Genetic diversity analysis of Maize (*Zea mays* L.) inbred lines under drought stress using grain yield and yield related traits

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ABSTRACT

Genetic diversity is a key to the success of a breeding programme which helps breeder to develop novel cultivars which are adaptable to target environment. In the present study, one hundred and thirty three inbred lines were evaluated under controlled and drought stress environment at two locations Dharwad and Devihosur to assess their genetic diversity using morphological traits. Pooled ANOVA for all the measured traits were significant (p=<0.05). Environment variance was higher than genotype and genotype x environment variance suggesting the climatic conditions played a crucial role in expression of traits. Mahalanobis D^2 method identified seven and eleven cluster under controlled and drought stress environment respectively. Under drought stress environment DIL72, DIL10, DIL101 and DIL44 showed high mahalanobis distance values and low drought susceptibility index indicating their diverse and drought tolerance nature. These lines could be further used to develop heterotic hybrids or base population to generate new recombinants.

Keywords: ANOVA, D-square method, drought stress, genetic diversity, inbred lines, maize,

Genetic diversity in a population provides plants the ability to adapt to sudden changes in environmental conditions (Raza *et al*., 2019). The success of a breeding programme lies in identifying and incorporating the genetic diversity from various plant genetic resources. Climate change results may result in unfavorable conditions for crops, thus substantially disrupting food security over this century (Deutsch *et al*., 2018, FAO, 2017, Stokstad, 2019). Developing cultivars which would be adaptable to extreme and fluctuating environments is a unique challenge for plant breeders (Swarup *et al*., 2021). Drought stress is a major abiotic factor influencing the productivity of agricultural crops in the 20th century. In India, area irrigated are of cereals is 35 % whereas area under irrigation that of maize is only 26 % and rest of the area depends on seasonal rain (DAC&FW, 2020). For the year 2019, productivity of maize in India is 29.46 quintal/hectare and that of world is 57.50 quintal/hectare, this yield gap is mainly due to genetic potential of hybrids and crop management practices. In the rainfed regions, erratic rainfall especially during flowering stage makes plants to be susceptible to drought stress.

The expression of a trait under stress environment is significantly different than that of under optimal conditions. Thus, the genetic diversity among the lines under the stress environment helps us in selecting suitable parents to produce heterotic and adaptable progenies (Liu and Qin, 2021). Knowledge regarding the genetic diversity among the inbred lines during the initial stage of a breeding programme plays a significant role in developing potential heterotic hybrids (Hallauer *et al*., 1988). Heterosis is a function of two factors namely genetic diversity and gene action (Falconer, 1960), thus carefully chosen, geneticallydiverse parents can be manifested into heterotic hybrids/varieties. Among the various statistical methods to assess genetic diversity Mahalanobis D^2 method using morphological traits is widely used. D^2 is multivariate statistic given by P. C. Mahalanobis where the D^2value of a sample supplies a measure of divergence between two groups under comparison (Mahalanobis, 1936). The differential performance of genotypes under drought stress is an indicative of expression of its alleles it possesses. Thus, the present study was undertaken to evaluate the genetic diversity of maize inbred lines under two conditions i.e., irrigated (controlled) and drought stress condition across two locations.

MATERIALS AND METHODS

A hundred and thirty three yellow inbred lines derived from crosses between superior hybrids through pedigree breeding currently at F_6 generation were analyzed for their genetic divergence using morphological and yield related traits. The inbred lines were evaluated at two locations i.e., Maize research center and seed farm (MRC&SF), Devihosur and Botanical garden (MRS), University of Agricultural Sciences, Dharwad with two replications under drought stress and controlled environments during *Summer* 2019 in Randomized Complete Block Design (RCBD). The lines were sown in a plot of 5 meters length with 60cm

