative effects of heat and water-stress stresses have not been extensively investigated for traits related to grain yield and quality, particularly in hot and water stress affected areas globally including Pakistan. Furthermore, rather than using real field research, the majority of earlier studies relied on crop prediction models. The goal of the present field investigation is to clarify (1) the precise mechanism underlying heat and water-stress stress tolerance in both individuals and combinations. (2) to look into the causes of maize's resistance to heat and water-stress (3) to assess how well five different maize hybrids perform in relation to one another when exposed to heat and water-stress, as well as how these factors interact.

Methodology

The research study was carried out under natural field conditions for maize growing season 2022) at Maize and Millets Research Institute (MMRI), Yusafwala- Sahiwal, Pakistan. the seeds of six maize hybrids i.e., YH-5482, YH-5427, YH-5407, YH-1898, FH-1046, and YH-5399 were obtained from the MMRI while P-1543 was received from Pioneer seeds. Similarly, three maize hybrids were obtained from other national and multinational seed companies i.e., NK-8441 from Syngenta Seeds, JPL-1908 from local seed company Jullundur seeds Pvt Limited and SB-9663 from Sohna Bheej, Kissan Seed Corporation respectively. These seeds of these hybrids were planted in RCB design under split-plot arrangement in field conditions (a) Optimal growing conditions (b) heat treatment (c) Water deficient condition (d) combined water-stress and heat stress treatment. The Tandzi and Mutengwa (2020) method was employed to determine grain yield. Stomatal conductance (Ci), and transpiration rate (Tr) was measured using the infrared gas analyser named as CI-320. Spectroscopy was used to calculate the concentration of chlorophyll a and chlorophyll b. ROS and antioxidant accumulation was measured according the formula described by Sergiev et al. (1997) and applied by Yousaf et al. (2022).

Statistical Analysis

Sr. #

SPAD

Ear length (EL)

Ear width (EW)

Grains per ear (G/E)

Protein % (Protein)

1

2

3

4

5

6

7

8

9

10

Analysis of variance (ANOVA) and correlation coefficient analysis were performed on the data collected to determine the variations among maize hybrids and the extent and direction of the relationship between different plant traits (Steel and Torrie, 1997). Their mean values were used to draw bar graphs to illustrate the differences among local and multinational maize hybrids under individual and concurrent heat + drought stress conditions. Furthermore,

Table 1a: Plant tra

nt traits recorded/measured under current study			
Plant trait	Sr. #	Plant trait	
Days to 50% anthesis (DT)	11	Transpiration rate (Tr)	
Days to 50% silking (DS)	12	Stomatal conductance (Ci)	
Plant height (PH)	13	Water use efficiency (WUE)	
Ear height (EH)	14	Chlorophyll a (Chl a)	

19

Chlorophyll b (Chl b) 15 16 Proline contents (Proline)

17 Hydrogen peroxide (H₂O₂)

18 Superoxide dismutase (SOD) Grain yield (GY)

The ANOVA's result revealed the frequency of notable variations between maize hybrids under normal, individual, and combined heat and drought stress circumstances (Table 1). All the studied morpho-physiological, agronomic and biochemical parameters including days to 50% anthesis, days to 50% silking, plant height, ear height, ear length, ear

Thousand-grain weight (TGW)

width, grains per ear, thousand-grain weight, SPAD, Protein percentage, transpiration rate, stomatal conductance, water use efficiency, chlorophyll a, chlorophyll b, proline contents, hydrogen peroxide, superoxide dismutase and grain yield showed significant variations for treatments, hybrids and their interactions.

Table 1b. ANOVA of	nlant traits of mains k	whatda undan individual as wal	l as concurrent heat and water stress
Table ID: ANOVA OF	plant traits of maize i	lybrids under marvidual as we	h as concurrent neat and water stress

Traits	Replication (R)	Treatments (T)	Error (R×T)	Hybrids (H)	Interaction (T×H)	Error (R×T×H)
Plant traits/df	2	3	6	9	27	108
Days to 50% anthesis (DT)	0.07	687.23**	0.19	35.26**	12.67**	0.27
Days to 50% silking (DS)	0.59	697.64**	0.21	27.44**	11.49**	0.37
Plant height (PH)	52.1	5427.9**	32	923.2**	153.8**	5.3
Ear height (EH)	2.34	956.34**	1.72	899.2**	89.47^{**}	2.1
Ear length (EL)	0.72	31.21**	0.08	6.66^{**}	2.89^{**}	0.09
Ear width (EW)	2.35	268.66^{**}	0.69	39.79**	7.14^{**}	0.71
Grains per ear (K/E)	612.3	68643.7**	82.4	3925.2**	3122.3**	109.8
Thousand grain weight (TGW)	221.3	22489.9**	10.24	20857.2^{**}	1487.1^{**}	21.0
SPAD	0.76	342.11**	0.09	29.45**	9.92^{**}	0.31
Protein % (Protein)	0.45	6.75**	0.019	0.21^{**}	0.39**	0.058
Transpiration rate (Tr)	0.008	4.85^{**}	0.007	0.299^{**}	0.141^{**}	0.005
Stomatal conductance (Ci)	78.5	48647.2**	37.3	8598.4^{**}	473.1**	16.2
Water use efficiency (WUE)	0.28	42.24**	0.04	1.85^{**}	1.68^{**}	0.05
Chlorophyll a (Chl a)	0.020	1.024**	0.002	0.112^{**}	0.071^{**}	0.004
Chlorophyll b (Chl b)	0.012	0.054^{**}	0.001	0.72^{**}	0.042^{**}	0.003
Proline contents (Proline)	0.41	896.3**	0.0365	3.92^{**}	2.89^{**}	0.076
Hydrogen peroxide (H ₂ O ₂₎	0.019	29.16**	0.009	0.011^{*}	0.21	0.009
Superoxide dismutase (SOD)	9.67	3124.5**	0.48	51.36**	21.33**	0.106
Grain yield (KY)	87689	27658972**	4967	2868547**	4465324**	19546

