INT. J. BIOL. BIOTECH., 17 (2): 367-375, 2020.

CHARACTERIZATION OF MAIZE INBRED LINES FOR GRAIN YIELD AND RELATED TRAITS UNDER HEAT STRESS CONDITIONS

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ABSTRACT

The current experimental study was conducted in tunnel conditions at Maize and Millets Research Institute (MMRI), Yusafwala, Sahiwal to characterize one hundred (100) maize inbred lines for their heat tolerance ability on the basis of morpho-phonological traits. Data was recorded for different heat stress related primary and secondary plant traits i.e. days to 50% tasseling, days to 50% silking, anthesis-silking interval, plant height, ear height, ear length, 1000-grain weight and grain yield per hectare. The results revealed high genetic divergence among maize inbred lines for their high temperature stress tolerance capacity. Correlation analysis unveiled the significantly positive association of plant height with grain yield (0.683^{**}) and thousand grain weight (0.974^{**}) while strong negative association with ear height (-0.831^{**}) and ear length (0.631^{**}) under heat stress conditions. Cluster analysis classified maize inbred lines into four clusters in which cluster consisted of highly productive inbred lines under heat stress. Similarly, Principal component analysis categories maize inbred lines for their heat stress tolerance and susceptibility. Based on these results, best parent lines will be selected for the improvement/development of climate resilient maize genotypes.

Key-words: Principal component analysis, Biplot analysis, Genetic diversity, Climate change

INTRODUCTION

Maize is the most productive and widely adopted field crop in the World. Its cultivation of is constantly increasing in the world due to its wide utilization as food, feed and fodder for human, poultry and livestock industry. Furthermore, it is also broadly used in wet and dry milling industry to provide raw material for artificial sweetener, cooking oil, pharmaceutical product, alcohol- based beverages, biofuels, baby food, cosmetics and bakery products (Serna-Saldivar, 2019). Maize contributes 0.5% in GDP and 2.6% to value addition. It was cultivated on an area of 1318 thousand hectares with a production of 6309 thousand tons with an average yield of 4787 kg/ha (Economic Survey of Pakistan, 2018-19). In Pakistan, maize is cultivated in spring and Autumn seasons. Spring sown maize is much productive than autumn sown crop because its vegetative growth period (60-73 days) is more than autumn sown maize (50-50). Furthermore, in autumn season, grain filling and maturation period comes in the month of October-November when there is sufficiently low temperature which reduces the efficiency of seed setting and grain development. However, reproductive stage of maize crop in spring season coincides with high temperature stress (above 40 °C) which severely affects fertilization and grain development by increasing the anthesis-silking interval (Dass *et al.*, 2010 and Cicchino *et al.*, 2010). Although, maize is a temperature loving C4 plant, however, very high temperature stress could reduce seed setting up to 80% due to abrupt pollen shedding over a short period of time (Fonseca *et al.*, 2005). Grain yield is highly associated with days to 50% tasseling, days to 50% silking, grain weight and number of grains per ear which are significantly reduced under high temperature stress conditions (Jones *et al*., 1984). Therefore, development of heat resilient maize hybrids is the most essential thing to do for sustainable maize production under changing climatic conditions. This could be achieved by two different ways i.e. (1) inducing heat tolerance in existing, high yielding maize hybrids (2) developing new hybrids having excellent heat tolerance (Yousaf *et al*., 2017). The strategy is generally adopted is adapted for monogenic or oligo-genecally controlled traits like disease resistance and is less effective against polygenic traits. Therefore, development of new hybrids through the hybridization of heat tolerant maize inbred lines is the most convenient and suitable approach.

Genetic diversity analysis is one of the most basic and imperative step in maize hybrid development. It can be studied through different traits including morpho-physiological, biochemical and molecular. Morpho-phonological characterization of available germplasm/inbred lines for heat tolerance related genetic diversity is believed to be the initial step in developing high temperature stress tolerant hybrids (Khan *et al.*, 2014). Researchers used different multivariate statistical approaches to categorize and characterize germplasm (Ignjatovic-Micic *et al*., 2015; Asare 2016; Yousaf *et al.*, 2017). However, principal component based biplot analysis and cluster analysis are the most frequently used multivariate analysis to characterize genotypes on the basis of genetic diversity of their traits. The current study was designed to characterize maize inbred lines for their genetic divergence under high temperature stress conditions. The results may, hopefully, be useful in the identifications of suitable parent inbred lines that could be selected to use in future breeding programs of heat tolerant maize hybrid development.

