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Reproductive response based diversity in genetically distant maize (*Zea mays* L.) germplasm under heat stress

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Abstract

Maize (*Zea mays* L.) is third main cereal after wheat and rice. It is grown across the world. Maize yield is affected by different stresses. Among these, high temperature is the major one in reducing the yield. High temperature influences the reproductive stage of spring crop in Pakistan. So there is a need to study the reproductive responses of genetically diverse germplasm under high temperature. Total 11 hybrids of maize were tested using randomized complete block design. Data were recorded on pollen germination percentage, pollen grain size, pollen tube length, leaf temperature, cell membrane thermostability, leaf chlorophyll content, cob length, cob diameter, number of rows per cob, number of grains per row, 100 grain weight and grain yield per plant. The data were analyzed statistically to study the correlation and path coefficient. The analysis of variance showed that all the genotypes were significantly different from each other for all the above parameters. Highly significant results showed that there was a strong variability present in the genotypes. The grain yield per plant had positive and significant correlation with pollen germination percentage, pollen grain size, pollen tube length, leaf chlorophyll contents, cob length, cob diameter, number of kernels per row and 100 grain weight. It had significantly negative correlation with leaf temperature and cell membrane thermostability which was expressed as relative cell injury percentage. Path coefficient analysis showed that pollen germination percentage, pollen grain size, cell membrane thermostability, cob diameter and 100 grain weight had positive direct effects on grain yield. Pollen tube length, leaf temperature, leaf chlorophyll contents, cob length, number of rows per cob and number of grains per row had negative direct effects on yield per plant. 100 grain weight had the highest positive direct effect on grain yield. So, it can be used as selection criteria for yield improvement.

Keywords: Maize, high temperature, grain yield, correlation
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Introduction

Maize (*Zea mays* L.) is one of the major cereals after wheat and rice, grown around the globe. It is the only cultivated member of the genus *Zea*. Maize is grown all around the world ranging from temperate to tropical regions and geographically from 58° North latitude to 40° South latitude. In Pakistan it is grown on the fields of Punjab and hilly areas of Khyber Pakhtunkhwa. Maize is an income resource in developing countries (Tange *et al.*, 2008).

Maize is highly nutritious as its grain contains 72.4% starch, 12.2% moisture, 9.1% protein, 3.45% fat, 1.5% ash and 1.4% fiber (Ahmed *et al.*, 2014).It is a multipurpose crop and used in several forms. Maize grain is a rich source of starch, proteins, vitamins and minerals. Maize is C4 plant. Heat stress is one of the main environmental stresses that affect the growth and crop yield in various regions of the world (Ulukan, 2009). It is a monoecious plant with pistilate and staminate flowering pattern and is much prone to heat stress at reproductive stage. In Pakistan, maize is cultivated in both spring and summer seasons. The spring season crop faces high temperature stress at reproductive stage. While autumn season crop faces high temperature at planting time that affects seed germination and seedling growth.

Different biotic and abiotic stresses are seriously effecting the maize crop at different growth stages in different regions across the world (Aslam *et al.*, 2014; Naveed *et al.*, 2014; Aslam *et al.*, 2015 *a, b, c*). Temperature stress reduces the plant growth, affects photosynthesis, pollination and average grain yield. In maize, leaf growth is more from 0 to 35°C, but reduces at 35 to 40°C. More than 30ºC temperature reduces the Rubisco activation (Steven *et al.*, 2002). Leaf temperature above 38°C hinders the net photosynthesis due to thermal inactivation of enzymes. High temperature results in flower abortion and reduction in seed size (Talwar *et al.*, 1999). High temperature stress affects pollination by silk desiccation and pollen abortion. Heat stress results in too early development of anther and block its cell development (Oshino *et al.*, 2007). It also results male sterility in some plant species (Sato *et al.*, 2006; Abiko *et al.*, 2005). Heat stress reduced grains density whereas, grains dry weight decreased from 79 to 95% in field conditions under heat stress (Commuri and Jones, 2001).

The degree of damage is dependent on the time period and intensity of high temperature. The

temperatures more than 35°C during the period of pollination and grain filling decreases the grain yield by 1% under rain-fed conditions and by 1.7% under drought conditions (Lobell *et al.*, 2011).
The present study was carried out to evaluate 11 maize hybrids under heat stress condition. The information so derived may be helpful for developing selection criterion and future maize breeding programs to develop heat tolerant genotypes.

