Characterization of maize (*Zea Mays* L.) hybrids for physiological attributes and grain quality traits under heat stress

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Abstract

Heat stress has become one of the major constraints in maize production worldwide. The current research was planned to define the heat stress-related characteristics of indigenous and exotic maize hybrids based on morpho-physiological and grain quality traits. The research was conducted at Maize and Millets Research Institute (MMRI), Yusafwala, Sahiwal during spring 2019. Hybrids were sown under two heat regimes: (i) optimal sowing and (ii) sowing under heat stress (late sowing). Hybrids differed significantly ($P<0.05$) in grain yield and related traits under both conditions. Correlation analysis showed a positive correlation of grain yield with net photosynthetic rate ($r = 0.393^*$), days to 50% anthesis ($r = 0.437^*$), and days to 50% silking ($r = 0.429^*$), and a negative association with ear leaf angle ($r = -0.420^*$) under heat stress. Cluster analysis categorized maize hybrids into three clusters based on their mean performance under optimal and heat stress conditions. It further showed that indigenous hybrids (cluster 3), especially KSC-9663, YH-5519, YH-5482, YH-1898, and YH-5507 were more productive and heat tolerant than exotic hybrids i.e., MV-633, MV-600-4, Maxima, SHG-43, and MV-600-2 (cluster 1). Principal component analysis (PCA) and biplot graphs showed that the first five principal components contributed to 72% of the total variability among genotypes, and that the main sources of variation were days to 50% anthesis and silking, plant height, percentage of protein and oil contents, stomatal conductance, and net photosynthetic rate.

Keywords: Climate change; cluster analysis; biplots; photosynthesis

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Introduction

Maize, one of the most extensively cultivated cereal crops worldwide, is used as food, feed, fodder, and biofuel. It is the principal food in many countries, especially in Sub-Sharan Africa, where 208 million people depend on maize for their food and livelihood (Macauley, 2015). Maize crops are cultivated in both spring and autumn seasons in Pakistan. The area devoted to spring sowing of maize is increasing constantly due to higher per-

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hectare yields compared to autumn crops. However, it was observed that grain yield per hectare of maize in Pakistan (5.19 tons' ha^{-1}) is comparably lower than United State (10.51 tons' ha^{-1}), Turkey (11.54 tons ha^{-1}), Canada (9.24 tons ha^{-1}), Argentina (8.10 tons ha^{-1}), Egypt (8.0 tons ha^{-1}), Ukraine (7.19 tons ha^{-1}), European Union (7.51 tons ha^{-1}), China (6.32 tons ha^{-1}) and Brazil (5.51 tons' ha^{-1}) (USDA, 2021). The main reasons for lower grain yields are high temperatures, less irrigation water availability, disease infestation (stalk rot and leaf blight), insect-pest attack (shoot fly and maize borers), high input rates, poor management of crop, and use of substandard seed.

Maize, being a C4 crop, efficiently uses solar radiation to produce photosynthesis. However, high temperatures can impair plant growth by affecting many vital metabolic and physiological processes. High temperatures during flowering and grain development can cause drastic reductions in grain yield by affecting fertilization and seed setting during hot summer seasons. Increased anthesis–silking interval is the main cause of lower grain yield under heat stress in maize, as it reduces seed setting by 80% due to sudden pollen shedding in a short period (Dass et al., 2010). Furthermore, heat stress decreases the number of grains per ear by reducing the number of flowers per plant, pollen availability and pollen viability, and by limiting pollen tube growth – all of which ultimately result in lower grain yields (Rezaei et al., 2014).

It is reported that maize grain yield increases with higher temperatures up to 29 ℃; beyond this, final grain yield decreases by 1% for every 1 ℃ increase (Lobell et al., 2011). Another study found that every 1 ℃ increase over the optimum temperature (30 ℃ to 34 ℃) during the reproductive phase can result in a 3% to 4% decrease in grain yield (Shaw, 1983). Record drops in maize crop grain yield due to heat stress were recorded in many regions of the world in last few years (Van der Velde et al., 2010). The most severe conditions are in South Asia, where 15% to 50% reductions in maize grain yields are predicted due to rising temperatures in the future (Kumar et al., 2011). These factors create an urgent need to develop heat-resilient, climate-smart maize genotypes to feed immensely growing populations worldwide. Suitable genotypes can be obtained by evaluating available germplasms under heat stress to explore their genetic diversity for heat tolerance. This would facilitate the selection of parents and plant traits with greater precision and accuracy, for use in efficient breeding programs to develop heat-tolerant maize genotypes.

Genetic variations present in cultivated germplasms are a basic resource for crop improvement. Germplasm from tropical climatic regions is fairly more heat tolerant as compared to germplasm from temperate climatic regions (Kugblenu et al., 2013). Hence, such genotypes could serve as a source population for the development and improvement of heat tolerant genotypes. Higher levels of heat resilience have not yet been achieved in maize due to the lack of studies designed to explore the genetic diversity present in warmer regions of the world (Paran and Van, 2007). Exploring the genetic diversity in available germplasms could maximize crop yields and minimize crop losses due to unfavorable environmental conditions such as heat and drought stress (Gepts, 2010). Therefore, it is pivotal to evaluate germplasms from warmer areas of the world to select heat stress tolerant germplasm in the development of heat-resilient maize inbred lines and hybrids. Hence, the present study was designed to evaluate and characterize maize germplasm for their heat tolerance ability based on the morpho-physiological, phonological, and grain quality-related parameters under elevated temperature conditions.