MATERIALS AND METHODS

Experimental material, design and location

The current study was executed at Maize and Millets Research Institute (MMRI), Yusafwala-Sahiwal during spring, 2016. One hundred maize inbred lines of temperate, tropical and temp-trop origin were collected from MMRI Yusafwala, Sahiwal-Pakistan Table (4.1). The treatments were laid out under strip plot design having 20 cm plant-plant and 75cm row-to-row distance, respectively. Net plot size per line was maintained at 3 m^2. The inbred lines were sown under high tunnel on March 16, 2016 and before flowering, tunnel was covered with plastic sheet to provide maximum temperature (> 45 $^{\circ}$C) at flowering and post flowering stages. Sowing was carried out with the help of dibbler @ 2 seeds per hill and at 3-4 leaf stage, thinning was done to insure plant population.

Data acquisition and statistical analysis

Data for different phonological traits i.e. days to 50% tasseling (DT), days to 50% silking (DS) and anthesis-silking interval (ASI) was recorded at suitable phenophases. At physiological maturity, data was also recorded for plant height (PH), ear height (EH), ear length (EL), thousand kernels weight (TKW) and grain yield per hectare (GY). Data was statistically analyzed for analysis of variance (ANOVA), correlation coefficient (Steel *et al*., 1997), principal component analysis (PCA), cluster analysis (ACA) and biplot analysis (Sneath and Sokal, 1973) to figure out the relationship among different plant traits and to classify maize inbred lines for their heat stress tolerance. Two statistical packages, Statistix 8.1 and XLSTAT 16.0 were used to analyzed the data and compute biplots.

RESULTS AND DISCUSSION

Mean comparisons under heat stress conditions

Under heat stressed conditions in tunnel, mean data showed range of variation among maize inbred lines for all studied heat stress associated plant traits. Days to 50% tasseling is the the main factor determining the rate of success of fertilization. Data presented in Table 1 showed that maize inbred line ILY-5 has the highest value (65.0) for DT followed by ILY-12 (64.7) and DDRIL-4 (64.7) whereas inbred line ELIL-1 showed lowest value (57.7) for DT. Days to 50% silking is an important trait with respect to heat stress response as the inbred lines with lower days to 50% silking are generally early maturing which helps the plants to avoid heat stress. Maize inbred line ELIL-1 took minimum DS (61.0) while DDRIL-4 took maximum DS (68.7) followed by ILY-5, ILY-12 and YIL-16 (68.0).

Another important trait correspondent to heat stress is anthesis silking interval. Higher the value of ASI, lesser the chances of synchronization between male and female flowers causing failure of fertilization (Zhang *et al.*, 2013). Maximum value for ASI was observed in inbred line YIL-23 (4.3) whereas minimum value (3.0) was observed in more than 50% inbred lines. Highest value for plant height was observed in maize inbred line DDRIL-14 (183 cm) while lowest plant height was shown by ILY-3 (98.3 cm). Similarly, ear height was found maximum in inbred line DDRIL-14 (57.0 cm) while minimum in ILY-17 (40.1 cm). It was observed that plant and ear height get reduced under heat stress conditions. This could be due to the reduction in inter-nodal elongation as suggested by Weaich *et al.*, (1996) and Cairns *et al.*, (2012). Heat stress much influence the length of ear (EL) and thousand grain weight (1000GW) in maize. Generally, EL and 1000GW get reduced in maize under high temperature stress as a result of the disturbance in source-sink relationship and changes in distribution is assimilates as reported by Edreira *et al.* (2011).

In this screening experiment, maximum ear length was found in YIL-28 (17.1 cm) whereas minimum EL was observed in ILY-3 and ILY-4 (10.6 cm). Similarly, thousand grain weight was found to be the maximum in DDRIL-14 (206.3 g) while minimum 1000GW was in YIL-28 (123.7 g). Above all these plant traits; grain yield is the most essential trait for sustainable maize production under high temperature stress conditions (Yousaf *et al.*, 2018). As a combination of complex interactions of different plant traits, it is thought to be one of the most vulnerable traits to high temperature stress. In current study, highest grain yield was recorded in YIL-28 (2231 kg/ha) followed by DDRIL-13 (2206 kg/ha) while lowest grain yield was observed in DDRIL-14 (1038 kg/ha) followed by YIL-16 (1046 kg/ha). The lower grain yield under heat stress conditions is generally due to the lethal effects of heat stress on yield associated traits including ASI, plant high, number of grains per ear, photosynthetic rate, chlorophyll contents, assimilate distribution and biomass etc (Wahid *et al.*, 2007).