x 20 cm spacing containing single row. Drought stress was induced by with holding irrigation a week before anthesis whereas as controlled plots were irrigated as per package of practice. No rainfall were received during flowering stage of the crop. The stress and controlled plots were separated by a gap of four meters and bunds were formed in order to avoid seepage of water. Twelve grain yield and yield related traits were recorded at appropriate growth stages of the crop i.e., days to pollen shed (days), days to silking (days), days to maturity(days), plant height (centimeter), ear height (centimeter), cob length (centimeter), cob girth (centimeter), number of kernel rows (count), number of kernels per row (count), hundred seed weight(gram), shelling per cent (per cent) and grain yield (kg ha^{-1}). To evaluate inbred lines for their drought tolerance, Drought Susceptibility Index(DSI) was calculated for grain yield as given by Fisher and Maurer (1978).

Data analysis

The data was subjected to variance analysis considering four environments *i.e.* combination of two water regimes (stress and controlled) and two environments (Devihosur and Dharwad). Two data sets stress and controlled were obtained by pooling data i.e., by averaging values across two locations Devihosur and Dharwad and analyzed for genetic diversity using Mahalanobis D^2 method (Mahalanobis, 1936). The data is assumed to follow multivariate gaussian distribution and D^2 value is calculated as the deviation fromcentroid of the data. D^2 value is calculated as,

$$D_i^2 = (x_i - \mu)' \Sigma^{-1} (x_i - \mu)$$

where, D_i^2 = D square values of observation i, $x_i = i^{th}$ observation, μ = overall mean of the data, Σ^{-1} = inverse of variance-covariance matrix. The D^2 value indicates, a measure of how far the observation (x) is from the center of the distributions of all values taking into account all the variables being considered and their covariances. Mahalanobis distance for each of inbred line was calculated using '*mahalanobis*' function of R program (R core team, 2020). Based on the mahalanobis distance the lines were classified into different distinct clusters as perTocher method and inter and intra-cluster distances were calculated as given by Singh and Chaudhary (1985) using 'biotools' package of R program.

RESULTS AND DISCUSSION

Range and mean values for stress and controlled environments is given in Table 1. Grain yield ranged from 2,218 kg

Table 1: Combined ANOVA with mean sum of square (MSS) for morphological and yield related traits across four environments

Traits	df	Days to 50% pollen shed (days)	Days to 50% silking (days)	Days to Maturity (days)	Plant Height (cm)	Ear height (cm)	Cob length (cm)	Cob girth (cm)	Number of kernel rows per cob (count)	Number of kernels per row (count)	Hundred seed weight (g)	Shelling percentage (per cent)	Grain Yield (kg ha^{-1})
Replication within Environment	4	347.21*	422.9*	388.58*	22840.2*	8218.96*	117.43*	28*	69.16*	531.48*	115.84*	2041.51*	100629*
Environment	3	3082.69*	2897.74*	18929.5*	115811*	35987.7*	1927.64*	70.84*	1000.81*	9569.53*	4072.69*	43700.1*	2.6E+08*
Genotype	132	19.96*	19.72*	47.07*	1011.81	393.11*	8.61	0.37*	6.49*	42.27*	30.6*	159.99*	2442978*
Genotype x Environment	396	10.82*	13.98*	29.58*	424.1*	189.27*	3.81*	0.33*	3.09*	31.24*	19.31*	108.72*	1545067*
Error	528	0.91	0.96	0.84	31.23	19.98	0.25	0.11	0.32	0.5	0.29	1.81	32.36
Broad sense Heritability (%)	-	58.1	48.58	52.5	65.78	61.97	64.19	44.07	62.29	46.27	52.2	49.49	51.95

*Note: *, significant at 5% level*

Table 2 : Minimum, Mean and Maximum of the morphological and yield related traits of inbred lines under controlled and stress conditions