Significant at $P \le 0.05 = *$; Significant at $P \le 0.01 = **$; Non-significant at P > 0.05

Physiological paraments

The way that various plant physiological parameters react to heat- and water-deficient conditions as well as their combined stress was extremely serious in almost every

single maize cultivar. The results revealed from the bar table based on mean values are given in Figure 1 (A-F) and Figure 2G. The result revealed a significant reduction in SPAD (35 to 14 μ g/cm²), water use efficiency (10 to 6 mmole m⁻² s⁻¹)

[Citation: Hussain, S., Ghani, A., Khalid, M., Nayab, S.F., Hassan, I.U., Sattar, A., Kausar, S., Saleem, M., Asif, M., Akram, F., Imran, M., Ullah, R., Sarwar, M.I., Kamal, N., Khan, M.A., Sajjad, M.H., Yousaf, M.I. (2023). Morphometric, physiological and biochemical charactrization of local and exotic maize hybrid under heat and water-stress stress conditions at postanthesis stage. Biol. Clin. Sci. Res. J., 2023: 548. doi: https://doi.org/10.54112/bcsrj.v2023i1.548]

3

cluster analysis was implied to characterize maize hybrids into different groups, based on their productivity, stability and stress tolerance capabilities. Different statistical packages including Statistix 8.1, XLSTAT 16.0 and OriginPro were used to carry out statistical data analysis. Microsoft Excel was used for graphical illustrations

Results

Analysis of Variance (ANOVA)

and chlorophyll a (2.8 to 2.0 mg g⁻¹ FW) and chlorophyll b (1.7 to 1.1 mg g⁻¹ FW), respectively. However, in transpiration rate and stomatal conductance, there was a significant increase under heat stress conditions from 2.5 to 3.9 mmole m⁻² s⁻¹ in transpiration rate while 220 to 310 mmole m⁻² s⁻¹ in stomatal conductance, respectively (Figure 1 c-d). Concerning hybrid performance, YH-5427, YH-5482 and YH-5399 showed significant tolerance to physiological damage under all the stress.

Biochemical and associated traits

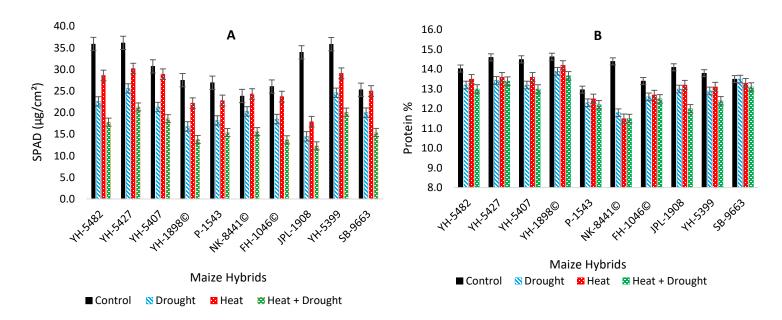
functions and tolerance to stresses-related Plant biochemical traits showed significant changes in their activity and accumulation. The proline contents were seen to be significantly increased under all stress conditions (Figure 2H). Nonetheless, combined heat + water stress conditions exhibited a significant accumulation of protein, where proline contents increased from 6 μ g g⁻¹ FW in control to 24 µg g⁻¹ FW in combined stress conditions. The second-highest accumulation was observed under waterstress stress conditions, where proline contents were increased from 6 μ g g⁻¹ FW in control to 17 μ g g⁻¹ FW in water-stress stress conditions. The accumulation of hydrogen peroxide (H₂O₂), which is an important reactive oxygen species, was significantly accelerated under the stress conditions imposed as a result of heat, water-stress and their combined effects (Figure 2I). Water-stress stress alone as well as combined with heat stress significantly increased the activity of H2O2 from 1 µmole g-1 FW to 6 µmole g-1 FW in maize hybrid YH-1898. Similarly, the accumulation of superoxide dismutase (SOD), which is an important enzymatic antioxidant, was pointedly improved under all stress conditions, especially water-stress stress either alone or in combination with heat stress (Figure 2J). The highest accumulation was observed in YH-5427 (from 38 to 70), YH-5399 (from 41 to 72) and SB-9663 (from 32 to 64) under combined water-stress + heat stress conditions.