Table 1. Names of Inbred lines under study and mean values for different plant traits under heat stress.

S. No.	Inbred line Name	DT	DS	ASI	EH	PH	EL	TGW	GY
1	ILY-1	59.3	62.3	3.0	48.5	104.3	11.3	140.7	2008.7
2	ILY-2	62.3	65.3	3.0	47.8	101.0	10.8	137.3	2072.0
3	ILY-3	60.3	64.0	3.7	46.8	98.3	10.6	143.0	1993.7
4	ILY-4	60.3	63.3	3.0	45.7	102.0	10.6	140.7	2016.7
5	ILY-5	65.0	68.0	3.0	46.3	106.7	13.7	137.0	2153.7
6	ILY-6	63.0	66.7	3.7	54.1	148.0	14.3	177.0	1637.7
7	ILY-7	58.0	61.3	3.3	42.4	104.3	12.1	157.7	1787.7
8	ILY-8	62.7	65.7	3.0	46.4	112.0	12.7	161.7	1769.7
9	ILY-9	59.7	63.3	3.7	54.4	133.7	14.7	175.7	1672.7
10	ILY-10	60.3	63.7	3.3	45.1	106.3	11.4	138.7	2050.7
11	ILY-11	60.0	63.0	3.0	46.5	119.0	13.6	157.3	1807.7
12	ILY-12	64.7	68.0	3.3	45.8	109.0	10.7	144.0	1978.7
13	ILY-13	63.0	66.0	3.0	47.2	111.3	13.5	158.0	1799.7
14	ILY-14	64.0	67.7	3.7	56.2	153.3	15.8	186.0	1546.7
15	ILY-15	63.7	66.7	3.0	54.9	157.3	14.7	174.0	1692.7
16	ILY-16	62.0	63.0	3.0	46.8	112.0	12.8	137.3	2152.7
17	ILY-17	63.7	67.3	3.7	40.1	98.7	12.1	140.7	2017.7
18	ILY-18	62.3	65.3	3.0	42.9	102.0	12.7	142.0	2000.7
19	ILY-19	61.3	65.0	3.7	45.1	113.7	13.2	151.7	1872.7
20	ILY-20	59.3	62.3	3.0	54.2	146.7	15.2	181.3	1559.7
21	ILY-21	59.0	62.0	3.0	44.8	106.7	14.5	130.0	2192.7
22	ILY-22	62.7	65.7	3.0	55.8	152.3	15.6	167.3	1753.7
23	ILY-23	58.3	62.0	3.7	56.5	157.7	16.7	178.0	1644.7
24	ILY-24	61.7	65.0	3.3	40.4	101.7	13.8	153.3	1847.7
25	ILY-25	63.7	66.7	3.0	53.9	145.3	14.6	160.7	1782.7
26	ELIL-1	57.7	61.0	3.3	46.2	111.7	11.8	144.7	1972.7
27	ELIL-2	60.0	63.3	3.3	55.7	175.7	15.8	200.7	1057.7
28	ELIL-3	61.3	65.3	4.0	52.5	157.0	14.4	171.3	1733.7
29	ELIL-4	60.3	63.3	3.0	43.6	143.3	11.6	137.0	2148.7
30	ELIL-5	63.7	66.7	3.0	48.8	148.3	12.4	148.3	1967.7
31	ELIL-6	60.3	63.3	3.0	42.6	145.3	12.9	134.7	2159.7
32	ELIL-7	62.7	65.7	3.0	51.1	149.0	13.3	164.0	1737.7
33	ELIL-8	64.3	67.3	3.0	55.5	163.3	15.2	180.7	1594.7
34	ELIL-9	63.0	66.3	3.3	52.5	155.0	14.6	168.0	1727.7
35	ELIL-10	62.0	65.0	3.0	45.6	145.7	12.0	144.3	1989.7
36	PEIL-1	63.7	66.7	3.0	40.9	141.0	12.5	132.7	2181.7
37	PEIL-2	60.0	63.3	3.3	41.3	138.0	10.8	141.3	2004.7
38	PEIL-3	62.3	65.3	3.0	41.5	134.0	11.4	131.7	2178.7
39	PEIL-4	62.7	65.7	3.0	44.9	139.3	11.3	137.0	2145.7
40	PEIL-5	58.3	62.3	4.0	53.2	162.7	14.8	192.7	1493.7
41	PEIL-6	59.3	62.3	3.0	42.4	143.3	11.3	133.3	2174.7