	Days to 50% pollen shed (days)	Days to 50% silking (days)	Days to Maturity (days)	Plant Height (cm)	Ear height (cm)	Cob length (cm)	Cob girth (cm)	Number of kernel rows per cob (count)	Number of kernels per row (count)	Hundred seed weight (g)	Shelling percentage (per cent)	Grain Yield (kg ha^{-1})
Controlled Environment												
Minimum	61.72	65.80	109.60	139.70	60.67	13.82	3.17	12.02	21.23	19.88	64.30	2218.00
Mean	66.87	70.13	117.70	174.90	88.84	16.31	3.99	14.14	30.13	25.73	76.32	3959.00
Maximum	70.54	74.66	123.80	206.90	116.81	19.24	4.59	16.66	36.61	32.19	86.11	5220.00
Stress Environment												
Minimum	61.22	62.75	100.10	111.30	50.26	9.74	2.32	8.21	12.39	9.88	21.14	738.50
Mean	65.10	67.76	109.60	139.10	68.85	12.29	3.15	10.80	19.96	18.73	57.58	2295.40
Maximum	65.36	68.02	109.00	176.10	86.73	15.83	3.79	13.60	25.91	23.05	73.21	3490.20
Per cent reduction	2.65 %	3.38 %	6.88 %	20.47 %	22.50 %	24.65 %	21.13 %	23.59 %	33.75 %	27.19 %	24.55 %	42.02 %

ha^{-1} to 5220 kg ha^{-1} under controlled environment whereas under drought stress condition it ranged from 738.50 kg ha^{-1} to 3490.20 kg ha^{-1}. The inbred lines were medium to late maturing with mean of 66.87 under controlled and 65.10 under drought stress environment. All the traits showed reduced mean under stress environment with grain yield (42.02%) recording highest reduction followed by number of kernels per row (33.75%) and hundred seed weight (27.19%). Days to pollen shed(2.65%) and days to silking (3.38%) showed minimum reduction in their mean values. Combined analysis of variance (Table2) revealed significant difference among the inbred lines for all the studied traits. Environment mean sum of squares was greater than both genotypic mean sum of squares and genotype x environment mean sum of squares further broad-sense heritability ranged from 44.07% to 64.19 % indicating the environmental conditions play a major role in the expression of all the measured trait. Selection of genotypes under target environment in this case stress environment improves genetic gain and adaptability of genotypes (Ceccarelli *et al*., 1998).

Mahalanobis analysis

Seven clusters were delineated under controlled environment and elven clusters were identified under drought stress environment indicating the performance of inbred line under stress condition varied more than under controlled condition (Table 3 and 4). Cluster 2 with 64 lines under controlled environment and cluster 2 with 37 lines of drought stress environment was the largest cluster and with higher intra cluster distance. Cluster 7 and Cluster 11 contained only single inbred line of controlled and drought stress environment respectively.

Among the non-singleton cluster, for controlled environment Cluster 10 showed lowest intra-cluster distance and for droughtstress environment Cluster 4 showed lowest intra cluster distance. Highest inter-cluster distance for controlled environment was between Cluster 5 & 6 followed by Cluster 5 & 4, Cluster 6 & 3 whereas for drought stress environment was between Cluster 8 & 10 followed by Cluster 7 & 8, Cluster 9 & 10 (Table 5 and 6).The inter-cluster distance was greater than intra cluster distance which is conformity as per Alam, *et al*. (2013), Najar, *et al*. (2018)

Grain yield, plant height and number of kernel rows per cob contributed towards the divergence of lines under water drought environment indicating their variable expression under drought stress environment. Similarly, Kumawat, *et al*., 2020 observed greater divergence for plant height and number of kernel rows per cob under stress condition (water logged) whereas Singh and Chaudhari (2003), Beyene *et al*., 2005

Table 3: Inter and intra cluster distance based on mahalanobis D-square under controlled environment

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Cluster 1	303.11						
Cluster 2	889.41	284.64					
Cluster 3	1549.24	660.71	182.43				
Cluster 4	539.83	1428.02	2088.06	65.31			
Cluster 5	2176.75	1287.98	628.03	2715.62	114.46		
Cluster 6	2816.65	1927.81	1267.69	3355.53	640.10	254.62	
Cluster 7	1060.07	1948.82	2608.92	521.40	3236.52	3876.45	0