Correlation Coefficient Analysis

The correlation coefficients analysis revealed the presence of positive and significant correlation of grain yield with proline (r = 0.98^{**}), SOD (r = 0.93^{**}), Chl a (r = 0.92^{**}), Chl b (r = 0.86^{**}), transpiration rate (r = 0.83^{**}) and stomatal conductance $(r = 0.81^{**})$ in ten maize hybrids under normal/control conditions (Figure 3a-d). However, under water-stress stress conditions, grain yield was positively correlated with transpiration rate ($r = 0.79^{**}$), Chl b (r = 0.78^{**}), Chl a (r = 0.75^{**}) and SPAD value (r = 0.69^{**}). Under heat stress conditions, grain yield was positively correlated with stomatal conductance $(r = 0.85^{**})$ transpiration rate ($r = 0.66^{**}$) and Proline ($r = 0.64^{**}$) (Figure 3a-d). However, under heat stress, grain yield was negatively correlated with hydrogen peroxide ($r = 0.58^{NS}$). Lastly, under combined heat and water-stress stress conditions, grain yield was significantly and positively associated with SPAD value ($r=0.88^{**}$), Proline ($r=0.79^{**}$), transpiration rate (r= 0.78**), superoxide dismutase (r= 0.73^{**}), stomatal conductance (r= 0.72^{**}) while negatively correlated with hydrogen peroxide (r= -0.87**) (Figure 3ad).

Cluster Analysis

Cluster analysis was applied to the recorded data to characterize the maize hybrids into different groups based on the relative strength of their homogeneity and heterogeneity. In the current study, cluster analysis classified ten maize hybrids into three groups or clusters or classes under control, water-stress stress, heat stress and combined heat + water-stress stress which makes them forty entries with respect to stress treatments (Tabel 2-3 & Figure 4). The first group/cluster was comprised of twelve entries (12) and this group was recognized as the cluster of most productive hybrids



[Citation: Hussain, S., Ghani, A., Khalid, M., Nayab, S.F., Hassan, I.U., Sattar, A., Kausar, S., Saleem, M., Asif, M., Akram, F., Imran, M., Ullah, R., Sarwar, M.I., Kamal, N., Khan, M.A., Sajjad, M.H., Yousaf, M.I. (2023). Morphometric, physiological and biochemical charactrization of local and exotic maize hybrid under heat and water-stress stress conditions at post-anthesis stage. *Biol. Clin. Sci. Res. J.*, **2023**: *548*. doi: <u>https://doi.org/10.54112/bcsrj.v2023i1.548</u>]

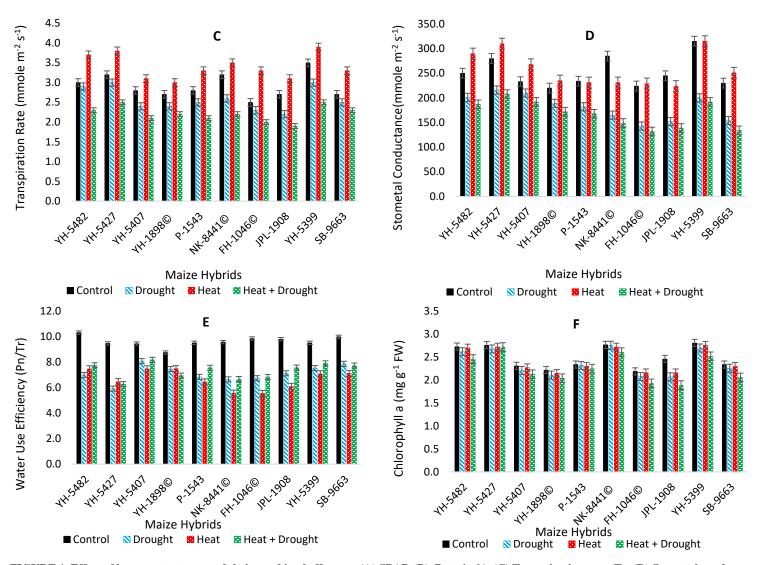
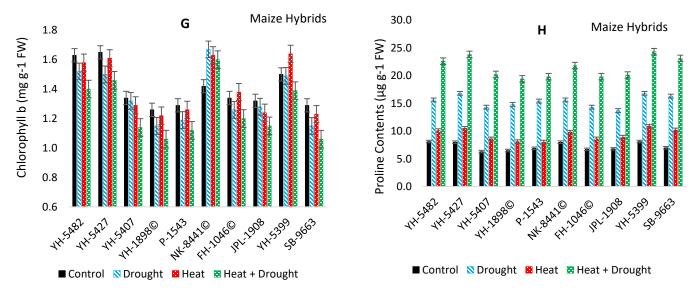


FIGURE 1 Effect of heat, water stress and their combined effects on (A) SPAD (B), Protein %, (C) Transpiration rate; Tr, (D) Stomatal conductance; Ci, (E) Water Use Efficiency; WUE, (F) Chlorophyll *a* in ten maize hybrids.



