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MORPHOMETRIC AND PHENOLOGICAL CHARACTERIZATION OF MAIZE (*ZEA MAYS* L.) GERMPLASM UNDER HEAT STRESS

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ABSTRACT

Maize is an important food, feed and industrial crop that is severely affected by adverse climatic conditions, especially heat stress. An experimental study was designed to characterize maize hybrids of different origin for morphological and phenological traits under heat stress. The research material consisted of 21 maize hybrids of different origin. Data were recorded for different plant traits related to heat tolerance: days to 50% tasseling, days to 50% silking, plant height, cob height, plant harvested, cob harvested, cob length, number of grains per cob, thousand grain weight, shelling percentage and grain yield per hectare. The results showed high genetic variability among maize hybrids for yield-related traits under elevated temperature stress. Principal component and biplot analysis were performed to assess heat tolerance in hybrids of different ecological areas. Locally bred maize hybrids FH-988, FH-922, FH-949, YH-1898, YH- 5402 and YH-5133 were found to be the most heat tolerant and possessed high genetic divergence for yield and yield-related morphological and phonological traits under heat stress. The results also showed that days to 50% heading, days to 50% silking, grain yield per plot and cob length could be used as efficient selection criteria for the production of heat-resilient maize hybrids.

Keywords: Principal component analysis, Biplot analysis, Genetic diversity, Climate change

INTRODUCTION

Maize (*Zea mays* L.), the third most important cereal crop worldwide after wheat and rice, is cultivated on a total area of 184.80 million hectares with a total production of 1037.79 million metric tons and an average yield of 5.62 metric tons per hectare (FAO, 2014). It is predominantly grown for poultry and livestock feed (65%) and for human consumption (35%) (Halidu *et al.*, 2014). In Sub-Saharan Africa about 208 million people depend on maize as a source of food and livelihood (Macauley, 2015).

In Pakistan, maize is sown on an area of 1144 thousand hectares producing 4920 thousand tons, with an average yield of 4301 kg per hectare. It contributes 0.4% of the GDP and 2.2% in value addition (Anonymous, 2015-2016). The per-hectare yield of maize in Pakistan (4.29 metric tons/ha) is much lower than in other maize-producing countries such as the USA (10.73 metric tons/ha), Canada (9.36 metric tons/ha), Turkey (8.73 metric tons/ha), Argentina (8.20 metric tons/ha) and China (5.81 metric tons/ha). However, Pakistan's average maize yield is almost double than that of India (2.63 metric tons/ha) (USDA, 2016). The reasons for low maize yield in Pakistan are high temperature, stalk rot infestation, maize borer and shoot fly infestation, poor crop management, high input rates and use of low quality, substandard seed. Heat stress in the flowering and grain filling periods due to elevated temperatures drastically affect crop productivity and is considered a major constrain to maize production in changing climatic conditions. A record drop in maize production was reported in many maize-growing areas of the world (Van der Velde *et al.*, 2010). It is predicted that maize yield might be reduced be 4% to 42% due to increasing temperatures (EPA, 1998).

In Pakistan, maize is cultivated in both spring and autumn seasons. The reproductive stage of maize in both seasons, especially the spring season, coincides with high temperature stress (above 32 °C) which drastically affects fertilization and seed setting by increasing the anthesis-silking interval (Dass *et al.*, 2010, Struik *et al.*, 1986; Cicchino *et al.*, 2010). Seed setting can be drastically reduced by as much as 80% during high temperatures due to sudden pollen shedding over a very short time (Fonseca *et al.*, 2005). Grain yield is highly correlated with grain weight, which is markedly reduced under heat stress due to the reduction in endosperm size/number, which decreases sink volume (Jones *et al.*, 1984). Heat stress severely affects the vegetative phase in maize plants by reducing leaf elongation, leaf area, shoot biomass and the rate of photosynthetic CO_2 assimilation, which ultimately reduces grain yield by limiting source capacity (Watts, 1972). Thus, there is an urgent need for achieving tolerance in existing maize genotypes or for breeding new genotypes with high tolerance to cope with heat stress. These targets could be achieved by selecting heat-tolerant maize germplasms and intercrossing them to produce heat-tolerant maize genotypes.