42	PEIL-7	61.3	64.7	3.3	53.3	155.7	13.7	168.0	1722.7
43	PEIL-8	60.7	63.7	3.0	41.3	131.7	10.8	139.3	2092.7
44	PEIL-9	62.3	66.0	3.7	53.7	148.7	13.9	166.3	1746.7
45	PEIL-10	61.3	65.0	3.7	49.3	136.3	11.7	158.7	1790.7
46	PEIL-11	62.0	65.3	3.3	55.8	155.7	14.2	185.0	1543.7
47	PEIL-12	59.3	62.3	3.0	52.8	157.7	14.9	170.7	1709.7
48	PEIL-13	59.7	63.0	3.3	42.2	133.3	11.9	156.7	1779.7
49	PEIL-14	60.3	63.3	3.0	55.6	166.0	13.4	197.0	1206.7
50	PEIL-15	59.7	63.0	3.3	51.5	151.0	13.1	176.3	1654.7
51	DDRIL-1	62.3	66.0	3.7	56.8	168.7	15.6	201.0	1065.7
52	DDRIL-2	64.0	67.3	3.3	45.1	142.0	12.0	145.0	1957.7
53	DDRIL-3	63.7	66.7	3.0	41.4	138.0	11.6	135.7	2155.7
54	DDRIL-4	64.7	68.7	3.3	40.7	141.0	11.8	132.7	2176.7
55	DDRIL-5	58.3	61.3	3.0	47.1	145.3	12.0	148.7	1905.7
56	DDRIL-6	64.3	67.7	3.3	44.5	148.0	11.7	141.3	1999.7
57	DDRIL-7	62.0	65.3	3.3	53.2	164.7	15.1	179.0	1623.7
58	DDRIL-8	60.3	63.3	3.0	50.5	159.0	14.8	174.3	1694.7
59	DDRIL-9	62.7	65.7	3.0	46.6	143.7	12.2	155.0	1859.7
60	DDRIL-10	62.7	66.0	3.3	42.7	143.0	11.9	147.0	1996.7
61	DDRIL-11	58.3	61.3	3.0	45.8	145.7	12.1	156.7	1816.7
62	DDRIL-12	61.3	64.3	3.0	42.3	137.7	14.9	135.7	2158.7
63	DDRIL-13	60.3	63.7	3.3	41.9	140.7	15.7	128.3	2205.7
64	DDRIL-14	58.7	61.7	3.0	57.0	183.7	15.7	206.3	1037.7
65	DDRIL-15	63.3	67.0	3.7	47.7	140.3	12.8	165.3	1770.7
66	YIL-1	60.3	63.7	3.3	42.9	141.3	11.3	143.0	1997.7
67	YIL-2	62.7	65.7	3.0	45.5	146.3	12.1	164.7	1759.7
68	YIL-3	59.7	62.7	3.0	42.5	133.0	11.6	155.0	1883.7
69	YIL-4	63.0	66.3	3.3	51.6	157.0	14.3	161.7	1767.7
70	YIL-5	62.3	65.3	3.0	46.0	130.0	11.8	150.7	1874.7
71	YIL-6	60.3	63.3	3.0	52.3	163.3	15.7	183.7	1571.7
72	YIL-7	63.7	67.3	3.7	52.9	158.3	15.3	178.7	1633.7
73	YIL-8	58.3	62.3	4.0	49.8	133.7	12.7	165.7	1745.7
74	YIL-9	62.7	66.0	3.3	41.8	124.3	11.6	145.0	1994.7
75	YIL-10	60.3	63.3	3.0	44.7	131.3	11.8	151.3	1905.7
76	YIL-11	61.0	64.3	3.3	55.7	168.3	16.4	197.7	1082.7
77	YIL-12	59.7	63.0	3.3	47.3	124.3	12.3	157.3	1775.7
78	YIL-13	58.7	61.7	3.0	48.2	133.0	12.6	165.0	1745.7
79	YIL-14	60.3	63.7	3.3	48.3	137.7	12.4	159.3	1776.7
80	YIL-15	62.7	65.7	3.0	56.3	173.7	16.1	159.3	1784.7
81	YIL-16	64.3	68.0	3.7	56.8	171.0	15.6	202.3	1045.7
82	YIL-17	61.7	64.7	3.0	46.3	127.3	12.6	149.3	1909.7
83	YIL-18	63.3	66.7	3.3	45.9	132.7	12.3	153.7	1793.7
84	YIL-19	64.3	67.7	3.3	56.8	167.3	16.6	201.0	1109.7