[Citation: Hussain, S., Ghani, A., Khalid, M., Nayab, S.F., Hassan, I.U., Sattar, A., Kausar, S., Saleem, M., Asif, M., Akram, F., Imran, M., Ullah, R., Sarwar, M.I., Kamal, N., Khan, M.A., Sajjad, M.H., Yousaf, M.I. (2023). Morphometric, physiological and biochemical charactrization of local and exotic maize hybrid under heat and water-stress stress conditions at post-anthesis stage. *Biol. Clin. Sci. Res. J.*, **2023**: *548*. doi: <u>https://doi.org/10.54112/bcsrj.v2023i1.548</u>]

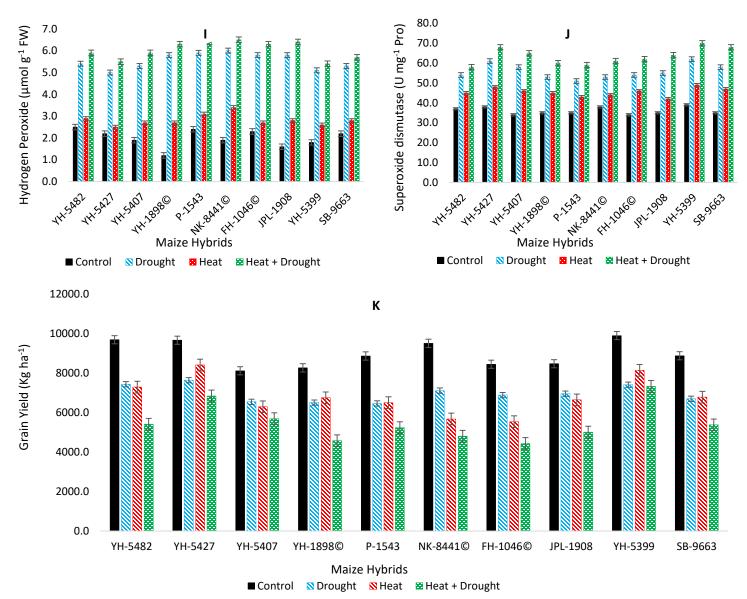
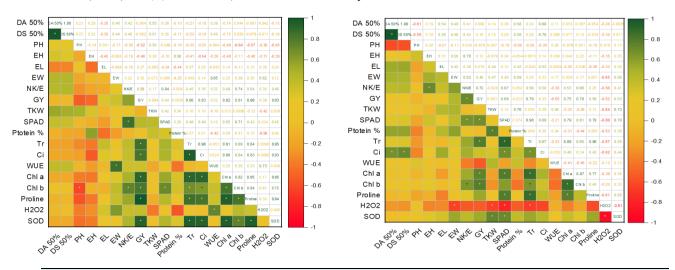


FIGURE 2 | Effect of heat, water stress and their combined effects on (G) Chlorophyll b (H), Proline contents, (I) Hydrogen peroxide; H₂O₂, (J) Superoxide dismutase; SOD, and (K) Grain Yield; KY ha⁻¹ inn ten maize hybrids.



[Citation: Hussain, S., Ghani, A., Khalid, M., Nayab, S.F., Hassan, I.U., Sattar, A., Kausar, S., Saleem, M., Asif, M., Akram, F., Imran, M., Ullah, R., Sarwar, M.I., Kamal, N., Khan, M.A., Sajjad, M.H., Yousaf, M.I. (2023). Morphometric, physiological and biochemical charactrization of local and exotic maize hybrid under heat and water-stress stress conditions at post-anthesis stage. *Biol. Clin. Sci. Res. J.*, **2023**: *548*. doi: <u>https://doi.org/10.54112/bcsrj.v2023i1.548</u>]

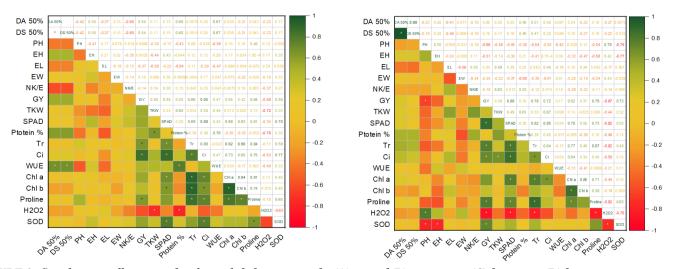
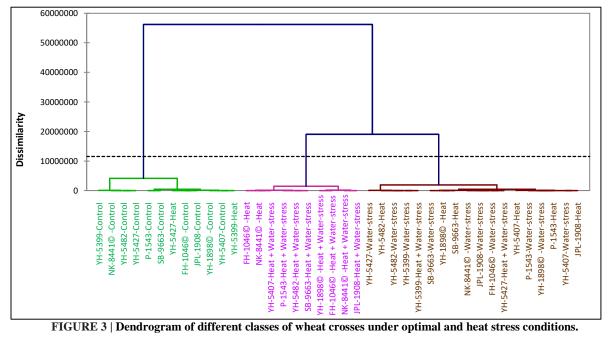


FIGURE 3 Correlation coefficient graphs of recorded plant traits under (A) control (B) water stress (C) heat stress (D) heat + water-stress

In this cluster, all the hybrids of control treatment YH-5482-Control, YH-5427-Control, YH-5407-Control, YH-1898© -Control, P-1543-Control, NK-8441© -Control, FH-1046© -Control, JPL-1908-Control, YH-5399-Control and SB-9663-Control along with two hybrids from the heat stress group i.e., YH-5427-Heat and YH-5399-Heat, depicting that these two hybrids were most heat tolerant hybrids from their group (Table 3).