Materials and methods

The plant material was comprised of 11 hybrids of maize TG-4557, P-1543, TG-46B90, TG-4560, TG-4265, 15BJSF6, DK-6103, FH-988, FH-985, FH-963 and FH-1046. The germplasm was collected from multiple origins. The hybrids were evaluated against high temperature stress. Hybrids were planted in the field following replicated randomized complete block design (RCBD). Plant to plant distance was kept 18 cm and row to row distance 76 cm. Sowing was done at the end of March to expose reproductive stage to heat stress. Total five plants were selected randomly from each row and data was recorded at reproductive stage on parameters like leaf temperature, cell membrane thermostability, leaf chlorophyll content, pollen germination percentage, pollen grain size, pollen tube length, cob length, cob diameter, number of rows per cob, number of grains per row, 100 grain weight and grain yield per plant.

Statistical analysis

Data recorded for different parameters was statistically analyzed by analysis of variance following the method described by Steel *et al.* (1997) and further correlation analysis was computed to study association among traits following Kwon and Torrie (1964). Direct and indirect effects were analyzed by Path coefficient analysis following by Dewey and Lu (1959).

Results and discussion

Analysis of variance was performed for all traits. Analysis of variance described highly significant differences among 11 hybrids for all the pollen related parameters, physiological parameters and morphological parameters. Mean square comparison (Table-1) exhibited highly significant differences among genotypes. Naveed et al., (2014) also reported the significant effects of heat stress on pollen related traits of maize genotypes. Pollen germination, pollen grain size and pollen tube length are highly affected by high temperature stress. Pollen germination percentage was reduced in heat stress. Genotypes FH-988 and 15BJSF6 had the highest pollen germination values while TG-4557 and TG-4265 had the lowest values. Hybrids with high pollen germination percentage have high seed setting. Poor pollen production and pollen germination results in reduction in yield. Heat stress also affected the pollen grain size. Genotypes DK-6103 and TG-4560 had the highest pollen grain size values while FH-1046 and TG-4557 had the lowest values. For pollen tube length genotypes FH-988 and TG-46B90 had the highest values while TG-4265 and FH-963 had the lowest values. Prasad *et al*. (2000) explained that pollen germination and pollen tube growth rate were extremely sensitive to high temperature stress. Signal transfer mechanism was damaged due to high temperature. Due to this damage pollen tube growth in style and ovule was inhibited. Due to high temperature, almost 21% seed setting was decreased (Sakata *et al*., 2000).

Genotypes FH-963 and P-1543 had the highest mean leaf temperature while 15BJSF6 and FH-988 had the lowest mean leaf temperature values. Photosynthetic apparatus is highly sensitive to heat stress. In high temperature stress leaf temperature increase and it affects photosynthesis and respiration. Due to reduced photosynthesis, yield of maize affected. Steven *et al.* (2002) described sensitivity of photosynthetic apparatus of maize to heat stress. Total photosynthetic activity was reduced when temperature reaches above 38°C. Genotypes P-1543 and FH-963 had the highest cell membrane thermostability values while FH-988 and 15BJSF6 had the lowest values. High temperature reduced the cell membrane thermostability. Due to heat stress electrolyte leakage increases and membranes became instable. Huang and Gao (2002) reported that membrane stability and integrity of bent grass was poorer at high temperature. Leaf chlorophyll contents are higher in only heat tolerant varieties. Heat stress reduced the chlorophyll contents. Genotypes TG-4560 and 15BJSF6 had the highest chlorophyll contents values respectively while TG-4557 and FH-1046 had the lowest values.