Genetic diversity analysis is imperative in crop improvement and can be studied through morphological, biochemical and molecular markers. Morphological characterization for genetic divergence among genotypes is considered an initial step (Khan *et al.*, 2014). Many researchers have used principal component analysis to assess genetic variability among maize genotypes because it retrieves small numbers of components that account for most of the variations in the data (Ignjatovic-Micic *et al*., 2015, Llaurado and Gonzales, 1993, Dao 2014, Asare 2016). Biplot analysis has been used to estimate the relationship between different morphological traits and grain yield under heat stress. It has also been used to classify maize germplasms for heat tolerance and susceptibility.

We designed the present study to characterize maize germplasms comprised of local and exotic hybrids from different ecological areas for genetic diversity under heat stress. The results are expected to be useful in efforts to select heat-tolerant hybrids for use in future breeding programs to produce heat-resilient maize cultivars.

MATERIALS AND METHODS

The experiment was conducted in the research area of the Maize and Millets Research Institutes, Yusafwala-Sahiwal during spring, 2016. A total of 21 maize hybrids were evaluated for heat tolerance, of which 16 maize hybrids were indigenous and obtained from the Maize and Millets Research Institute, Yusafwala-Sahiwal and 5 hybrids were collected from different multinational companies (Pioneer, Monsanto, Agroman and Jalandhar Pvt. Limited). The experiment was laid out in a randomized complete block design with three replications, and a net plot size of 22.5m^2. The crop was sown on 16 March, 2016 so that the reproductive stage of the plants coincided with maximum temperature stress. Sowing was done with the help of a dibbler to ensure a 15-cm plant-to-plant distance. Data were collected for heat-related plant parameters: days to 50% tasseling (DT), days to 50% silking (DS), plant height (PH), cob height (CH), plants harvested per plot (P.Hr), cobs harvested per plot (C.Hr), cob length (CL), number of grains per cob (NG/Cob), thousand grain weight (TGW), shelling percentage (S%) and grain yield per hectare (GY). The data were statistically analyzed for analysis of variance, correlation coefficients (Steel et al., 1997) and principal component analysis (PCA) (Sneath and Sokal, 1973) with two statistical applications: XLSTAT v. 15 and SPSS v. 16.0.

RESULTS AND DISCUSSION

Metrological data showed the effect of heat stress on the maize hybrids we tested during their life span, especially in the flowering and grain filling periods (Fig 1). Maximum mean temperature was observed in May (43.13 °C) and June (44.1 °C), which coincided with the flowering and grain filling periods.

Analysis of variance disclosed highly significant differences among genotypes for all traits under study (Table 1). Correlation analysis was conducted to identify associations among different traits themselves and with grain yield under heat stress. The greatest positive association was found between days to 50% tasseling and days to 50% silking (r = 0.993**) followed by plants harvested and cobs harvested (r = 0.867**), thousand grain weight and grain yield per hectare (0.688**). Negative associations were found between cobs harvested per plot and number of grains per cob ($r = -0.352^{NS}$), cobs harvested per plot and grain yield per hectare ($r = -0.325^{NS}$) and cob height and plants harvested per plot ($r = -0.311^{NS}$). Grain yield per hectare was positively and significantly associated with thousand grain weight (r = 0.688**), cob height (r = 0.643**), plant height (r = 0.451*) and days to 50% tasseling (r = 0.448*) as shown in Table 2.

Principal component analysis based on eleven morphological and phenological traits extracted five PCs with an eigenvalue greater than 1 (Table 3). The contribution of these PCs to total variability was 87.42%. The contribution of PC1 to total divergence in the population studied here was 33.685%, of which the traits accounting for the largest contributions were cob height (0.863), days to 50% (0.820), days to 50% silking (0.794), grain yield per hectare (0.767) and plant height (0.519). Principal component 2 explained 17.25% of the total variability in the data. In PC2, plants harvested per plot (0.722) and cobs harvested per plot (0.709) made positive contribution while number of grains per cob (−0.512) and thousand grain weight (−0.417) made negative contributions to this component. The third PC contributed 14.32% of the total variability in yield-related traits and morphological traits with the largest contributions to PC3 were cob height (0.821) and number of grains per cob. The traits plants harvested per plot (0.380), cobs harvested per plot (0.344), shelling percentage (0.293) and plant height (0.281) also contributed positively to PC3 but at lower magnitudes. Total variability due to PC4 was 13.16%. This component comprised mainly positive loading from thousand grain weight (0.862). Principal component 5 accounted for 9.00% of the variability in total genetic divergence and the main contribution was from the variation in shelling percentage (0.531).