The mean grain yield of cluster-I was 8867.3 kg ha⁻¹ and all the others showed the highest values in this cluster except proline (7.817 μ g g⁻¹ FW), hydrogen peroxide (2.092 μ mol g⁻¹ FW) and superoxide dismutase (38.083 U mg⁻¹ Pro) (Table 2). Cluster II consisted of eighteen (18) entries including YH-5482-Water-stress, YH-5427-Water-stress, YH-5407-Water-stress, YH-1898© -Water-stress, P-1543-Water-stress, NK 8441© -Water-stress, FH-1046© -Waterstress, JPL-1908-Water-stress, YH-5399-Water-stress, SB-9663 Water-stress, YH-5482-Heat, YH-5407-Heat, YH-

1898© -Heat, P-1543-Heat, JPL-1908-Heat, SB 9663-Heat, YH-5427-Heat + Water-stress and YH-5399-Heat + Waterstress (Table 3). The mean grain yield of cluster-II was 6896.5 kg ha⁻¹ and this cluster had minimum mean values for plant height (173.570 cm), ear height (82.237 cm) and number of grains per ear (573.824) (Table 2). The last cluster, cluster-III comprised of NK-8441© -Heat, FH-1046[°] -Heat, YH-5482-Heat + Water-stress, YH-5407-Heat + Water-stress, YH-1898© -Heat + Water-stress, P-1543-Heat + Water-stress, NK-8441[©] -Heat + Water-stress, FH-1046© -Heat + Water-stress, JPL-1908-Heat + Waterstress and SB-9663-Heat + Water-stress. The cluster is characterized as the group of hybrids with the least productivity with a grain yield 5177.1 kg ha⁻¹ and three biochemical traits showed the highest mean values including proline (18.520 µg g⁻¹ FW), hydrogen peroxide (5.550 µmol g⁻¹ FW) and superoxide dismutase (58.700 U mg⁻¹ Pro) (Table 3).



[Citation: Hussain, S., Ghani, A., Khalid, M., Nayab, S.F., Hassan, I.U., Sattar, A., Kausar, S., Saleem, M., Asif, M., Akram, F., Imran, M., Ullah, R., Sarwar, M.I., Kamal, N., Khan, M.A., Sajjad, M.H., Yousaf, M.I. (2023). Morphometric, physiological and biochemical charactrization of local and exotic maize hybrid under heat and water-stress stress conditions at post-anthesis stage. *Biol. Clin. Sci. Res. J.*, **2023**: *548*. doi: <u>https://doi.org/10.54112/bcsrj.v2023i1.548</u>]

Traits/Class	Class 1	Class 2	Class 3
Days to 50% anthesis (DT)	70.461	66.196	60.777
Days to 50% silking (DS)	73.489	69.122	64.110
Plant height (PH)	192.186	173.570	177.947
Ear height (EH)	90.983	82.237	86.967
Ear length (EL)	19.194	17.437	16.740
Ear width (EW)	47.282	45.397	44.390
Grains per ear (K/E)	631.084	573.824	603.328
Grain yield (KY)	8867.349	6896.518	5177.125
Thousand-grain weight (TGW)	317.117	286.287	253.093
SPAD	30.117	21.622	17.058
Protein % (Protein)	13.886	13.107	12.517
Transpiration rate (Tr)	3.067	2.794	2.390
Stomatal conductance (Ci)	261.750	206.167	173.300
Water use efficiency (WUE)	9.160	7.088	7.045
Chlorophyll a (Chl a)	2.534	2.385	2.225
Chlorophyll b (Chl b)	1.441	1.344	1.274
Proline contents (Proline)	7.817	14.200	18.520
Hydrogen peroxide (H ₂ O ₂)	2.092	4.628	5.550
Superoxide dismutase (SOD)	38.083	53.611	58.700

Table 2: Mean values of three classes from cluster analysis plant traits in ten maize hybrids under heat, water stress and	
combined stresses	

Table 3: Cluster analysis-based	assification of maize hybrids under four different stress trea	tments

Class	1	2	3
Objects	12	18	10
	YH-5482-Control	YH-5482-Water-stress	NK-8441© -Heat
	YH-5427-Control	YH-5427-Water-stress	FH-1046© -Heat
	YH-5407-Control	YH-5407-Water-stress	YH-5482-Heat + Water-stress
	YH-1898© -Control	YH-1898© -Water-stress	YH-5407-Heat + Water-stress
	P-1543-Control	P-1543-Water-stress	YH-1898© -Heat + Water-stress
	NK-8441© -Control	NK-8441© -Water-stress	P-1543-Heat + Water-stress
	FH-1046© -Control	FH-1046© -Water-stress	NK-8441© -Heat + Water-stress
	JPL-1908-Control	JPL-1908-Water-stress	FH-1046© -Heat + Water-stress
	YH-5399-Control	YH-5399-Water-stress	JPL-1908-Heat + Water-stress
	SB-9663-Control	SB-9663-Water-stress	SB-9663-Heat + Water-stress
	YH-5427-Heat	YH-5482-Heat	
	YH-5399-Heat	YH-5407-Heat	
		YH-1898© -Heat	
		P-1543-Heat	
		JPL-1908-Heat	
		SB-9663-Heat	
		YH-5427-Heat + Water-stress	
		YH-5399-Heat + Water-stress	

Discussion

Heat and water-stress stresses, whether experienced individually or in combination, have a significant impact on maize productivity and sustainability. Maize is a crucial staple crop globally, and its susceptibility to these stresses has far-reaching consequences. When faced with high temperatures, maize plants often exhibit reduced photosynthesis rates and impaired pollen viability, leading to decreased yields. Water-stress, on the other hand, limits water availability for the plant, affecting growth and development. When these stresses occur together, the negative effects are compounded, resulting in even more severe yield reductions. Furthermore, the sustainability of maize cultivation is compromised by these stresses. Soil degradation and increased vulnerability to pests and diseases are common consequences, as stressed plants are less able to defend themselves. To address these challenges, sustainable agricultural practices such as precision irrigation, crop rotation, and the development of heat and water-stress-tolerant maize varieties are essential. These efforts can help mitigate the impact of heat and water-stress stresses, safeguarding maize productivity and ensuring the long-term sustainability of this critical crop.