Materials and Methods

Experimental material and location

The research was executed at Maize and Millets Research Institute (MMRI) in Yusafwala, Pakistan (Latitude N 30° 41' 6.8511" and Longitude: E 73° 12' 54.8821") during spring 2018. Thirty (30) local and multinational single cross maize hybrids (Table 1) were screened and characterized for their heat tolerance ability based on morpho-physiological, phonological as well as grain quality parameters under both optimal and heat stress conditions. Hybrids were sown under two heat regimes (i) optimal sowing (10th February, 2019)

Table 1
Names and origin of the 30 local and exotic maize hybrids used in the study

Sr.	Hybrids	Origin	Sr.	Hybrids	Origin
1	YH-5493	MMRI, Pakistan	16	KSC-9633	Kissan Seed Corporation
2	YH-5532	MMRI, Pakistan	17	KSC-9618	Kissan Seed Corporation
3	YH-5516	MMRI, Pakistan	18	KSC-5971	Kissan Seed Corporation
4	YH-5494	MMRI, Pakistan	19	KSC-9617	Kissan Seed Corporation
5	YH-5519	MMRI, Pakistan	20	P-1543	Pioneer Seeds
6	YH-5507	MMRI, Pakistan	21	YH-1898	MMRI, Pakistan
7	YH-5521	MMRI, Pakistan	22	DK-6724	Monsanto Seeds
8	YH-5491	MMRI, Pakistan	23	NK-8711	Syngenta Seeds
9	YH-5496	MMRI, Pakistan	24	HC-2040	ICI, Pakistan
10	YH-5482	MMRI, Pakistan	25	HC-9091	ICI, Pakistan
11	YH-5524	MMRI, Pakistan	26	SHG-43	Exotic hybrid
12	YH-5487	MMRI, Pakistan	27	MV-600-4	Agroman, Pakistan
13	YH-5490	MMRI, Pakistan	28	MV-633	Agroman, Pakistan
14	YH-5518	MMRI, Pakistan	29	Maxima	Agroman, Pakistan
15	YH-5480	MMRI, Pakistan	30	MV-600-2	Agroman, Pakistan

MMRI: Maize and Millets Research Institute, Yusafwala, Sahiwal

and (ii) sowing under heat stress (22nd March, 2019) to provide high temperature at anthesis and grain development stages. Treatments were allocated under RCBD in triplicate with a split-plot arrangement. Each hybrid was sown in 4 rows, each of 4-meter length and 75 cm distance was maintained between these rows. However, the distance between plants was kept 20 cm. Sowing was done with the help of dibbler at the rate of 2 seeds per hill and at the 4-5 leaf stage, and thinning was done to ensure an optimum plant population. Standard agronomic practices were carried out for both treatments. The optimal treatment crop was harvested on 22nd June, 2019 while heat stress treatment (late sowing) crop was harvested on 11th July, 2019.

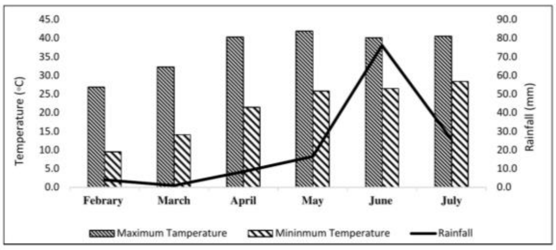


Fig. I. Metrological data during the crop season (Spring, 2019)

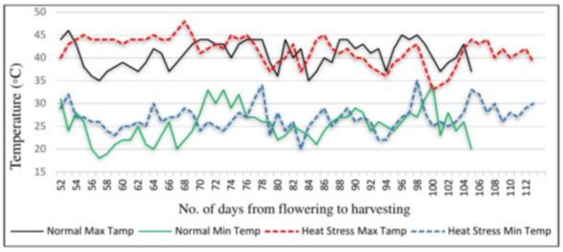


Fig. II. Minimum and maximum temperatures during the crop growth period in optimal (Solid lines, black and gr

Meteorological conditions

Meteorological data were recorded for both treatments throughout the cropping season (Fig. I). During the cropping season, the optimal-sown crop faced 36.7 ℃ average maximum daily temperature, whereas the late-sown (heat stress) crop faced a much higher average maximum daily temperature of 41.0 ℃. Mean data for minimum and maximum temperatures showed that the late sown crop experienced severe heat shock during its flowering stage, which was above the threshold level for maize crops (31 ℃ to 34 ℃) (Fig. II). At this stage, maize hybrids were exposed to deadly heat shock (45 ℃) for 11 consecutive days from 53 to 63 days after sowing. Because the reproductive phase of maize is very sensitive to high temperatures, such a high temperature can drastically reduce grain yield (Dass et al., 2010). Alternatively, average maximum temperature during the flowering period for optimal crop remained between 35 ℃ and 38 ℃, which was a bit higher than required for optimum reproductive growth (31 ℃ to 34 ℃) (Sánchez et al., 2014).