Table 4 : Inter and intra cluster distance based on mahalanobis D-square under drought stress environment

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8	Cluster 9	Cluster 10	Cluster 11
Cluster 1	105.25										
Cluster 2	519.52	119.74									
Cluster 3	682.47	1201.33	87.71								
Cluster 4	251.23	270.32	932.42	49.77							
Cluster 5	906.79	388.24	1588.81	656.93	105.22						
Cluster 6	352.02	870.43	331.69	601.62	1257.87	120.01					
Cluster 7	890.58	1409.52	209.43	1140.56	1797.01	539.56	58.04				
Cluster 8	1347.02	828.25	2029.05	1097.08	440.98	1698.13	2237.24	124.63			
Cluster 9	1124.35	605.49	1806.41	874.41	218.46	1475.46	2014.63	223.90	61.56		
Cluster 10	1198.57	1717.52	516.83	1448.56	2105.02	847.48	308.87	2545.26	2322.64	176.90	
Cluster 11	712.58	194.43	1394.60	462.76	194.90	1063.65	1602.82	634.83	411.91	1910.82	0

Table 5 : List of inbred lines under each of the seven clusters of controlled environment

Clusters	Inbred lines
Cluster 1 (43)	DIL46, DIL90, DIL95, DIL109, DIL74, DIL93, DIL16, DIL69, DIL82, DIL129, DIL86, DIL88, DIL70, DIL102, DIL122, DIL72, DIL91, DIL104, DIL89, DIL118, DIL125, DIL124, DIL100, DIL84, DIL114, DIL116, DIL96, DIL76, DIL41, DIL97, DIL94, DIL117, DIL2, DIL40, DIL131, DIL103, DIL61, DIL121, DIL75, DIL101, DIL79, DIL17, DIL105
Cluster 2 (64)	DIL113, DIL120, DIL23, DIL28, DIL78, DIL81, DIL5, DIL110, DIL67, DIL19, DIL119, DIL108, DIL15, DIL22, DIL71, DIL42, DIL65, DIL18, DIL92, DIL45, DIL38, DIL3, DIL51, DIL44, DIL14, DIL24, DIL31, DIL7, DIL30, DIL111, DIL60, DIL132, DIL29, DIL47, DIL39, DIL21, DIL80, DIL83, DIL127, DIL106, DIL34, DIL123, DIL56, DIL107, DIL77, DIL27, DIL59, DIL32, DIL115, DIL10, DIL133, DIL49, DIL48, DIL9, DIL26, DIL25, DIL99, DIL53, DIL85, DIL57, DIL112, DIL35, DIL62, DIL12
Cluster 3 (16)	DIL13, DIL33, DIL130, DIL126, DIL52, DIL128, DIL1, DIL66, DIL11, DIL63, DIL36, DIL68, DIL20, DIL54, DIL64, DIL43
Cluster 4 (4)	DIL4, DIL50, DIL87, DIL73
Cluster 5 (3)	DIL55, DIL58, DIL37
Cluster 6 (2)	DIL6, DIL8
Cluster 7 (1)	DIL98

observed highest variation for grain yield under normal environment.

The mahalanobis distance for inbred lines which represents overall deviation of the lines from the centroid of data. Top ten inbred lines with highest mahalanobis distance under drought stress environment is given in Table 7along with their respective drought susceptible index (DSI)value and cluster number. The mahalanobis distanceof all the top ten hybrids were significant (p =< 0.01). Inbred lines DIL72, DIL10, DIL101 and DIL44 showed low DSI indicating their ability for drought tolerance. Cross between DIL72 x DIL10 and DIL101

Table 6 : List of inbred lines under each of the eleven clusters of drought stress environment