85	YIL-20	58.7	61.7	3.0	56.3	160.3	16.5	197.3	1170.7
86	YIL-21	64.3	67.3	3.0	48.4	129.0	11.8	150.7	1890.7
87	YIL-22	58.7	62.0	3.3	49.7	133.0	12.4	143.3	2008.7
88	YIL-23	59.7	64.0	4.3	44.5	128.0	12.0	136.0	2142.7
89	YIL-24	60.3	63.3	3.0	56.7	181.3	13.6	201.7	1124.7
90	YIL-25	62.3	65.7	3.3	41.2	123.0	11.3	137.7	2116.7
91	YIL-26	60.0	63.3	3.3	53.3	158.0	15.3	186.7	1525.7
92	YIL-27	59.7	63.3	3.7	52.6	148.0	14.5	178.7	1614.7
93	YIL-28	60.7	64.0	3.3	40.2	136.7	17.1	123.7	2230.7
94	YIL-29	59.3	62.7	3.3	56.4	173.3	14.2	199.3	1100.7
95	YIL-30	60.3	63.7	3.3	55.6	168.0	16.4	194.3	1194.7
96	YIL-31	61.7	65.0	3.3	44.5	137.3	11.6	151.0	1905.7
97	YIL-32	63.3	66.3	3.0	51.8	154.7	13.8	167.0	1756.7
98	YIL-33	62.3	65.3	3.0	44.7	136.7	11.2	143.7	1984.7
99	YIL-34	60.3	63.7	3.3	45.8	145.7	11.7	148.7	1916.7
100	YIL-35	61.7	65.3	3.7	55.9	164.0	16.7	186.0	1534.7
LSD 0.05		2.56	2.59	0.56	9.23	29.51	2.2	32.4	401.3

Table 2. Correlation among plant traits under heat stress conditions.

Characters	DT	DS	ASI	EH	PH	EL	TGW
DS	**0.980****						
ASI	-0.072	0.095					
CH	-0.029	-0.003	0.176				
PH	0.004	0.030	0.069	0.690**			
CL	-0.022	0.004	0.164	0.710**	0.621**		
TGW	-0.108	-0.065	0.219*	0.880**	0.717**	0.677**	
GY	0.116	0.078	-0.183	-0.831**	0.683**	-0.631**	0.974**

* = Significant (< 0.05), ** = Highly Significant (< 0.01)

Table 3. Mean values of morpho-phonological traits for all four clusters.

Cluster No.	DT	DS	ASI	EH	PH	EL	TGW	GY
Cluster-1	61.5	64.8	3.2	45.0	127.3	11.8	146.5	1952
Cluster-2	61.6	64.7	3.2	43.1	129.2	12.6	134.5	2152
Cluster-3	61.3	64.6	3.3	51.3	146.7	14.1	169.9	1700
Cluster-4	60.9	64.2	3.3	56.3	171.2	15.4	199.9	1109

Table 4. Eigen values, total and cumulative variance of eleven traits under heat stress conditions.