Data acquisition

Data were collected for 14 morpho-physiological, phenological, and grain quality traits, viz., days to

Table 2
Results of analysis of variance of 14 morpho-physiological and grain quality traits in maize hybrids under optimal and heat stress conditions

SOV	df	DT	DS	PH	EH	ELAn	ELAr	NGE	EL	TGW	Prot	Oil	Pn	C	GY
Replication	2	6.9^{NS}	7.3^{NS}	142.7^{NS}	120.1^{NS}	2.0^{NS}	3004.0^{NS}	92.5^{NS}	0.165^{NS}	456.6^{NS}	0.015	0.065^{NS}	1.69^{NS}	248.5^{NS}	427026^{NS}
Treatments (T)	1	13554.7**	13798**	19364.9*	2074.0*	21.4*	15773.5*	32616.3**	264.5**	10982.4*	3.87*	0.43*	5.78*	38252.1**	308800000**
Error (a)	2	10.3	11.7	636.1	42.7	0.51	282.7	21.8	0.94	131.4	0.065	0.02	0.26	187.8	1083406
Hybrids (H)	29	44.3**	42.8**	948.2**	701.6**	297.7*	30056**	37836.6**	12.4**	2559.9**	4.69**	1.115**	307.36**	26686**	9458530**
(H×T)	29	16.8**	15.6**	704.3**	457.5**	5.6*	378.2^{NS}	113.9^{NS}	0.25^{NS}	541.2**	0.13^{NS}	0.0118^{NS}	21.98**	1794.2**	3434652**
Error (b)	11	1.6	1.6	217.0	18.88	1.54	779.0	333.8	2.07	76.7	0.104	0.017	0.16	126.5	1050428

** = Highly significant at 1%, * = Significant at 5%, NS = Not significant, DT = days to 50% anthesis (days), DS = days to 50% silking (days), PH = plant height (cm), EH = ear height (cm), ELAn = ear leaf angle, NGE = number of grains per cob, EL = ear length (cm), TGW = 1000 grain weight (g), Prot. = grain protein content percentage (%), Oil = grain oil content percentage (%), Pn = Net photosynthetic rate (μmol m^{-2} s^{-1}), C = stomatal conductance (mmol m^{-2} s^{-1}), GY = grain yield (tons/ha)

50% anthesis, days to 50% silking, plant height (cm), ear height (cm), ear leaf angle, ear leaf area (cm^2), number of grains per ear, ear length (cm), thousand grain weight (g), grain protein content percentage, grain oil percentage, net photosynthetic rate (μmole m^{-2} s^{-1}), stomatal conductance (mmole m^{-2} s^{-1}), and grain yield (tons/ha). Days to 50% anthesis and days to 50% silking were calculated from the date of sowing to the emergence of 50% tassels and 50% silks. Plant and ear heights were recorded at maturity from 10 guarded plants with a measuring rod in centimeters. Ear leaf angle was determined in 10 random ear leaves per hybrid with the help of the Android mobile application Clinometer (available at google play store (https://play.google.com/store/apps/details?id=com.plaincode.clinometer). Laser leaf area meter (CI-203) was used to measure the ear leaf area in cm^2 from 10 random ear leaves per hybrid. Grain protein and grain oil content percentages were recorded by near infrared spectrometry (NIR-Inframatic 9200). The physiological parameters, net photosynthetic rate (μmole m^{-2} s^{-1}), and stomatal conductance (mmole m^{-2} s^{-1}) were recorded through an infrared gas analyzer (IRGA) model CI-340 (CID Bio-Science) during the grain development in 10 random ear leaves per hybrid between 9:00 am and 11:30 am. Grain yield per hectare was calculated according to the given formula (Tandzi and Mutengwa, 2020):

Grain yield (Kg ha^{-}) = Field grain weight × Moisture factor (at 15% grain moisture level)

where the moisture factor $= (1 - \frac{\text{Moisture}}{85})(\frac{80}{100})$.

Statistical Data Analysis

The analysis of variance (Kwon and Torrie, 1964) was applied to the recorded data for the detection of differences among maize hybrids, and correlation coefficient analysis was used to compute correlation between grain yield and associated parameters (Steel et al., 1997). Furthermore, maize hybrids were classified and characterized on the account of their mean performance through principal component (Sneath and Sokal, 1973). Statistical package, XLSTAT 16 was used to compute different statistical analysis.

Results

Analysis of variance (ANOVA)

Analysis of variance unveiled substantial differences among treatments and maize hybrids for studied parameters (Table 2). Similarly, genotype × environment interactions were also significant for days to 50% anthesis and silking, plant height, ear height, ear leaf angle, thousand grain weight, net photosynthetic rate, stomatal conductance, and grain yield. However, genotype × environment interactions were non-significant for leaf area, grains per ear, ear length, grain protein content percentage, and grain oil percentage.

Correlation coefficient analysis

Table 3
Correlation of morpho-physiological and grain quality traits in maize hybrids under optimal and heat stress conditions

Variables		DT	DS	PH	EH	ELAn	ELAr	NGE	EL	1000GW	Prot	Oil	Pn	C
GY	r_n	-0.115	-0.138	0.121	-0.060	-0.095	0.455	0.380	0.242	0.092	-0.312	-0.204	0.634	0.228
	r_h	0.437	0.429	0.097	0.265	-0.420	0.142	0.031	-0.126	-0.031	-0.037	0.268	0.393	-0.170

r_n = Correlation coefficient under optimal sowing, r_h = Correlation coefficient under heat stress treatment; values in bold indicate significant associations at alpha=0.05; DT = days to 50% anthesis (days), DS = days to 50% silking (days), PH = plant height (cm), EH = ear height (cm), ELAn = ear leaf angle, NGE = number of grains per cob, EL = ear length (cm), TGW = 1000 grain weight (g), Prot = grain protein content percentage (%), Oil = grain oil content percentage (%), Pn = net photosynthetic rate (µmol m^{-2} s^{-1}), C = stomatal conductance (mmol m^{-2} s^{-1}), GY = grain yield (tons/ha)

Table 4
Class/Cluster means for morpho-physiological and grain quality traits in maize under normal and heat stress conditions