Biplot analysis was used to visualize the interrelationships among different traits and thus facilitate parent selection for breeding purposes. In our PC biplots, the variables are superimposed as vectors, and their relative lengths represent the relative amounts of variability. In PC1 and PC2 biplot, plant height (PH), cob height (CH), number of grains per cob (NG/cob) and shelling percentage (S%) predominated, whereas days to 50% tasseling (DT), days to 50% silking (DS), grain yield per hectare (GY) showed minimum differences (Fig 2). On the other hand, in PC1 and PC3 biplot, cob length (CL), number of grains per cob (NG/cob) and cob height (CH) showed maximum differences (Fig 3). The traits number of cob harvested, plant height, cob height, cob length and number of grains per cob had the most discriminating power due to their relatively longer vector length. The results of PCA and Biplot analysis suggested high variability for days to 50% tasseling, days to 50% silking, plant height, cob height and grain yield in maize hybrids FH-988, FH-922, FH-949, YH-5402 and YH-5133 under conditions of high temperature stress.

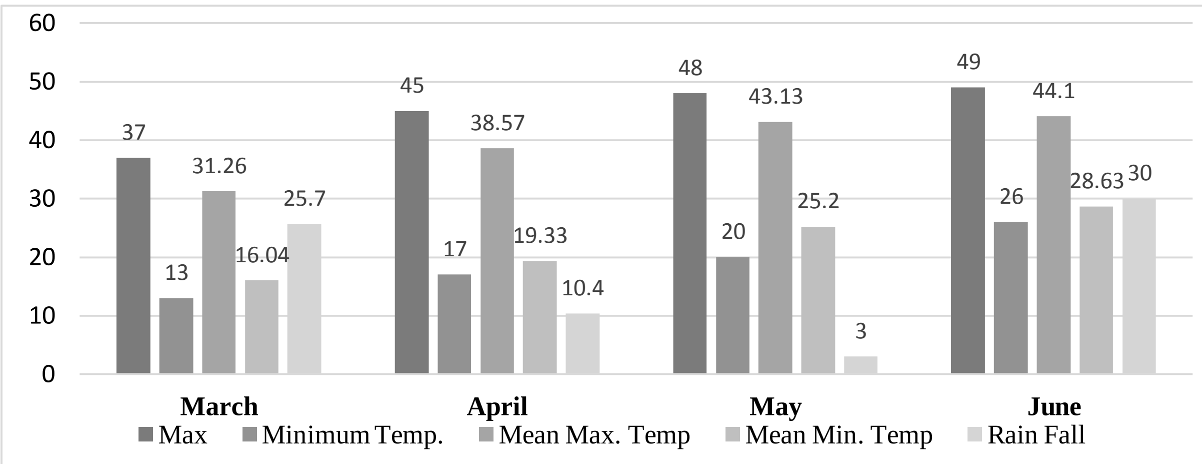
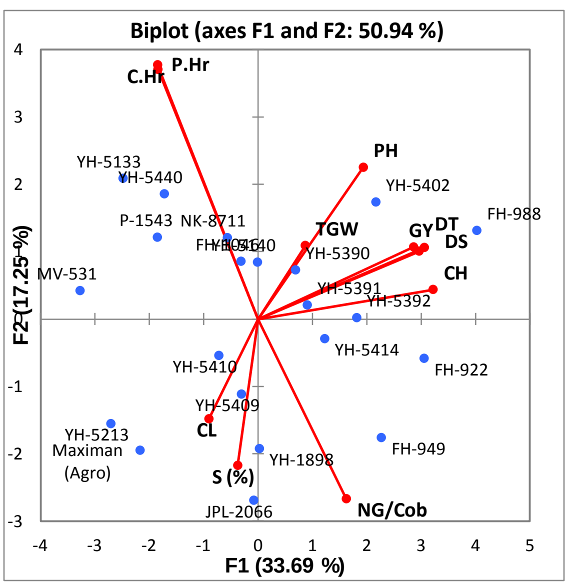


Fig. 1. Metrological data (temperature, °C and rainfall, cm) for the spring season maize crop.