Even though maize is one of the most adaptable grain crops in the world, it is highly susceptible to heat stress when it comes to reproduction, especially in its initial blooming phases. The purpose of the present investigation was to assess the effects of heat and water-stress stress alone or in combination with each other on commercial hybrids produced locally and internationally, as well as to clarify the significance of morpho-physiological and biochemical traits-particularly photosynthesis-related traits, reactive oxygen species and accumulation of enzymatic antioxidants- when figuring out heat endurance. ANOVA revealed the existence of very substantial differences between the maize hybrid, illustrating their genetic potential as well as productivity variations under control and three stress conditions i.e., heat stress, water-stress stress and combined heat + water-stress stress conditions. Comparable outcomes were also described by Yousaf et al. (2017) and Yousaf et al. (2022b), who revealed the existence of substantial variations in maize hybrids under individual heat and water-stress stress as well as their combined effects. Correlation coefficient analysis was used to predict the correlation of grain yield with plant morpho-physiological and biochemical-related traits under different abiotic stress conditions.

The results from correlation analysis showed a substantially positive correlation of grain yield with chlorophyll a, chlorophyll b, stomatal conductance, transpiration rate, proline contents and hydrogen peroxide under control conditions. However, under individual heat and water-stress stress conditions, grain yield had a significant positive correlation with chlorophyll a, chlorophyll b, stomatal conductance, transpiration rate, proline contents and superoxide dismutase while a considerably negative correlation with hydrogen peroxide. Under combined heat + water-stress stress conditions, grain yield was found to be highly correlated with SPAD value, stomatal conductance, transpiration rate, proline contents and superoxide dismutase with a strong negative correlation with hydrogen peroxide. Similar findings were reported by many scientists that abiotic stresses, individual and/or combined had a very strong correlation with grain yield with photosynthesisrelated traits like SPAD value, stomatal conductance, transpiration rate, chlorophyll a, chlorophyll b and superoxide dismutase (Ghani et al., 2020; Yousaf et al., 2022a; Yousaf et al., 2022b; Rehman et al., 2022). In order to ensure the transfer of stem reserves to sink and promote optimal photosynthetic activity in maize during heat stress, larger amounts of chlorophyll a and b must be produced (Khajeh-Hosseini et al., 2016). More biomass is delivered to the sink over the course of the silking process, and eventually, a high yield is reached (Yousaf et al., 2017; Shehzad et al., 2019).

Several investigators employed cluster analysis to identify cultivars under different environmental scenarios such as water and heat stress conditions and found it quite useful in screening and classifying germplasm to run a successful hybrid breeding program (Saeed et al., 2018; Bhatti et al., 2020; Khalid et al., 2020). Ghani et al. (2023) evaluated and categorized ten maize hybrids using cluster analysis, demonstrating the effectiveness of cluster analysis grouped into three clusters depending upon their productivity and stress tolerance. In the current study, cluster analysis grouped maize hybrids concerning their genetic diversity under different water and heat stress conditions. In the current study, cluster analysis categorizes maize hybrids into three groups under control, heat, water-stress and their combined stresses. The highest tolerance against abiotic stresses was observed in the hybrids of group-A. Therefore, to select hybrids with the highest tolerance and productivity, hybrids present in group-A must be considered to run a successful breeding program.

Stress-tolerant hybrids can produce higher yields in challenging environmental conditions due to a combination of key factors. First, their elevated photosynthetic rate allows them to efficiently convert light energy into vital carbohydrates, even in adverse conditions. This increased efficiency stems from their higher concentrations of chlorophyll a and b, pigments essential for absorbing and utilizing light energy. These pigments enable these hybrids to capture more sunlight and convert it into essential plant energy.

Furthermore, stress-tolerant hybrids often exhibit the trait of producing more grains per ear, especially in grain crops (Sabagh et al., 2020). This characteristic directly contributes to an enhanced overall yield, as more grains translate into higher productivity per plant (Wagas et al., 2021). In regions prone to stressors like water-stress or extreme temperatures, this trait becomes particularly advantageous. Moreover, these hybrids have developed a robust defence mechanism against the damaging effects of environmental stressors through the significant accumulation of enzymatic antioxidants, particularly superoxide dismutase (SOD) (Tiwari and Yadav, 2019). When exposed to stress, plant cells may generate harmful reactive oxygen species (ROS), causing oxidative stress and cellular damage (Riaz et al., 2021). The presence of high levels of SOD and other antioxidants allows stress-tolerant hybrids to neutralize these ROS, preserving cellular integrity and function. This protection ensures that they can continue with

photosynthesis and grain production even when other plants might succumb to stress-related damage (Hussain et al., 2019). Considering the performance of hybrids, three maize hybrids YH-5427, YH-5482 and YH-5399 were the most productive and stress-tolerant maize hybrids. As a result, these traits might be utilized in selecting parents for developing maize hybrids for harsh climatic conditions. Similar findings were detected by Yousaf et al., (2018) and Yousaf et al. (2022b), who reported that the hybrids developed from the germplasm having stress-tolerant genes, could show more stress tolerance from the hybrids developed in temperate climates.