Principal Component	Eigen value	% Total Variance	Cumulative Eigenvalue	Cumulative %
PC1	4.036	50.445	4.036	50.445
PC2	1.984	24.804	6.020	75.249
PC3	0.983	12.284	7.003	87.533
PC4	0.444	5.548	7.447	93.080
PC5	0.361	4.514	7.808	97.595
PC6	0.167	2.092	7.975	99.687
PC7	0.019	0.242	7.994	99.928
PC8	0.006	0.072	8.000	100.000

Table 5. Factor loading of plant traits in principal component analysis.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
DT	-0.110	***0.987***	-0.091	-0.030	-0.035	-0.004	0.002	-0.053
DS	-0.065	***0.994***	0.069	-0.030	-0.001	-0.019	-0.001	0.053
ASI	0.242	0.046	***0.965***	0.031	0.083	0.010	-0.004	-0.009
EH	***0.923***	0.064	-0.041	-0.069	-0.138	0.343	-0.018	0.002
PH	***0.816***	0.100	-0.187	0.120	0.524	0.009	-0.004	-0.002
EL	***0.805***	0.073	-0.033	0.539	-0.217	-0.092	-0.002	0.000
TGW	***0.962***	-0.012	0.002	-0.223	-0.073	-0.089	0.107	0.000
GY	***-0.933***	0.029	0.025	0.286	0.085	0.180	0.086	0.002

Note: Values in Bold are significantly important in the respective PC's

Correlation Analysis

Correlation analysis is generally applied to measure the direction and intensity of relationship among two or more variables. Correlations coefficient for maize inbred lines under heat stressed condition were calculated between morphological, phonological and grain yield related parameters under high temperature stress (Table 2).

Under high temperature stress, DT was positive and highly correlated with DS (0.980**). However, its correlation with grain yield was non-significant but positive (0.116), showing that increase in DT could increase grain yield in maize but non-significantly as described by Yousaf *et al.* (2018). However, Yousaf *et al.* (2017) described a positively significant association between days to 50% tasseling and grain yield. Days to 50% tasseling were also observed to be negatively and non-significantly correlated with ASI (-0.072), ear height (-0.029), ear length (-0.022) and 1000-GW (-0.108). Noor *et al.* (2019) detected highly significant differences among maize hybrids in complete accordance with results. Day to 50% silking was reported to have positive yet non-significant relationship with ASI (0.095), plant height (0.030), ear length (0.004) and grain yield (0.078) while negative and non-significant correlation with ear height (-0.003) and thousand grain weight (-0.065). Highly significant but negative correlation was revealed between grain yield and other plant traits like ear height (-0.831**) and ear length (-0.631**). However, a strong, positive correlation of grain yield with plant height (0.683**) and 1000-GW (0.972**) was observed. Maize inbred lines are much sensitive to heat stress than hybrids due to their consecutive selfing which make them more susceptible towards any stress. The results were in agreement with Yousaf *et al.* (2018) and Yousaf *et al.* (2017) who revealed that grain yield had a positive correlation for PH and 1000-GW but negative with ear length under heat stress.

Cluster Analysis under Heat Stress

Cluster analysis is a type of multivariate analysis which is used to classify genotypes on the basis of different studied traits. In current study, hundred (100) maize inbred lines were characterized on the basis of eight (8) plant traits under heat stress conditions. Inbred lines were grouped into four clusters (Table 3) as shown by dendrogram (Fig 1). Significant variances in dendrogram were detected among maize inbred lines for traits expression under heat stress.

High temperature at reproductive stage cause severe yield loss in maize (Wahid *et al.,* 2007). Dissimilarity index of maize inbred lines from cluster analysis resulted in a dendrogram by using 100 inbred lines in four diverse clusters under heat stress (Fig 1). First cluster was comprised of twenty-eight inbreds, Cluster-II of nineteen, Cluster-III of forty-two and thirty-seven inbreds were in cluster-IV. The larger number of maize inbred lines was observed in cluster-III. Highest estimates of genetic diversity were obtained in Cluster-I and cluster-III while cluster-II and cluster-IV have lesser number of inbred lines. For selection against heat stress tolerance in clusters, cluster with highest value for grain yield per hectare and lowest value for DS, ASI, EH should be selected (Table 3).

Cluster-I comprised of 28 maize inbred lines and was the second largest cluster. It had lowest mean values for ASI and ear length while intermediate values for all the other studied traits including grain yield. Cluster-II comprised of nineteen inbred lines and this cluster was appeared to be the most productive cluster in terms of grain yield per hectare (2152 kg/ha). However, thousand grain weight (134.5 g) and ear height (43.1) were reported to had lowest mean values. From this cluster, female inbred lines with highest grain yield were selected. Similarly, cluster-III was the largest cluster in terms of maize inbred lines and comprised of forty-two (42) inbred lines. This cluster has intermediate values for all studied plant traits. The cluster-IV was the smallest cluster, consisting of eleven (11) inbred lines. This cluster has highest mean values for ASI, PH, EH, EL, 1000-GW while lowest mean values for DT, DS and GY. Male inbred lines for mating were selected from this cluster.