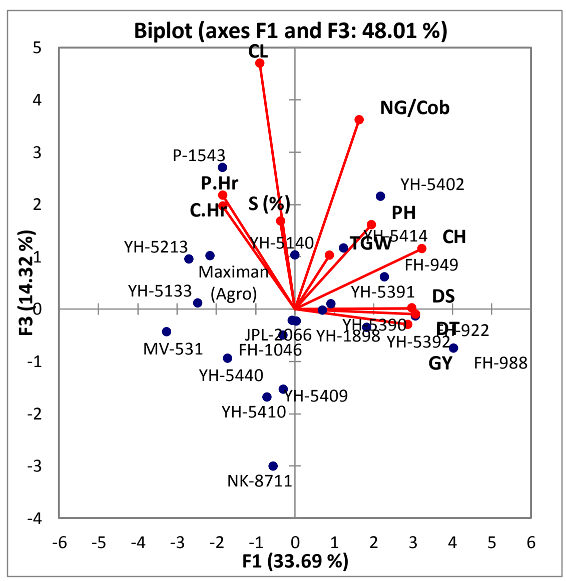


Fig.2. Biplot of PC1 and PC2.

Fig.2. Biplot of PC1 and PC2.

Table 1. Analysis of variance (ANOVA) for morphological and phenological characters in maize hybrids.

SOV	Df	DT	DS	PH	CH	P.Hr	C.Hr	CL	NG/Cob	TGW	S (%)	GY
Replications	**2**	0.397	0.206	23.190	13.635*	6.683	49.00	0.009	0.302	134.81	0.778	62372
Genotypes	**20**	21.33**	20.56**	355.53**	289.22**	100.18**	139.37**	0.884**	36.68**	1381.18**	15.45**	5105835**
Error	**40**	0.814	0.939	14.52	7.002	37.02	38.78	0.0089	1.468	100.56	0.611	117698
Overall mean		**73.8**	**76.59**	**210.67**	**109.06**	**148.68**	**152.38**	**7.896**	**46.397**	**302.92**	**86.41**	**10622**

* Significant at 5% probability level, ** Significant at 1% probability level, NS = Not significant
(DT = Days to 50% tasseling, DS = Days to 50% silking, PH = Plant height, CH = Cob height, P.Hr = Plant harvested, C.Hr = Cob harvested, CL = Cob length, NG/Cob = Number of grains per cob, TGW = Thousand grain weight, S (%) = Shelling percentage, GY = Grain yield per hectare)

Table 2. Trait associations for morhological and phenological characters in terms of correlation coefficient (r) in maize hybrids.

Trait	DT	DS	PH	CH	P.Hr	C.Hr	CL	NG/Cob	TGW	S (%)
DS 50%	0.993**	1								
PH	0.311	0.281	1							
CH	0.604**	0.580**	0.641**	1						
P.Hr	-0.233	-0.208	0.145	-0.311	1					
C.Hr	-0.145	-0.119	0.016	-0.302	0.867**	1				
CL	-0.264	-0.255	-0.072	-0.082	0.141	0.169	1			
NG/Cob	0.299	0.299	0.157	0.434*	-0.280	-0.352	0.496*	1		
TGW	0.041	0.026	0.025	0.130	0.041	0.001	0.146	-0.048	1	
SP (%)	-0.134	-0.063	-0.151	-0.105	-0.076	-0.036	0.138	0.242	0.005	1
GY	0.448*	0.419	0.451*	0.643**	-0.289	-0.325	-0.272	0.108	0.688**	-0.090

* Significant at 5% probability level, ** Significant at 1% probability level, NS = Not significant

Table 3. Eigenvalue, factor variability (%) and cumulative variability (%) of different factors in maize hybrids under heat stress.