Conclusion

The current experimental study revealed the presence of highly significant variations among maize hybrids for morphometric, physiological, biochemical and qualityrelated traits under control and stress conditions i.e., heat stress, water-stress stress, hat + water-stress stress combined. The correlation coefficient analysis showed a strong correlation of grain yield with chlorophyll a, chlorophyll b, stomatal conductance, transpiration rate, proline contents and superoxide dismutase while a strong negative correlation with hydrogen peroxide. Furthermore, cluster analysis divided the maize hybrids into three clusters based on their performance, diversity and stress tolerance ability. The cluster analysis showed that locally developed maize hybrids especially YH-5482, YH-5427 and YH-5399 had more heat and water-stress stress tolerance than the commercially cultivated multinational maize hybrids under the given circumstances.

Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate Approved by the department Concerned. Consent for publication Approved Funding Not applicable

Conflict of interest

The authors declared absence of conflict of interest.

References

- Murdia, L. K., R. Wadhwani, N. Wadhawan, P. Bajpai, and S. Shekhawat. "Maize utilization in India: an overview." *American Journal of Food and Nutrition* 4, no. 6 (2016): 169-176.
- Kumar, K., Singh, J., Singh, B. R., Chandra, S., Chauhan, N., Yadav, M. K., & Kumar, P. (2021). Consumption and processing patterns of maize (Zea mays): A review. US Department of Agriculture, 30000, 4-7.
- Erenstein, O., Jaleta, M., Sonder, K., Mottaleb, K., & Prasanna, B. M. (2022). Global maize production, consumption and

trade: Trends and R&D implications. Food Security, 14(5), 1295-1319.

- USDA. 2023. United State Department of Agriculture: World Agricultural Production. United States Department of Agriculture, Circular series. WAP. 08-22. United State.
- Waqas, M. A., Wang, X., Zafar, S. A., Noor, M. A., Hussain, H. A., Azher Nawaz, M., & Farooq, M. (2021). Thermal stresses in maize: effects and management strategies. Plants, 10(2), 293.
- Luan, X., & Vico, G. (2021). Canopy temperature and heat stress are increased by compound high air temperature and water stress and reduced by irrigation–a modeling analysis. Hydrology and Earth System Sciences, 25(3), 1411-1423.
- Ozturk, M., Turkyilmaz Unal, B., García-Caparrós, P., Khursheed, A., Gul, A., & Hasanuzzaman, M. (2021). Osmoregulation and its actions during the water-stress stress in plants. Physiologia plantarum, 172(2), 1321-1335.
- Zandalinas, S. I., Mittler, R., Balfagón, D., Arbona, V., and Gómez-Cadenas, A. (2018). Plant adaptations to the combination of water-stress and high temperatures. Physiol. Plant 162, 2–12. doi: 10.1111/ppl.12540
- Farooq, M., Gogoi, N., Barthakur, S., Baroowa, B., Bharadwaj, N., Alghamdi, S. S., et al. (2017). Water-stress stress in grain legumes during reproduction and grain filling. J. Agron. Crop Sci 203, 81–102. doi: 10.1111/jac.12169
- Hussain, H. A., Men, S., Hussain, S., Chen, Y., Ali, S., Zhang, S., et al. (2019). Interactive effects of water-stress and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. Sci. Rep. 9:3890. doi: 10.1038/s41598-019-40362-7
- Nelimor, C., Badu-Apraku, B., Tetteh, A. Y., Garcia-Oliveira, A. L., and N'guetta, A. S. P. (2020). Assessing the potential of extra-early maturing landraces for improving tolerance to water-stress, heat, and both combined stresses in maize. Agronomy 10:318. doi: 10.3390/agronomy10030318
- Riaz, M. W., Yang, L., Yousaf, M. I., Sami, A., Mei, X. D., Shah, L., et al. (2021).Effects of heat stress on growth, physiology of plants, yield and grain quality of different spring wheat (Triticum aestivum 1.) genotypes. Sustain 13, 1–18.doi: 10.3390/su13052972
- Lipiec, J., Doussan, C., Nosalewicz, A., and Kondracka, K. (2013). Effect of water-stressand heat stresses on plant growth and yield: a review. Int. Agrophysics 27,463–477. doi: 10.2478/intag-2013-0017
- Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., et al.(2017). Crop production under waterstress and heat stress: plant responsesand management options. Front. Plant Sci 8:1147. doi: 10.3389/fpls.2017.01147
- Tandzi, L. N., and Mutengwa, C. S. (2020). Estimation of maize (Zea mays L.)yield per harvest area: appropriate methods. Agronomy 10:29. doi: 10.3390/agronomy10010029
- Yousaf, M. I., Bhatti, M. H., Maqbool, M. A., Ghani, A., Akram, M., Ibrar, I., et al.(2021). Heat stress-induced responses in local and exotic maize hybridsfor morphophysiological and grain quality traits. Pakistan J. Agric. Sci. 58,1511– 1521. doi: 10.21162/PAKJAS/21.424
- Lichtenthaler, H. K. (1987). Chlorophylls and Carotenoids: Pigments ofPhotosynthetic biomembranes. Methods Enzymol 148, 350–382. doi: 10.10160076-6879(87)48036-1
- Peng, Y. S., and Liu, E. (1992). A comparative study of methods of extracting
 - chlorophyll. Acta Agric. Univ. Pekin. 18, 247-250.
- Sergiev, I., Alexieva, V., and Karanov, E. (1997). Effect of spermine, atrazine and combination between them on some endogenous protective systems and stressmarkers in plants. Proc. Bulg. Acad. Sci. 51, 121–124.