To advance grain yield in maize for heat stressed environment, one has to select inbred line parents from cluster-1 and cluster-2 because these clusters had higher means for yield and its associated traits. Furthermore, male and female parents must have maximum diversity between them. This is the point where cluster analysis could play a constructive role in selection of parent inbred lines. Kandel *et al.* (2018) and Rani *et al.* (2018) also used cluster analysis to characterize maize inbred lines under high temperature stress and divide maize inbred lines in 4 and 6 cluster, respectively. Saeed *et al.* (2018) also used cluster analysis to study maize hybrids under high temperature stress conditions.

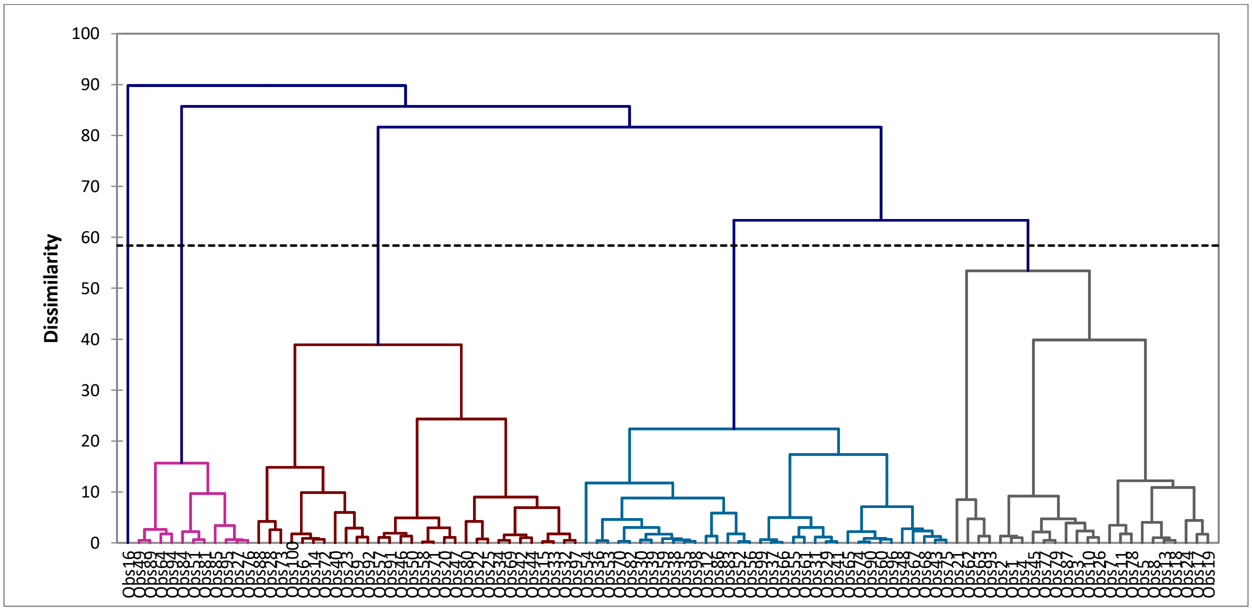

Fig. 1. Dendrogram of 100 maize inbred lines under heat stress conditions using Ward's Method and Elucidation Distancing.