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Eigenvalue	3.705	1.898	1.576	1.447	0.990	0.824	0.241	0.209	0.079	0.028	0.003
Variability (%)	33.685	17.251	14.324	13.158	9.000	7.489	2.189	1.900	0.722	0.255	0.027
Cumulative %	33.685	50.936	65.260	78.418	87.418	94.907	97.096	98.995	99.717	99.973	100.000

Table 4. Principal component analysis of 11 morphological and phenological traits in maize hybrids under heat stress.

Trait	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
DT	0.426	0.147	-0.014	-0.308	0.324	-0.205	-0.043	-0.200	-0.026	-0.105	0.708
DS	0.412	0.140	0.003	-0.326	0.385	-0.162	-0.057	-0.184	-0.101	0.147	-0.679
PH	0.270	0.313	0.224	-0.033	-0.503	0.432	-0.025	-0.527	0.101	-0.217	-0.061
CH	0.448	0.060	0.160	-0.020	-0.249	0.137	-0.345	0.662	-0.355	-0.070	0.008
P.Hr	-0.257	0.524	0.303	-0.072	0.063	0.077	0.459	0.066	-0.489	0.301	0.087
C.Hr	-0.256	0.515	0.274	-0.136	0.220	-0.003	-0.248	0.299	0.577	-0.205	-0.048
CL	-0.126	-0.207	0.654	0.083	-0.097	-0.356	-0.441	-0.247	-0.068	0.327	0.059
NG/Cob	0.226	-0.372	0.503	-0.155	-0.047	-0.098	0.610	0.194	0.260	-0.203	-0.053
TGW	0.121	0.151	0.143	0.717	0.286	-0.200	0.046	-0.096	-0.204	-0.497	-0.084
S (%)	-0.052	-0.303	0.234	0.031	0.534	0.732	-0.130	-0.047	-0.071	0.034	0.061
GY	0.398	0.148	-0.042	0.478	0.037	0.087	0.130	0.086	0.403	0.622	0.084

Table 5. Contribution of maize hybrids of different origin to genetic diversity for yield and yield-related morphological and phenological traits under heat stress.

Genotype	Origin of Hybrid	F1	F2	F3	F4	F5
FH-1046	MMRI Yusafwala	0.125	1.823	0.763	20.286	0.005
FH-949	MMRI Yusafwala	6.612	7.804	1.130	6.094	2.814
FH-922	MMRI Yusafwala	12.001	0.867	0.056	0.847	6.417
FH-988	MMRI Yusafwala	20.824	4.304	1.706	0.288	8.762
YH-1898	MMRI Yusafwala	0.001	9.318	0.166	20.791	0.504
YH-5213	MMRI Yusafwala	9.406	6.082	2.742	2.291	24.459
YH-5390	MMRI Yusafwala	0.613	1.316	0.002	3.333	0.705
YH-5391	MMRI Yusafwala	1.057	0.104	0.029	2.074	2.416
YH-5392	MMRI Yusafwala	4.242	0.001	0.378	0.172	1.038
YH-5402	MMRI Yusafwala	6.044	7.523	13.991	0.070	7.519
YH-5409	MMRI Yusafwala	0.119	3.138	7.140	4.556	0.505
YH-5410	MMRI Yusafwala	0.666	0.745	8.597	2.327	0.027
YH-5414	MMRI Yusafwala	1.936	0.220	4.090	0.104	7.623
YH-5140	MMRI Yusafwala	0.000	1.767	3.210	15.716	0.338
YH-5133	MMRI Yusafwala	7.916	10.888	0.038	0.457	6.832
YH-5440	MMRI Yusafwala	3.811	8.612	2.698	0.028	3.191
NK-8711	Syngenta	0.408	3.626	27.336	0.695	0.662
P-1543	Pioneer	4.401	3.668	22.080	0.505	7.464
Maxima	Agroman	6.044	9.557	3.123	11.540	2.255
MV-531	Agroman	13.765	0.436	0.579	4.088	0.068
JPL-2066	Julandhar Pvt. Limited	0.008	18.202	0.147	3.740	16.398

Table 6. Contribution of different plant characteristics to genetic diversity for yield and yield-related morphological and phenological traits in maize under heat stress.