Class	Treatment	Tass	Silk	PH	CH	L. An	L. Ar	GC	EL	TGW	Pro	Oil	Pn	C	GY
Class 1	Optimal	74.5	77.6	201.7	104.5	34.9	558.6	567.3	19.0	311.9	13.4	4.2	7.6	143.4	9240.6
	Heat stress	55.7	58.6	180.7	94.5	37.4	550.9	543.7	16.6	295.6	12.9	3.9	9.9	205.3	5625.3
Class 2	Optimal	73.0	76.0	206.7	99.1	32.5	639.3	575.8	18.6	324.5	12.8	4.1	15.6	169.6	10698.
	Heat stress	55.5	58.5	182.5	93.3	37.4	577.4	594.7	17.1	303.9	12.8	3.8	10.3	189.7	7219.0
Class 3	Optimal	73.9	76.9	201.2	100.7	34.6	620.8	636.1	19.3	314.3	13.2	4.1	21.2	175.1	12321.
	Heat stress	58.0	61.0	182.8	98.2	31.8	578.6	555.3	16.2	300.4	13.0	4.3	14.8	164.4	9034.3

DT = days to 50% anthesis (days), DS = days to 50% silking (days), PH = plant height (cm), EH = ear height (cm), ELAn = ear leaf angle, NGE = number of grains per cob, EL = ear length (cm), TGW = 1000 grain weight (g), Prot = grain protein content percentage (%), Oil = grain oil content percentage (%), Pn = net photosynthetic rate (µmol m^{-2} s^{-1}), C = stomatal conductance (mmol m^{-2} s^{-1}), GY = grain yield (tons/ha)

Table 5
Eigenvalue, individual and cumulative variability of 12 principal components (PCs) under optimal and heat stress conditions

Principal Factors		PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14
Eigenvalue	Optimal	4.013	2.112	1.733	1.579	1.060	0.816	0.731	0.530	0.466	0.376	0.319	0.161	0.103	0.001
	Heat	3.794	2.077	1.581	1.368	1.290	0.883	0.756	0.636	0.571	0.388	0.306	0.236	0.114	0.001
Variability %	Optimal	28.66	15.09	12.38	11.28	7.57	5.83	5.23	3.79	3.33	2.68	2.28	1.15	0.74	0.005
	Heat	27.10	14.84	11.29	9.77	9.22	6.31	5.40	4.54	4.08	2.77	2.19	1.68	0.81	0.01
Cumulative %	Optimal	28.66	43.75	56.13	67.41	74.98	80.81	86.03	89.82	93.1	95.83	98.11	99.26	99.99	100.00
	Heat	27.10	41.94	53.23	63.00	72.21	78.52	83.92	88.46	92.54	95.31	97.50	99.18	99.99	100.00

Correlation coefficient analysis was used to elucidate the relationships of each trait with grain yield under both optimal and heat stress conditions. Grain yield showed a significantly positive relationship with days to 50% anthesis ($r = 0.437^*$) and days to 50% silking ($r = 0.429^*$) under heat stress conditions, whereas a non-significant negative correlation was noticed between these parameters under optimal growing conditions (Table 3). However, under optimal sowing, grain yield shared a significantly positive relationship with ear leaf area ($r = 0.455^*$) and number of grains per ear ($r = 0.380^*$). The results showed that ear leaf angle had a significant negative correlation with grain yield under high temperature stress ($r = -0.420^*$), indicating that hybrids with a larger ear leaf angle were more heat susceptible. However, under optimal conditions, a non-significant correlation was identified between leaf angle and grain yield ($r = -0.095^{NS}$). Net photosynthetic rate was found to be positively correlated with grain yield under both optimal ($r = 0.634^{**}$) and heat stress ($r = 0.393^*$) conditions. However, the association was much stronger in optimal sowing than high temperature stress. Grain yield was further reported to have non-significant but negative correlation with ear height ($r = -0.126^{NS}$), thousand grain weight ($r = -0.031^{NS}$), stomatal conductance ($r = -0.170^{NS}$), and grain protein content percentage ($r = -0.037^{NS}$) under elevated temperature. Days to 50% anthesis and silking, ear leaf angle, and net photosynthetic rate had substantially strong correlation with grain yield under heat.

Table 6
Association between principal components (PCs) and plant traits under optimal and heat stress conditions

Plant traits	Optimal Conditions		Heat stress conditions	
	PC1	PC2	PC1	PC2
Days to 50% anthesis	0.734	0.404	0.864	0.042
Days to 50% silking	0.744	0.386	0.860	0.065
Plant height	-0.686	-0.001	0.411	0.400
Ear height	-0.487	0.002	0.546	0.332
Ear leaf angle	-0.210	-0.511	-0.490	-0.274
Ear leaf area	-0.351	0.574	-0.036	0.406
Number of grains per ear	-0.416	0.182	-0.288	0.596
Ear length	-0.225	0.201	-0.427	0.127
1000 grain weight	-0.447	0.021	0.012	-0.186
Grain protein content	0.652	-0.062	0.583	-0.552
Grain oil content	0.794	0.155	0.611	-0.207
Net photosynthetic rate	-0.191	0.752	0.213	0.640
Stomatal conductance	-0.643	0.075	-0.579	0.425
Grain yield	-0.342	0.732	0.491	0.466

Values in bold indicate significant associations between PCs and traits.