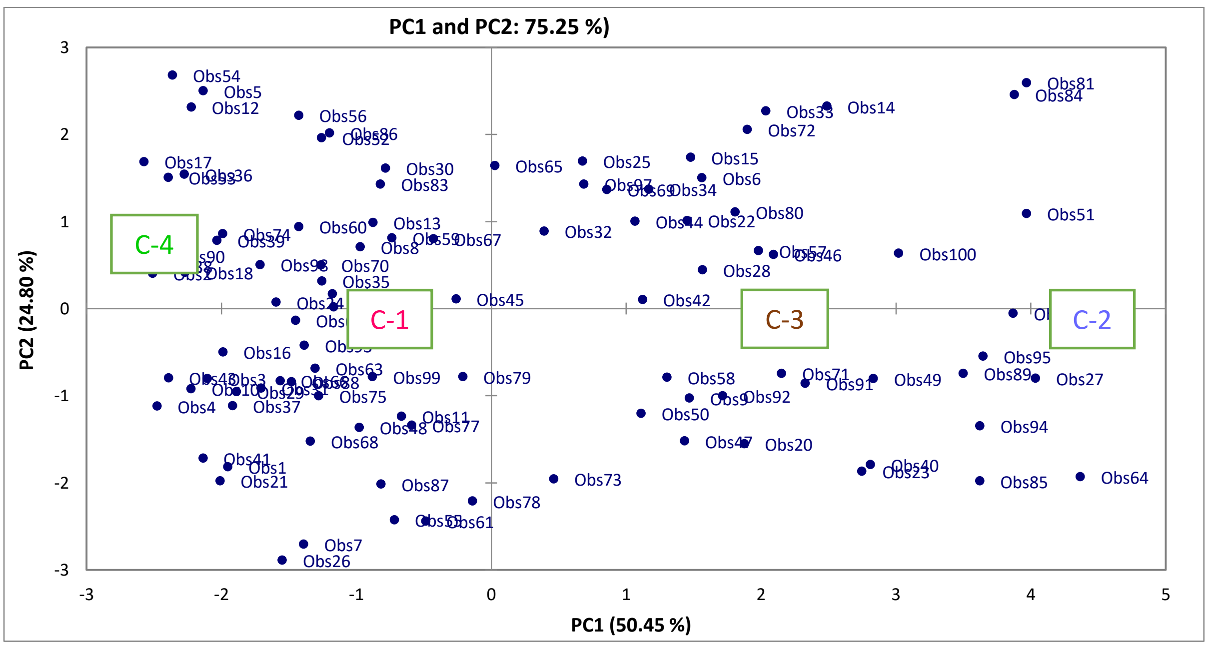

Fig. 2. Biplot distribution of 100 maize inbred lines.

3 Principal Component Analysis of Maize Inbred Lines under Heat Stress

PCA was also applied to categorize hundred maize inbred lines for tolerance against heat stressed environments and extracted eight PCs. The Eigen values, total and cumulative variance of these eight PCs are represented in Table 4. From these 8 PCs, first two PCs (PC1 and PC2) showed more than one Eigen value that contributes significantly for yield and related traits (Fig. 2). Yousaf *et al.* (2017) and Yousaf *et al.* (2018) mentioned that high value of PC1 and PC2 were good for selection of genotypes in these PCs all other traits with low value from these genotypes based on PCs could be unproductive. In first PC (PC1), which contributed 50.45% in total genetic diversity, EH, PH, EL, 1000GW and GY were the most important plant traits (Table 5). In this PC, all values were positive attraction except DT, DS and GY. Highest factor loading was found between thousand grain weight and PC1 (0.962) followed by grain yield and PC1 (-0.933). Second PC (PC2) contributed 24.8% variations in total variations with eigen value 1.98. In this PC, DT and Ds were the major contributors and maximum factor loading were detected between days to 50% silking and PC2 (0.994). Kandel, 2018 suggested that improvement in genetics of yield could be brought via genetic development of morpho-physiological traits to develop heat tolerant maize genotypes. PC3, PC4, PC5, PC6, PC7 and PC8 contributed to the total variation 87.53%, 93.08%, 97.60%, 99.69%, 99.93% and 100%, respectively.

Conclusion

High level of genetic variability was detected among maize inbred lines for morpho-phonological and yield associated traits under heat stress. Correlation analysis of grain yield was found significantly correlated with ear height, plant height, ear length and 1000-GW. Furthermore, cluster analysis categorized maize inbred lines into 4 distinct cluster having different mean values for studied plant traits. Principal component analysis classified the same 100 inbred lines into eight principal components (PCs). On the basis of these statistical approaches, ten best performing inbred lines were selected as female parent while five poor performing inbred lines were selected as male parents to be used in line development heat tolerant maize hybrids through line into tester analysis.

Acknowledgement:

This study is the part of Ph.D. work of Principal author, Mr. Muhammad Umer Khalid. We thank Khadim Hussain (Senior Scientist), Shahid Hussain (Scientific Officer), Aamir Ghani (Scientific Officer) for providing technical support throughout my research experiments study.

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(*Accepted for publication March 2020*)