Trait	F1	F2	F3	F4	F5
Days to 50% tasseling	0.820	0.203	-0.018	-0.370	0.322
Days to 50% silking	0.794	0.192	0.003	-0.392	0.383
Plant height	0.519	0.431	0.281	-0.039	-0.501
Cob height	0.863	0.083	0.201	-0.024	-0.248
Plant harvested/plot	-0.494	0.722	0.380	-0.087	0.063
Cob harvested/plot	-0.493	0.709	0.344	-0.164	0.219
Cob length	-0.242	-0.285	0.821	0.100	-0.097
Number of grains per cob	0.436	-0.512	0.632	-0.186	-0.047
Thousand grain weight	0.233	0.208	0.179	0.862	0.284
Shelling (%)	-0.100	-0.417	0.293	0.037	0.531
Grain yield per hectare	0.767	0.204	-0.052	0.575	0.037

Heat stress is a major factor in climate change that severely limits the productivity of almost all crops including maize, Worldwide (Lobell and Gourdji, 2012). It is projected that maize production will decrease by as much as 18.5% in South Asia by 2050 (ADB, 2009). This makes it necessary to produce heat-tolerant maize hybrids for sustainable maize production to fulfill consumers' demands. To this end, explorations of genetic variability in existing germplasms is potentially useful for maximizing crop yield and minimizing crop failure under unfavorable environmental conditions such as heat stress (Gepts, 2010). Germplasms collected from heat-stressed areas of the world possess adaptive traits such as leaf hairiness, leaf angle, thicker waxy cuticle, leaf layering and shorter life cycles, which enable them to cope with high temperature stress. To assess genetic diversity in the different hybrids, PCA was used because it identifies a minimum number of components that contribute maximum variability to a given data set. Heat stress delays tasseling and silking time in maize by increasing the anthesis-to-silking interval, which ultimately reduces grain yield as a result of poor fertilization and seed setting (Struik *et al.* 1986, Cicchino *et al.,* 2010). Heat stress reduces plant height, kernel weight (Traore *et al.,* 2000), number of seeds per cob (Singh *et al.,* 2016), ear length (Shrestha *et al.,* 2014) and grain yield (Giaveno and Ferrero, 2003). Our findings were in complete agreement with the results of these earlier studies.

Principal component and biplot analysis revealed that the greatest variation under heat stress occurred in maize hybrids FH-922, FH-988, FH-949, YH-5404 and YH-5133. These genotypes were furthest away from the origin in the biplot of discriminating traits. Similarly, plant traits days to 50% tasseling, days to 50% silking, plant height, cob height, and grain yield made the largest contributions to variability in the data, as shown by their longer vector length. Principal component and biplot analysis showed that maize hybrids

FH-988, FH-922, YH-1898, YH-5402 and YH-5390 produced the largest grain yields, so these hybrids can be considered heat tolerant. On the other hand, hybrids Maxima, MV-531 and YH-5140 produced the lowest yields under heat stress and can be considered heat susceptible. Hybrids were considered tolerant or susceptible on the basis of discriminating traits such as plant height, cob height, number of grains per cob and cobs harvested per plot. Local maize hybrids were much more heat tolerant than hybrids from multinational companies (Pioneer, Monsanto and Syngenta) because the former were bred under heat stress conditions and are more adaptable to heat stress. We selected contrasting genotypes as candidates for further study to develop heat-tolerant germplasms. It is clear from our PCA and biplot analyses that the maize germplasms we investigate have remarkable genetic variability in their response to heat stress and this variability may prove to be a valuable source in breeding programs aimed at obtaining heat-tolerant maize hybrids.

CONCLUSION

The current study documents a high level of genetic divergence for yield and yield-related morphological and phenological traits among the maize germplasms here under heat stress conditions. Heat stress-tolerant maize hybrids were identified through principal component and biplot analysis. According to our findings, hybrids FH-988, FH-922, FH-949, YH-1898, YH-5402 and YH-5133 were the most heat tolerant and had the highest diversity for the traits. These hybrids could be exploited in breeding programs to further develop heat-tolerant maize hybrids.

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