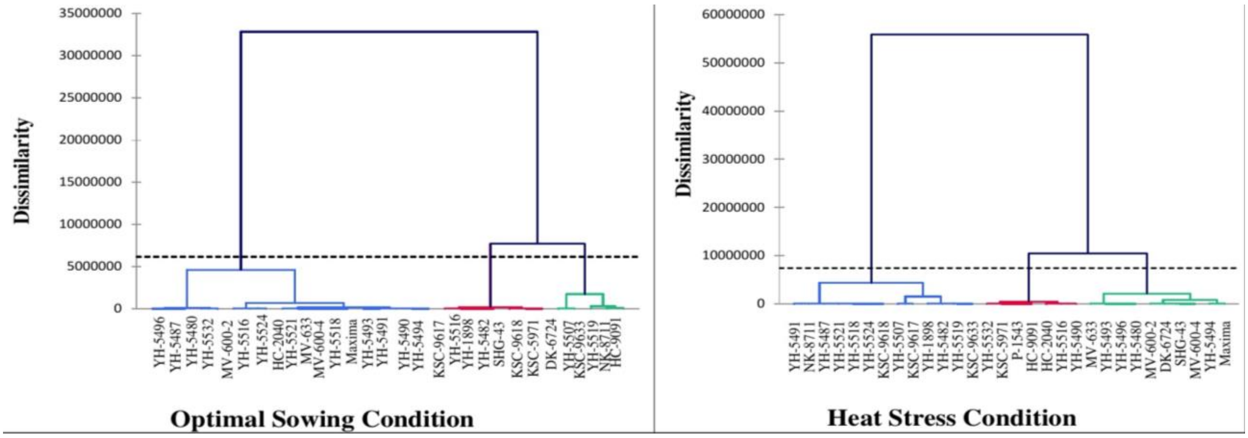


Fig. III. Dendrogram of maize hybrids under optimal and heat stress

Cluster analysis

Cluster analysis was used to categorize genotypes into different groups with high homogeneity within the cluster and high heterogeneity between clusters on the basis of their mean performance. Results identified three clusters of maize hybrids under optimal sowing conditions (T_n) (Table 4). Cluster-1 comprised of 18 hybrids including YH-5496, YH-5487, YH-5480, YH-5532, MV-600-2, YH-5516, YH-5524, HC-2040, YH-5521, MV-633, MV-600-4, YH-5518, Maxima, YH-5493, YH-5491, YH-5490, YH-5494, and KSC-9617 (Fig. III). These hybrids were late maturing with higher ear placement, larger ear leaf angle, higher grain protein content percentage, and grain oil percentage. However, hybrids included in this cluster were low yielding (9.2 t/ha) (Table 4). Cluster-2 comprised of 7 hybrids including YH-5516, YH-1898, YH-5482, SHG-43, KSC-9618, KSC-5971, and DK-6724, and can be classified as intermediate producing hybrids with a mean yield of 10.8 t/ha (Table 4). Similarly, cluster 3 consisted of 5 hybrids; YH-5507, KSC-9633, YH-5519, NK-8711, and HC-9091. This group had the highest mean values for days to 50% anthesis and silking, plant height, ear height, ear leaf area, grain protein content percentage, grain oil percentage, net photosynthetic rate, and grain yield. This group can be characterized as the most productive hybrids under optimal growing conditions, due to its highest mean values for grain yield (12.3 t/ha) and closely related traits (Table 4).

Table 7
Association between principal components (PCs) and maize hybrids under optimal and heat stress conditions

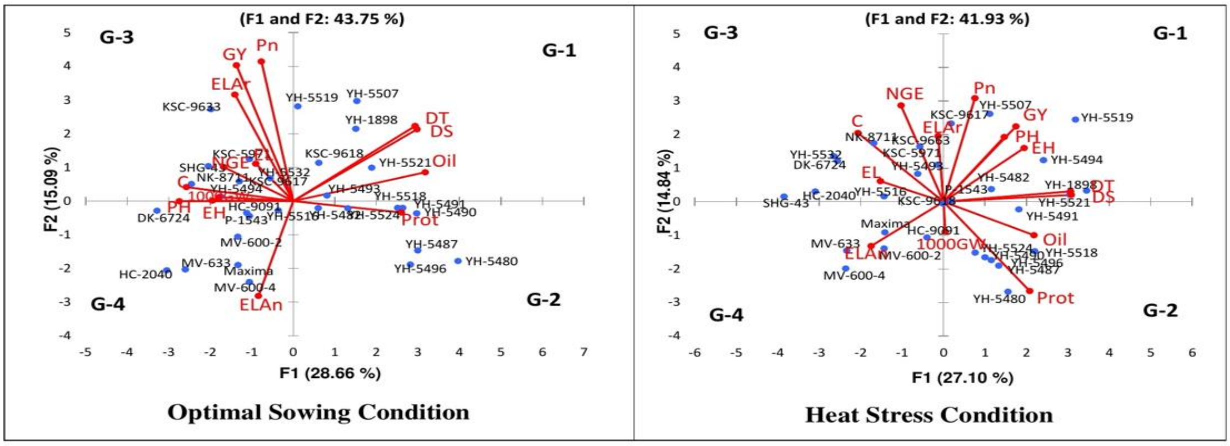
Sr.	Hybrids	Optimal Conditions		Heat stress conditions	
		PC1	PC2	PC1	PC2
1	YH-5493	0.811	0.169	-0.623	0.833
2	YH-5532	-0.559	0.675	-2.644	1.354
3	YH-5516	-0.356	-0.280	-1.436	0.157
4	YH-5494	-1.747	0.133	2.413	1.236
5	YH-5519	0.114	2.812	3.185	2.451
6	YH-5507	1.535	2.972	1.113	2.617
7	YH-5521	1.893	0.994	3.043	0.214
8	YH-5491	2.516	-0.204	1.819	-0.229
9	YH-5496	2.819	-1.884	1.155	-1.742
10	YH-5482	0.601	-0.211	1.151	0.378
11	YH-5524	1.318	-0.213	0.764	-1.518
12	YH-5487	2.997	-1.467	1.330	-1.905
13	YH-5490	2.974	-0.368	0.997	-1.652
14	YH-5518	2.636	-0.201	2.199	-1.475
15	YH-5480	3.970	-1.781	1.559	-2.684
16	KSC-9633	-1.977	2.731	-0.571	1.648
17	KSC-9618	0.613	1.138	-0.006	-0.056
18	KSC-5971	-1.056	1.244	-0.149	1.099
19	KSC-9617	-1.296	0.588	0.184	2.327
20	P-1543	-1.051	-0.443	0.190	0.018
21	YH-1898	1.510	2.148	3.452	0.332
22	DK-6724	-3.282	-0.287	-2.556	1.211
23	NK-8711	-2.453	0.508	-1.685	1.744
24	HC-2040	-3.046	-2.057	-3.091	0.303
25	HC-9091	-1.119	-0.355	-0.401	-1.060
26	SHG-43	-2.044	1.034	-3.849	0.154
27	MV-600-4	-1.057	-2.406	-2.361	-1.993
28	MV-633	-2.596	-2.029	-2.333	-1.459
29	Maxima	-1.331	-1.901	-1.414	-0.912
30	MV-600-2	-1.336	-1.059	-1.436	-1.391

