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Genetic diversity in Maize inbred lines under Water deficit conditions

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ABSTRACT
Hundred maize inbred lines at different stage of selfing were evaluated for obtaining information regarding genetic divergence through multivariate analysis using Mahalanobis'D^2 statistic for a set of twenty-three divergent characters. Analysis of variance for dispersion revealed that the "V" statistics, was significant in the individual and pooled analysis for maturity, morphological, physiological, yield, quality and seedling traits indicating diversity among the lines. Estimates of genetic divergence grouped maize lines into different clusters irrespective of their geographical diversity. Study confirmed divergent nature of eighty two maize lines, out of which fifteen lines mostly grouped in cluster-I in Y1, Y2 and pooled over years analyses were identified to be of elite nature exhibiting desired per se performance for drought related traits. Elite identified lines performed well under moisture stress conditions showing inbuilt tolerance towards moisture stress. Cluster means for maturity, morphological, physiological, yield and quality traits revealed substantial variability for all the traits. In pooled analysis these mean values were desirable mostly in cluster-I & IV and selection of lines from most divergent clusters, having high cluster means and showing high per se performance can be used in hybridization programme for development of high yielding varieties with tolerance to drought and better quality. Highest per cent contribution of divergence was recorded for traits viz., protein content (%) recorded, chlorophyll content before flowering (SPAD units), 100 grain weight (g).
Key Words: D^2 Statistic, Drought, Ears Per Plant, Maize, Stress.

INTRODUCTION

Maize belonging to the family of grasses is cultivated globally and is one of the most important cereal. Moisture stress or water deficit is an inevitable and recurring feature of global agriculture. About one-third of the world's potentially arable land suffers due to water shortage, and most of the crops production is often reduced by drought [1]. Evaluation of genotypes for relative drought tolerance is a tough task, since it is difficult to predict the stage at which the moisture stress is encountered under rainfed conditions. In order to develop genotypes with desirable traits, the breeders choose genetically distant parents, as genetic diversity plays an important role in plant breeding because hybrids derived from the lines of diverse origin display a greater heterosis than those between closely related strains. The concept of D^2 statistics was originally developed by P.C. Mahalanobis in 1928 [2]. The D^2 statistics gives a result based on the magnitude of divergence independent of the sample size. D^2 statistics is a powerful tool in quantifying the degree of divergence among biological populations and assessing the relative contribution of different components to the total divergence at intra- and inter- cluster levels. Rao [3] suggested the application of this technique for the assessment of genetic diversity in plant breeding. In plant breeding, genetic diversity plays an important role because hybrids between lines of diverse origin generally display a greater heterosis than those between closely related parents. This has been observed in maize, alfalfa, cotton and several other crops.

Murty and Arunachalam, [4] examined the nature of genetic divergence as measured through D^2 statistic and its relationship to components of genetic variation in some out breeding populations, self- fertilizing crops and crops showing variable degree of out crossing. Success in recombination breeding depends on the exploitation of genotypes as parents for heterotic crosses and transgressive segregants, therefore, the

situations warrants the exploitation of heterosis based on genetically diverse parents for further yield enhancement. In the present study, 100 maize lines were evaluated for two years to estimate genetic divergence and identify putative parents as per Mahalanobis D^2 statistics. Treating D^2 as a generalized statistical distance, the criteria used by Toucher [3] was applied for determining the group constellation and clustering was done accordingly. The character-wise rank totals were used to calculate the per cent contribution of each character to the total divergence. Average inter cluster and intra cluster distances were estimated as per the method given by Singh and Chaudhary [5].In order to develop genotypes with desirable traits, the breeders choose genetically distant parents, as genetic diversity plays an important role in plant breeding because hybrids derived from the lines of diverse origin display a greater heterosis than those between closely related strains. Estimation of genetic divergence also allows breeders to eliminate some parents in downsizing the core collections maintained and concentrate their efforts in a smaller number of hybrid combinations [6].