Development of cob was highly affected by heat stress. Metabolism of carbohydrates reduced by high temperature and it reduced the seed development. Genotypes 15BJSF6 and TG-46B90 had the highest cob length values while DK-6103 and TG-4557 had the lowest values. Genotypes 15BJSF6 and FH-988 had the highest cob diameter values while TG-4265 and TG-4557 had the lowest values. Suwa *et al.* (2010) determined the effect of high temperature on development of ear and metabolism of carbohydrates in both dent and sweet corn genotypes. Due to heat stress cob weight and other parts of ears decreased. Heat stress reduced the number of rows per cob and total grain yield per plant. Genotypes 15BJSF6 and DK-6103 had the highest number of rows per cob with values while FH-1046 and TG-4265 had the lowest values. Genotypes 15BJSF6 and TG-4560 had the highest 100 grain weight values while TG-4557 and FH-

1046 had the lowest values. Genotypes 15BJSF6 and TG-4560 had the highest grain yield per plant values while TG-4557 and FH-1046 had the lowest values. Number of kernels were reduced by heat stress. Genotypes FH-985 and TG-46B90 had the highest number of kernels per row values while 15BJSF6 and DK-6103 had the lowest values. Hussain *et al*. (2010) explained that number of cobs, number of rows per cob, number of kernels per row and yield per plant were much reduced in spring season due to heat stress at reproductive stage. Khodarahmpour *et al.* (2010) reported that high temperature stress reduced 70% grain yield in inbred lines of maize. Supply of assimilates to grains was reduces which ultimately reduces net yield.

Correlation analysis

Correlation was used as a logical step to find out the relationship between seed yield and other parameters as well as among yield components. Pollen germination showed positive correlation with pollen tube length, 100 grain weight and grain yield per plant at both genotypic and phenotypic levels. Pollen grain size showed positive association with leaf chlorophyll contents and grain yield per plant at both genotypic and phenotypic levels. Pollen tube length was positively correlated with pollen germination, cob length, cob diameter, number of rows per cob, 100-grain weight and grain yield per plant at both genotypic and phenotypic levels. The results coincides with the findings of Cárcova and Otegui (2001). Kakani *et al*. (2005) reported no significant correlation between pollen parameters and membrane thermostability.

Leaf temperature showed highly negative association with pollen germination, pollen tube length, leaf chlorophyll contents, cob length, 100 grain weight and grain yield per plant at genotypic and phenotypic levels. Griffin *et al*. (2004) reported that process of photosynthesis affected due to high leaf temperature. It ultimately reduced the net yield per plant. Cell membrane thermostability showed negative correlation with pollen tube length, leaf chlorophyll contents, cob length, 100 grain weight and grain yield per plant at both levels. Leaf chlorophyll contents was positively correlated with pollen grain size, 100 grain weight and grain yield per plant at both genotypic and phenotypic levels (Table-2). Fokar *et al*. (1998) reported high kernel weight positively correlated with high chlorophyll contents. Reynolds *et al*. (1997) described leaf temperature and chlorophyll contents are highly negatively correlated.

Cob length showed positive correlation with pollen tube length, 100 grain weight and grain yield per plant at genotypic and phenotypic levels. Cob diameter was highly positively correlated with pollen tube length, number of rows per cob, 100 grain weight and grain yield per plant at both genotypic and phenotypic levels. Umakanth and Khan (2001) reported significant and positive correlation of grain yield with ear length, ear diameter, plant height and grain weight. Number of rows per cob showed positive association with pollen tube length, cob diameter, 100 grain weight and grain yield per plant at both genotypic and phenotypic levels (Table-2). Number of grains per row showed negative correlation with 100 grain weight at both genotypic and phenotypic levels. 100 grain weight was positively correlated with pollen germination, pollen tube length, leaf chlorophyll contents, cob length, cob diameter, number of rows per cob and grain yield per plant at both genotypic and phenotypic levels. Grain yield per plant was positively correlated with pollen germination, pollen grain size, pollen tube length, leaf chlorophyll contents, cob length, cob diameter, number of rows per cob and 100 grain weight at both genotypic and phenotypic levels. It was negatively correlated with leaf temperature and cell membrane thermostability at both levels (Table-2). Zarei *et al.* (2012) reported kernel length, total number of kernels per cob, 100-grain weight, cob percentage and ear length had positive correlation on grain yield.

Path coefficient analysis

Pollen germination had positive direct effect on yield per plant. It had positive indirect effects through leaf temperature, cob diameter and 100 grain weight. Pollen germination had negative indirect effects on grain yield through pollen grain size, pollen tube length, cell membrane thermostability, leaf chlorophyll contents, cob length, number of rows per cob and number of grains per row (Table-3). Path analysis indicated the positive direct effect of pollen grain size on yield per plant. It exerted positive indirect effects through leaf temperature, cob diameter, number of grains per row and 100 grain weight. Negative indirect effects exerted through pollen germination, pollen tube length, cell membrane thermostability, leaf chlorophyll contents, cob length and number of rows per cob. Results indicated that pollen tube length exerted negative direct effect on grain yield per plant. It had positive indirect effects on grain yield through pollen germination, pollen grain size, leaf temperature, cob diameter, number of grains per row and 100 grain weight.