Values in bold indicate significant associations between PCs and hybrids.

Under heat stress conditions (T_h), cluster analysis again categorized maize hybrids into three clusters (Table 4). Cluster-1 consisted of 10 hybrids: MV-633, YH-5493, YH-5496, YH-5480, MV-600-2, DK-6724, SHG-43, MV-600-4, YH-5494, and Maxima (Fig. III). Notably, this cluster contained the largest number of exotic hybrids, and can be categorized as the most heat-sensitive group of hybrids due to their low productivity under high temperature stress conditions (5.6 t/ha) (Table 4). Hybrids in Cluster-1 had larger ear leaf angles and the highest stomatal conductance, whereas plant height, ear leaf area, grains per ear, thousand grain weight, net photosynthetic rate, and grain yield had the lowest mean values. Cluster-2 can be classified as a moderately tolerant group comprising the 7 hybrids YH-5532, KSC-5971, P-1543, HC-9091, HC-2040, YH-5516, and YH-5490, most of which were developed locally (Fig. III). Cluster 2 hybrids had higher leaf angle, higher grains per ear, larger ear length and greater 1000-grain weight (Table 4). Cluster-3 comprised of 13 hybrids; YH-5491, NK-8711, YH-5487, YH-5521, YH-5518, YH-5524, KSC-9618, YH-5507, KSC-9617, YH-1898, YH-5482, YH-5519 and KSC-9633, 12 of which were developed locally (Fig. III). This cluster can be characterized as the most heat-tolerant group due to its maximum mean values for grain yield (9.0 t/ha) and associated traits under heat stress (Table 4). Maize hybrids in cluster 3 had maximum mean values for yield-contributing traits, i.e., days to 50% anthesis and silking, plant height, ear height, ear leaf area, grain oil content percentage, grain protein content percentage and grain yield.

Principal component analysis

Principal component analysis (PCA) is being extensively used to assess genetic divergence in populations, due to its precision and informativeness. In the present study, PCA extracted 14 principal components (PCs) on the basis of morphological, phonological, physiological and grain quality traits under optimal and heat stress conditions (Table 5). Of these 14 PCs, only 5 had an eigenvalue greater than 1 under optimal and heat stress conditions (Table 5). These 5 PCs contributed to 74.9% of the total variability under optimal conditions, and 72.2% under heat stress conditions.



Optimal Sowing Condition

Heat Stress Condition

Fig. IV. Biplot of traits and maize hybrids under optimal and heat stress conditions

PC1 accounted for 28.6% of total variability in the data under optimal sowing. In PC1, oil content percentage (0.794), days to 50% silking (0.744), day to 50% anthesis (0.734), and grain protein content percentage (0.652) made the greatest positive contributions to divergence, while plant height (−0.686) and stomatal conductance (−0.643) made negative contributions under optimal growing conditions (Table 6). PC2 explained 15.09% of total diversity, and the traits with the greatest positive contributions were net photosynthetic rate (0.752), grain yield (0.732), and leaf area (0.574) (Table 7). Some hybrids showed greater variability than others in PC1 and PC2. For example, the six locally bred hybrids YH-5480, YH-5487, YH-5490, YH-5496, YH-5518, and YH-5491 made the largest positive contributions to total divergence in PC1, whereas 5 exotic hybrids (DK-6724, MV-633, NK-8711, HC-2040, and SHG-43) along with one local hybrid (HC-2040) made negative contribution. In PC2, the 6 local hybrids YH-5507, YH-5519, KSC-9663, KSC-5971, and KSC-9618 made positive contributions, whereas two exotic hybrids (MV-600-4 and Maxima) contributed negatively to divergence.