Clusters	Inbred lines
Cluster 1 (29)	DIL11, DIL19,DIL35,DIL42,DIL9,DIL24,DIL59,DIL84,DIL15,DIL54,DIL52, DIL5, DIL126,DIL89,DIL94, DIL18,DIL109,DIL121,DIL91,DIL13,DIL118, DIL34, DIL4,DIL67, DIL82, DIL43, DIL131,DIL32,DIL40
Cluster 2 (37)	DIL36, DIL132, DIL45, DIL79, DIL108, DIL38, DIL21, DIL110, DIL53, DIL37, DIL48, DIL106, DIL10 0,DIL3, DIL104,DIL112,DIL77,DIL20,DIL83,DIL25,DIL8,DIL56 , DIL22, DIL66, DIL102, DIL29, DIL75, DIL47, DIL99, DIL103, DIL70, DIL76, DIL31, DIL133, DIL46, DIL117, DIL2
Cluster 3 (7)	DIL69, DIL74, DIL127, DIL87, DIL17, DIL95, DIL62
Cluster 4 (13)	DIL72,DIL88,DIL30,DIL7,DIL71,DIL90,DIL123,DIL120,DIL55,DIL33, DIL85,DIL114,DIL41
Cluster 5 (20)	DIL68,DIL130,DIL26,DIL111,DIL101,DIL81,DIL64,DIL6, DIL39, DIL60, DIL51, DIL115, DIL105, DIL28, DIL119, DIL27, DIL113, DIL92, DIL12, DIL23
Cluster 6 (11)	DIL50,DIL61,DIL129,DIL122,DIL97,DIL96,DIL116,DIL93, DIL49,DIL128,DIL57
Cluster 7 (5)	DIL124,DIL125,DIL98,DIL65,DIL16
Cluster 8 (6)	DIL1, DIL78,DIL10,DIL44,DIL58,DIL107
Cluster 9 (2)	DIL14,DIL80
Cluster 10 (2)	DIL73,DIL86
Cluster 11 (1)	DIL63

Table 7: Top ten inbred with highest D2 values along with DSI (Drought Susceptible Index) and Cluster group.

Inbred line	Mahalanobis distance	Drought Susceptibility Index	Cluster group
DIL99	19.91**	0.79	2
DIL72	20.30**	0.47	4
DIL10	20.80**	0.36	8
DIL28	21.07**	0.54	3
DIL106	21.25**	0.53	2
DIL101	21.40**	0.32	5
DIL86	22.07**	1.31	10
DIL44	22.92**	0.30	8
DIL131	27.79**	0.81	1
DIL55	46.82**	1.01	4

*Note: *, significant at 5% level*

x DIL44 and DIL72 x DIL 101 can produce potential hybrids for drought tolerance exploiting heterosis.

Cluster mean for morphological and yield related traits is given in Table 8 & 9. For controlled environment Cluster 5 showed highest mean grain yield followed by Cluster 6 and 3 whereas Cluster 5 also showed highest number of kernels per row and cob girth and Cluster 6 showed highest number of kernel row and shelling per cent. Under drought stress environment Cluster 8 recorded highest grain yield followed by Cluster 9 and Cluster 5 whereas among non-singleton Cluster 8 recorded highest ear height, cob girth, number of kernels per row and shelling per cent. Inbred lines with high D^2 value selected from clusters with high mean for yield attributing traits can be further recombined to pool the favorable allele and obtain novel recombinant. Singh *et al*., 2009 also suggested picking genotypes from clusters with high mean value for yield attributing traits to act as parents for base population or hybrids. The inbred lines DIL1,DIL78,DIL10,DIL44,DIL58 and DIL107

Table. 8 : Cluster mean of morphological and yield related traits of controlled environment