Pollen tube length had negative effects through cell membrane thermostability, leaf chlorophyll contents, cob length and number of rows per cob. Leaf temperature had negative direct effect on grain yield per plant. Positive indirect effects exerted through pollen tube length, cell membrane thermostability, leaf chlorophyll contents, cob length and number of rows per cob. It had negative indirect effects through pollen germination, pollen grain size, cob diameter, number of grains per row and 100 grain weight (Table-3).

Cell membrane thermostability exerted positive direct effect on grain yield per plant. Results showed positive indirect effects through pollen tube length, leaf chlorophyll contents, cob length and number of rows per cob. It had negative indirect effects through pollen germination, pollen grain size, leaf temperature, cob diameter, number of grains per row and 100 grain weight. Leaf chlorophyll contents had negative direct effect on grain yield per plant. It had positive indirect effects on grain yield through pollen germination, pollen grain size, leaf temperature, cob diameter, number of grains per row and 100 grain weight. It exerted negative indirect effects through pollen tube length, cell membrane thermostability, cob length and number of rows per cob.

Results indicated that cob length had a negative direct effect on grain yield per plant. It had positive indirect effects through leaf temperature, cob diameter and 100 grain weight. Cob length showed negative indirect effects through pollen, cell membrane thermostability, leaf chlorophyll contents, number of rows per cob and number of grains per row. Alvi *et al*. (2003) reported that ear length had negative direct effect on grain yield. Cob diameter had a positive direct effect on grain yield per plant. It had positive indirect effects through leaf temperature and 100 grain weight. Cob diameter showed negative indirect effects through pollen tube length, cell membrane thermostability, leaf chlorophyll contents, cob length, number of rows per cob and number of grains per row.

Umakanth and Khan (2001) reported ear diameter showed maximum positive direct genotypic effects as well as indirect contribution through other characters on grain yield. Number of rows per cob exerted negative direct effect on grain yield per plant. It had positive indirect effects through pollen germination, leaf temperature, cob diameter, number of grains per row and 100 grain weight. It had negative indirect effects through, cell membrane thermostability, leaf chlorophyll contents and cob length. Jayakumar *et al*. (2007) reported the maximum negative direct effect on grain yield was exhibited by kernel rows followed by days to 50% silking. Results indicated that number of grains per row had negative direct effect on grain yield. It had positive indirect effects through pollen germination, pollen tube length, cell membrane thermostability, leaf chlorophyll contents, cob diameter and number of rows per cob. 100 grain weight had positive direct effect on grain yield per plant. It had positive indirect effects through pollen germination, pollen grain size, leaf temperature, cob diameter and number of grains per row. 100 grain weight had negative indirect effects on grain yield through pollen tube length, cell membrane thermostability, leaf chlorophyll contents, cob length and number of rows per cob. El-Badawy *et al.* (2011) and Khan *et al.* (2013) reported highest positive direct effect of 100 grain weight on yield per plant (Table-3).

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Table-1: Mean square comparison for different traits in 11 hybrids of maize under high temperature stress

SOV	DF	PG%	PGS	PTL	LT	CMT	LC	CL	CD	NR/Cob	NK/Row	GW	GY/P
Replication	1	0.727	0.589	1.747	0.499	0.72	1.071	0.828	0.003	0.324	5.501	3.270	1.534
Genotypes	10	79.527*	8.285*	12.567*	2.305*	32.548*	25.038*	13.484*	0.017*	3.072*	56.729*	50.470*	451.58*
Error	10	1.927	1.545	1.987	0.086	1.900	1.032	0.46	0.0009	0.312	2.699	1.377	11.65
Total	21												

* = Significant at 5% probability

Table-2: Genotypic (bold) and phenotypic correlation between different traits in maize