Under heat stress conditions, PC1 explained 27.1% of total variability, with an eigenvalue of 3.79 (Table 5). The traits that made the largest positive contributions to diversity in PC1 were days to 50% anthesis (0.864), days to 50% silking (0.860), oil percentage (0.611), grain protein content percentage (0.583), ear height (0.546), and grain yield (0.491), whereas the contribution of stomatal conductance (−0.579) was negative. PC2 accounted for 14.8% of the variability, with net photosynthetic rate (0.640) making the largest positive contribution. In PC1 the local hybrids YH-1898, YH-5519, and YH-5521 made the greatest positive contributions, whereas the exotic hybrids SHG-43, MV-600-4, MV-633, and MV-600-2 made negative contributions to diversity. Of the hybrids in PC2, three local ones (YH-5519, KSC-9617, and KSC-5971) showed positive contributions to divergence, whereas four hybrids (YH-5480, YH-5487, YH-5490, and YH-5524) made negative contributions to genetic variability.

Biplot analysis

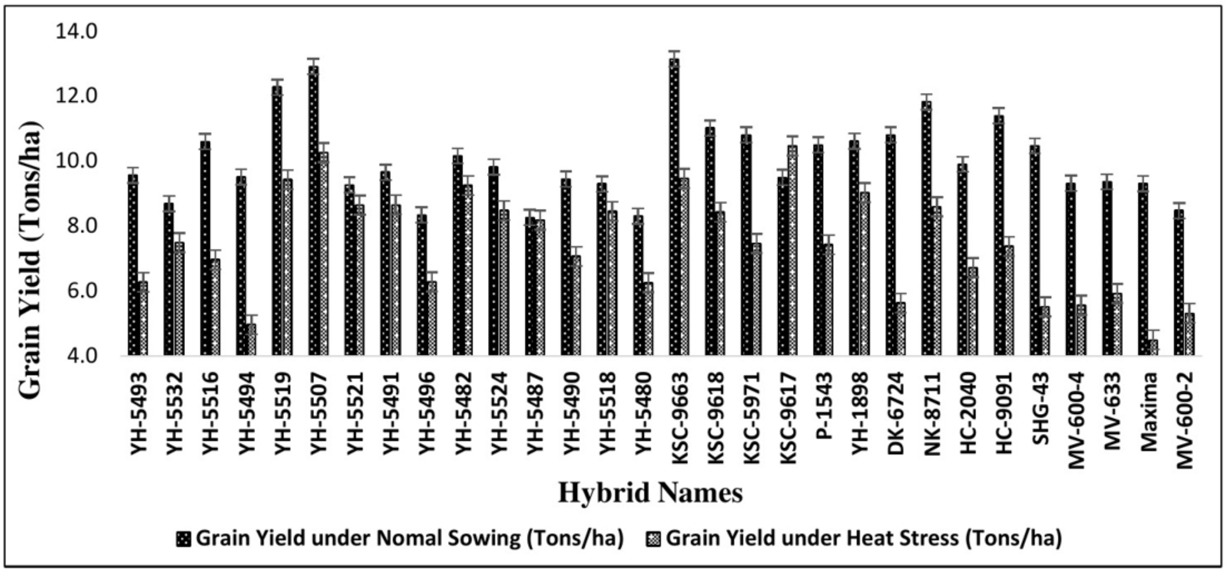


Fig. Mean values for grain yield in maize hybrids under optimal and heat stress conditions

Biplot graphs were generated from the PCA data for PC1 and PC2 under optimal (T_n) and heat stress (T_h) conditions. Vector length and cosine angle were used to group traits and hybrids. Biplots based on PC1 and PC2 under T_n (43.75% of total variability) showed that days to 50% anthesis and silking, ear leaf area, grain oil percentage, net photosynthetic rate, and grain yield were the traits with the greatest discriminating power due to their larger vector lengths (Fig. IV). Grain yield was very strongly related to net photosynthetic rate and ear leaf area, as shown by the appearance of their vectors within the same group (G-3). Similarly, under T_h (41.93% of total variability), the biplots for PC1 and PC2 showed that days to 50% anthesis and silking, grain protein content percentage, grain yield, net photosynthetic rate, stomatal conductance, and number of grains per ear were the most discriminating traits (Fig. IV). However, traits were more divergent in the T_h condition, as illustrated by the larger cosines of the angles between them. Grain yield was positively correlated with net photosynthetic rate, ear height, days to 50% anthesis, and days to 50% silking. Under optimal growth conditions, the number of grains per ear had little impact on grain yield, whereas under elevated temperature this trait had a significant impact on grain yield.

Discussion

Maize is one the most important and highly adaptive cereal crop of the world. It is primarily used for feed in poultry industry. Despite its high adaptability and C_4 nature, it is quite sensitive to high temperature at reproductive stages especially early flowering stage. The current study was designed to evaluate existing local and commercial hybrids for their heat tolerance and to elucidate the role of physiological traits in determining the heat tolerance in local and multinational maize hybrids. The results showed the presence of highly significant variations among maize hybrids, which depicts the differences among their performance and genetic potential under optimal and heat stress conditions. Similar results were reported by Yousaf et al. (2017) and Yousaf et al. (2020). The significance of the genotype × environment interaction suggests a differential response by maize hybrids for these traits under the two contrasting treatments which suggests that these traits are under minimal control of environmental states. Comparable results were also found by Shrestha et al., (2014) and Yousaf et al. (2018), who stated substantial differences between maize hybrids.