- Yousaf, M. I., Hussain, K., Hussain, S., Ghani, A., Bhatti, M. H., Mumtaz, A., Khalid, M.U., Mehboob, A., Murtaza, G., and Akram, M. (2022). Characterization of maize (Zea Mays L.) hybrids for physiological attributes and grain quality traits under heat stress. Iranian Journal of Plant Physiology 12(2), 4075- 4087.
- Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. Principles and Procedures of Statistics: A Biometrical Approach, 3rd Ed. McGraw Hill Book Co., New York.
- Yousaf, M. I., Hussain, K., Hussain, S., Shahzad, S., Ghani, A., Arshad, M. Mumtaz, A. andAkhtar, N. (2017). Morphometric and phenological characterization of maize (Zeamays L.) germplasm under heat stress. International Journal of Biology and Biotechnology 14(2), 271-278.
- Yousaf, M.I., Riaz, M.W., Jiang, Y., Yasir, M., Aslam, M.Z., Hussain, S., Shah, S.A.S., Shehzad, A., Riasat, G., Manzoor, M.A., and Akhtar, I. (2022). Concurrent Effects ofWater-stress and Heat Stresses on Physio Chemical Attributes, Antioxidant Status and Grain Quality Traits in Maize (Zea mays L.) Hybrids. Frontiers in Plant Science 13. 898823
- Ghani, A., Yousaf, M.I., Arshad, M., Hussain, K., Hussain, S., Hussain, D., Hussain, A., and Shehzad, A. (2020). YH-5427: A highly productive, heat tolerant, stalk rot and lodging resistance, yellow maize hybrid of Punjab, Pakistan. International Journal of Biology and Biotechnology 17 (3), 561-570.
- Rahman, S.U., M. Arif, K. Hussain, S. Hussain, T. Mukhtar, A. Razzaq and R.A. Iqbal. 2013. Evaluation of maize hybrids for tolerance to high-temperature stress in central Punjab. Amer. J. Bioeng. Biotech. 1:30-36
- Khajeh-Hosseini, M., Teixeira da Silva, J. A., and Siddique, K. H. M. (2016). Heat stress in crop plants: a review on morphological characteristics and possible mechanisms of tolerance. Frontiers in Plant Science 7, 930.
- Shehzad, A., M.I. Yousaf, A. Ghani, K. Hussain, S. Hussain and M. Arshad. 2019. Genetic analysis and combining ability studies for morphophonological and grain yield traits in spring maize (Zea mays L.). Int. J. Biol. Biotech., 16 (4): 925-931.
- Saeed, M., A. Mumtaz, D. Hussain, M. Arshad, M.I. Yousaf, M.S. Ahmad. 2018. Multivariate analysis-based evaluation of maize genotypes under high-temperature stress. I3 Biodiversity, 1.
- Bhatti, M. H., Yousaf, M. I., Ghani, A., Arshad, M., Shehzad, A., Mumtaz, A., Khalid, M.U.,Khalid, M.Z., Mushtaq, M.Z., and Shah, S.A.S. (2020). Assessment of genetic variability and traits association in upland cotton (Gossypium hirsutum L.). International Journal of Botany Studies 5(2), 148-151.
- Khalid, M. U., Akhtar, N., Arshad, M., and Yousaf, M.I. (2020). Characterization of maize inbred lines for grain yield and related traits under heat stress conditions. International Journal of Biology and Biotechnology 17(2), 367-375.
- 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
- Tiwari, Y. K., and Yadav, S. K. (2019). High-temperature stress tolerance in maize (Zea mays L.): Physiological and molecular mechanisms. Journal of Plant Biology 62, 93-102.
- Sabagh, A. E., Hossain, A., Iqbal, M. A., Barutçular, C., Islam, M. S., Çiğ, F., Erman, M., Sytar, O., Brestic, M., Wasaya, A., Jabeen, T., and Saneoka, H. (2020). Maize adaptability

toheat stress under changing climate. In Plant stress physiology. IntechOpen.

Yousaf, M. I., Hussain, K., Hussain, S., Ghani, A., Arshad, M., Mumtaz, A., and Hameed, R.A. (2018). Characterization of local and exotic maize hybrids for grain yield andquality traits under heat stress. International Journal of Agriculture and Biology 20(2), 333-337



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <u>http://creativecommons.org/licen</u> ses/by/4.0/. © The Author(s) 2023