Traits	PGS	PTL	LT	CMT	LC	CL	CD	GR/cob	G/ear row	GW	GY/P
PGS		**0.507***	**-0.196**	**-0.271**	**0.799****	**0.049**	**0.316**	**0.349**	**-0.314**	**0.379**	**0.597****
		0.178	-0.113	-0.239	0.583**	-0.001	0.247	0.194	-0.299	0.244	0.435*
PTL			**-0.715****	**-0.722****	**0.443***	**0.565****	**0.570****	**0.485***	**-0.387**	**0.619****	**0.809****
			-0.661**	-0.537*	0.378	0.461*	0.436*	0.466*	-0.321	0.532*	0.72**
LT				**1.0005**	**-0.574****	**-0.607****	**-0.336**	**-0.223**	**0.330**	**-0.654****	**-0.690****
				0.909**	-0.538**	-0.569**	-0.286	-0.200	0.313	-0.633**	-0.656**
CMT					**-0.672****	**-0.731****	**-0.299**	**-0.129**	**0.180**	**-0.634****	**-0.698****
					-0.539**	-0.681**	-0.314	-0.169	0.193	-0.545**	-0.605**
LC						**0.406**	**0.319**	**0.555****	**-0.494**	**0.773****	**0.795****
						0.378	0.255	0.406	-0.414	0.751**	0.759**
CL							**0.432***	**0.149**	**0.202**	**0.504***	**0.598****
							0.417	0.128	0.233	0.486*	0.569**
CD								**0.754****	**0.040**	**0.586****	**0.737****
								0.639**	0.041	0.548**	0.642**
GR/cob									**-0.412**	**0.800****	**0.709****
									-0.399	0.657**	0.648**
G/ear row										**-0.485***	**-0.351**
										-0.427*	-0.289
GW											**0.937****
											0.893**

* = Significant at 5% probability ** Significant at 1% probability

PG= Pollen germination, **PGS**= Pollen grain size, **PTL**= Pollen tube length,**LT**= Leaf temperature, **CMT**= Cell membrane thermostability, **LC**= Leaf chlorophyll contents, **CL**= Cob length, **CD**= Cob diameter, **GR/cob**= Grain rows per cob, **G/ear row**= Grains per ear row, **GW**= 100 Grain weight, **GY/ Plant**= Grain yield per plant

Table-3: Direct (diagonal) and indirect effects of different traits on grain yield per plant.

	PGS	PGS	PTL	LT	CMT	LC	CL	CD	NR/C	NK/R	GW
PGS	**0.126**	-0.024	-0.026	0.049	-0.025	-0.0609	-0.057	0.224	-0.134	-0.003	0.801
PGS	-0.006	**0.498**	-0.016	0.013	-0.009	-0.230	-0.002	0.092	-0.130	0.006	0.383
PTL	0.101	0.252	**-0.033**	0.047	-0.023	-0.127	-0.025	0.166	-0.181	0.008	0.625
LT	-0.094	-0.097	0.023	**-0.065**	0.032	0.165	0.027	-0.098	0.083	-0.007	-0.660
CMT	-0.097	-0.135	0.024	-0.065	**0.032**	0.193	0.032	-0.087	0.048	-0.004	-0.640
LC	0.027	0.398	-0.014	0.037	-0.022	**-0.288**	-0.018	0.093	-0.208	0.009	0.781
CL	0.163	0.024	-0.018	0.040	-0.024	-0.117	**-0.044**	0.126	-0.056	-0.004	0.509
CD	0.097	0.157	-0.019	0.022	-0.010	-0.092	-0.019	**0.291**	-0.282	-0.0008	0.592
NR/C	0.045	0.174	-0.016	0.015	-0.004	-0.159	-0.006	0.219	**-0.346**	0.008	0.808
NK/R	0.020	-0.157	0.013	-0.022	0.006	0.142	-0.009	0.012	0.154	**-0.020**	-0.490
GW	0.100	0.189	-0.020	0.043	-0.020	-0.222	-0.022	0.171	-0.299	0.009	**1.010**

PG= Pollen germination, **PGS**= Pollen grain size, **PTL**= Pollen tube length,**LT**= Leaf temperature, **CMT**= Cell membrane thermostability, **LC**= Leaf chlorophyll contents, **CL**= Cob length, **CD**= Cob diameter, **GR/cob**= Grain rows per cob, **G/ear row**= Grains per ear row, **GW**= 100 Grain weight, **GY/ Plant**= Grain yield per plan