Correlation analysis revealed a significant association of grain with different morpho-physiological traits under both optimal and heat stress conditions. Results showed that net photosynthetic rate, days to 50% tasseling, and silking were the most important traits for the selection of highly productive and heat tolerant maize hybrids due to its high correlation with grain yield under both conditions. The more days taken to tasseling and silking, the more the biomass/source of the plant that will increase the potential of the sink, and ultimately high yield will be achieved (Yousaf et al., 2017; Shehzad et al., 2019). Similarly, high photosynthetic efficiency will increase the potential of the source, that will ultimately increase the capacity of sink and grain yield (Cairns et al., 2013). However, the presence of significantly negative correlation between grain yield and leaf angle depicts that the more leaf area exposed to intense sunlight increases the tissue damage resulting in the reduced photosynthesis due to lower RuBisCO activity as explained by Wahid et al., (2007) and Cairns et al., (2013). Therefore, positive selection for net photosynthetic rate, days to 50% tasseling, and days to 50% silking, these parameters must be included in the process of selection of parents to develop/improve climate-smart maize hybrids.

Different multivariate analysis techniques including cluster and principal component analysis were applied to classify maize hybrids on the basis of their performance under optimal and heat stress conditions. The obtained results categorized maize hybrids into different classes based on the performance of their morpho-physiological and grain quality traits under both conditions. Cluster analysis disclosed that the performance of most hybrids was stable under both optimal (T_n) and heat stress (T_h) treatments. However, some hybrids showed evidence of shifting from heat-sensitive to heat-tolerant behavior and vice versa. In optimal sowing (T_n), all cluster 3 hybrids (the most productive cluster) were also among the most heat-tolerant hybrids (cluster 3) under the heat stress condition (T_h) due to greater number of days taken to 50% tasseling and silking, lower ear leaf angle, greater leaf area and highest net photosynthetic rate, stomatal conductance, and number of grains per ear which were reported to have significant correlation with grain yield under optimal and heat stress conditions (Shakoor et al., 2007; Bello et al., 2010). Hybrids in cluster 2 from T_n, YH-1898, YH-5482, KSC-9618 etc. showed large shifts from intermediate-producing hybrids (cluster 2) to highly heat-tolerant hybrids (cluster 3) under heat stress treatment (T_h). However, two hybrids (SHG-43 and DK-6724) moved to the heat-sensitive cluster in T_h, a change that revealed their sensitivity to heat stress. Similarly, the six hybrids YH-5487, YH-5524, YH-5521, YH-5518, YH-5491, and KSC-9617 changed from the poor-performing (Cluster 1) in T_n to the highly heat-tolerant cluster (cluster 3) in T_h. The reason for this shift might be the high heat tolerance ability due to lower leaf angle and high photosynthetic efficiency of these hybrids as suggested by Iqbal et al., 2015. Three hybrids (YH-5532, HC-2040, and YH-5490) showed moderately heat tolerant behavior (Cluster 2).

Cluster analysis effectively grouped the maize hybrids compared here into susceptible, reasonably tolerant, and highly tolerant to high temperature stress conditions for various traits. Many researchers have used cluster analysis to classify genotypes from different crop species under different environmental conditions including drought and heat stress and found this approach helpful for parent selection in hybrid breeding programs to develop heat-resilient genotypes (Saeed et al., 2018; Bhatti et al., 2020; Khalid et al., 2020). Al-Naggar et al., (2020) applied cluster analysis to evaluate and classify nineteen maize hybrids and showed that this method was able to effectively characterize maize hybrids based on their genetic diversity.

Principal component and biplot analysis also categorized maize hybrids into different groups. Similar trend was also observed in PCA and biplot analysis which showed that YH-5507, YH-5482, and YH-5519 were the most productive and heat tolerant hybrids under heat stress condition due to their strong correlation with net photosynthetic rate, plant height, days to 50% tasseling, and silking as shown by biplot graphs. This yield might be due to higher biomass based on plant height and leaf area; as discussed by Lambert et al., (2014), these traits are highly associated with higher grain yield in maize. Biplot analysis also revealed that most of the imported maize hybrids were mostly heat susceptible except NK-8711 and fall under group-4 of biplot. The highest value for ear leaf angle and the lowest value for net photosynthetic rate were the main causes of heat susceptibility and low productivity in cluster 1 hybrids. Ben-Asher et al., (2008) and Traore et al.,

(2000) also found that grain yield increased with net photosynthetic rate but was negatively associated with ear leaf angle under heat stress conditions. Furthermore, principal component analysis also depicted that days to 50% anthesis and silking, plant height, ear leaf angle, thousand grain weight, net photosynthetic rate, and grain oil content percentage were the most discriminating traits under heat stress conditions. Hence, these characters could be used in selection of parents for hybrid development in areas subject to heat stress. Comparable results were described by Yousaf et al., (2017) and Yousaf et al., (2018), who found that the indigenous hybrids YH-1898, FH-1046, FH-949, and YH-5133 were more heat tolerant than exotic ones (Maxima, MV-531, and P-1543). Ghani et al., (2017) also reported that the locally produced yellow maize hybrid YH-1898 had high grain yields under heat stress conditions.

Conclusion

The results showed the presence of highly significant differences among maize hybrids for grain yield and associated morpho-physiological and grain quality traits under optimal and heat stress conditions. Correlation analysis suggested that net photosynthetic rate, days to 50% silking, number of grains per ear, and leaf angle were the most significant traits for the choice of parents under heat stress conditions, as these traits showed significant relationships with grain yield. The results based on PCA, cluster analysis, and biplot graphs were highly consistent and showed that locally bred maize hybrids, especially YH-5507, YH-5519, KSC-9663, YH-5521, YH-5482, and YH-1898 and one multinational hybrid (NK-8441) were more heat tolerant than other exotic hybrids such as MV-600-4, MV-600-2, MV-633, and SHG-43, and displayed higher levels of diversity under both optimal and heat stress conditions.

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