	Days to 50% pollen shed (days)	Days to 50% silking (days)	Days to Maturity (days)	Plant Height (cm)	Ear height (cm)	Cob length (cm)	Cob girth (cm)	Number of kernel rows per cob (count)	Number of kernels per row (count)	Hundred seed weight (g)	Shelling percentage (per cent)	Grain Yield (kg ha^{-1})
Cluster 1 (43)	67.47	70.55	117.26	172.09	86.99	16.26	3.91	14.22	29.45	25.93	76.44	3277.84
Cluster 2 (64)	66.77	70.13	117.66	176.47	89.40	16.36	4.03	14.17	30.38	25.87	76.48	4166.70
Cluster 3 (16)	65.96	69.41	118.33	178.13	91.30	16.51	4.03	13.97	31.29	25.27	74.80	4826.84
Cluster 4 (4)	66.30	68.60	119.73	171.44	89.79	15.50	3.94	13.07	27.55	22.39	77.00	2738.94
Cluster 5 (3)	66.50	70.90	117.42	170.68	87.34	16.44	4.20	14.43	31.35	26.02	77.26	5454.47
Cluster 6 (2)	65.22	68.25	118.43	176.26	88.46	15.72	3.85	14.73	30.71	27.93	79.33	5094.41
Cluster 7 (1)	68.77	71.56	115.66	173.51	94.55	15.33	3.97	13.63	29.71	24.20	74.30	2217.98

Table. 9 : Cluster mean of morphological and yield related traits of drought stress environment

	Days to 50% pollen shed (days)	Days to 50% silking (days)	Days to Maturity (days)	Plant Height (cm)	Ear height (cm)	Cob length (cm)	Cob girth (cm)	Number of kernel rows per cob (count)	Number of kernels per row (count)	Hundred seed weight (g)	Shelling percentage (per cent)	Grain Yield (kg ha^{-1})
Cluster 1 (29)	65.24	68.08	109.62	137.91	69.62	12.43	3.15	10.41	19.77	18.54	55.49	2023.73
Cluster 2 (37)	65.23	67.86	108.39	137.05	67.56	12.33	3.14	11.33	19.75	19.51	59.21	2542.72
Cluster 3 (7)	65.86	67.77	108.09	137.08	69.18	11.79	3.19	9.82	18.13	15.91	52.12	1341.63
Cluster 4 (13)	65.82	68.22	109.33	141.62	68.20	12.52	3.16	10.97	20.44	19.07	56.11	2273.72
Cluster 5 (20)	65.28	68.23	108.72	141.75	69.80	12.25	3.17	11.20	21.41	19.77	61.21	2930.25
Cluster 6 (11)	65.24	68.43	110.54	134.66	66.84	11.56	3.05	10.46	18.86	16.73	55.05	1672.58
Cluster 7 (5)	65.08	67.78	108.97	139.43	70.16	11.20	3.10	10.14	17.10	17.07	50.60	1133.38
Cluster 8 (6)	65.75	68.39	109.88	151.14	73.21	13.62	3.29	10.38	21.79	19.61	65.07	3370.43
Cluster 9 (2)	65.38	66.57	105.87	142.58	67.60	14.16	3.19	11.31	21.19	19.79	64.94	3147.89
Cluster 10 (2)	66.20	66.69	111.23	134.98	68.07	11.67	2.82	9.49	20.91	15.40	49.21	825.40
Cluster 11 (1)	64.16	66.57	106.63	138.39	64.43	11.55	3.36	11.01	21.88	19.80	62.16	2736.08

which were grouped in Cluster 8 under stress condition recorded high grain yield and low DSI values, these lines can be used to develop a base population by allowing random mating for two to three generations. New trangressive segregants with novel genotype can be derived this base population.

Environment plays a dominant role in expression of traits especially under stress environments, thus the lines should be evaluated and selected under stress environment to obtain heterotic and stable progenies. DIL72, DIL10, DIL101 and DIL44 were identified as the most diverse inbred lines with tolerance to droughtstress. These lines could be further subjected to random mating to synthesize a new population with novel recombinants or could be used in production of synthetic varieties. The D^2 values helps us to identify diverse genotypes from the germplasm which helps in breeder to preserve and produce new heterotic varieties.

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