MATERIAL AND METHODS
The material for the present investigation consisted of 100 maize inbred lines. The lines were evaluated in factorial RBD with two replications over two years. Each inbred line was planted in two rows experimental plot of 1 meter length with inter and intra row spacing of 60 x 20 cm with recommended package of practices. The material was evaluated against four moisture management regimes viz; Well Watered (WW): Irrigated at knee height stage, flowering and grain filling stages. Intermediate Stress (IS): Irrigated at knee height stage and flowering stage. Mild Stress (MS): Irrigated at knee height stage and Stress (S): Rainfed. The meteorological data, including minimum and maximum temperatures, relative humidity (RH) and rainfall were collected throughout the experimental period for both the years [8]. Observations were recorded on various morphological, maturity, physiological, yield, quality and seedling related traits viz; days to 50 per cent tasseling, days to 50 per cent silking, anthesis-silking interval, days to maturity, plant height (cm), ear height (cm), leaf relative water content (%), canopy temperature before flowering (°C), canopy temperature before maturity (°C), stomatal count (mm-2), chlorophyll content before flowering (SPAD units), chlorophyll content before maturity (SPAD units), ears plant-1, kernels row-1, 100 grain weight (g), grain yield plot-1 (g) and protein content (%). Relative water content as per Barrs and Weatherly (1962) and was estimated using formula: RWC (%) = [(Fw - Dw)/ (Tw - Dw)] x 100. Where, Fw, Dw, and Tw stands for fresh weight, dry weight and turgid weight, respectively. Canopy temperature was measured by infra red thermometer Fluke 62 MAX – FLUKE Corporation, USA and chlorophyll content by Chlorophyll metre SPAD meter (Hanstech, Model CL-01) and protein content by NIR (Model CROPSCAN 2000G/2000B) at Seed Processing Unit SKUAST-K, J&K, India. Pooled analysis of variance (ANOVA) was used to quantify the genetic differences among the genotypes. The multivariate analysis was performed through INDOSTAT software version 9.2. using Mahalanobis' D2 statistics [7]. Treating D2 as a generalized statistical distance, the criteria used by Toucher [3] was applied for determining the group constellation and clustering was done accordingly (Fig.1). The character-wise rank totals were used to calculate the per cent contribution of each character to the total divergence. Average inter- and intra- cluster distances were estimated as per the method given by Singh and Chaudhary [5].

RESULTS
Genetic divergence was carried out for 100 maize inbred lines both in individual years and in data pooled over years as per Mahalanobis D^2 analysis employing Tocher's method [3]. Analysis of variance for dispersion revealed that the "V" statistics, which is a measure of Wilk's criterion was significant in the individual and pooled analysis for maturity, morphological, physiological, yield and quality indicating diversity among the lines (Table-1). Cluster analysis for morphological, maturity, yield, physiological and quality attributes grouped maize lines in 10 clusters in year-1 (Y1) with maximum number of lines (42) in cluster-III followed by 16 lines in cluster-IV, 14 lines in cluster-I, 9 lines in cluster-VII, 8 lines in cluster-VI and 2 lines in cluster-VIII, whereas cluster-II, cluster-V, cluster-IX and cluster-X were monogenotypic. In year-2 (Y2) lines were grouped in 12 clusters with maximum number of lines (53) in cluster-III, followed by cluster I (14), cluster-VIII (10), cluster-II (9), cluster-VII (6) and cluster X (2). Data pooled over years revealed 11 clusters with maximum number of genotypes in cluster-II (53). Cluster-I, VIII, IX and III accommodated 14, 11, 9 and 7 lines, respectively. Divergence analysis revealed similar grouping pattern for 61 lines whereas 39 lines exhibited dissimilar grouping pattern (Table-2,3). Inter and intra cluster distances, a measure of genetic divergence were estimated over pooled analysis (Table-4). Maximum inter cluster distance (D^2 value) was recorded between cluster-X and cluster-IV (580.89). This was followed by cluster-IV and cluster-XI (460.14) and cluster-IV and cluster-VI (447.50), cluster- IX and cluster-IV (444.71) and cluster-X and cluster-I (396.44). Minimum inter cluster distance was observed

between cluster-VII and cluster-VI (5.47) followed by cluster-IV and cluster-I (27.45) and cluster-V and cluster-VIII (32.98). Maximum inter cluster distance revealed that KDM-340A, CM-135, KDM-361A, KDM-372, KDM-932A, KDM-961, KDM-717, KDM-463, KDM-912A, KDM-343A, KDM-1051, KDM-402, KDM-918, KDM-1156, KDM-1236, KDM-372, CM-129, KDM-331, KDM-375, KDM-3008, KDM-439, KDM-926B, KDM-1124, KDM-909A, KDM-895, KDM-356A, KDM-916A, KDM-340A, KDM-131, KDM-362B, KDM-1196, KDM-940B, KDM-892A, KDM-958 and KDM-1095B exhibited maximum genetic divergence for maturity, morphological, physiological, yield and quality traits. Intra-cluster distance a measure of divergence among lines within the cluster recorded highest intra-cluster distance among lines. Estimation of the mean intra cluster distances among the 100 lines grouped on the basis of pooled performance across the years revealed that the cluster-IX exhibited the maximum distance (45.00) followed by cluster-VIII (23.48) and cluster- II (21.98).

Contribution of different maturity, morphological, physiological, yield and quality traits towards divergence revealed that cluster-I in pooled analysis accommodating KDM-932A, KDM-961, KDM-717, KDM-463, KDM-912A, KDM-343A, KDM-1051, KDM-402, KDM-918A, KDM-1156, KDM-1236, KDM-372, CM-129, KDM-331 exhibited desirable cluster means for days to 50% tasseling (69.24 days), days to 50% silking (72.24 days), days to maturity (137.6 days in pooled) and anthesis-silking interval (3 days), thereby indicating importance of these lines towards divergence for maturity traits. Line KDM-361A accommodated in cluster-IV in pooled analysis recorded desirable cluster means for morphological traits viz; plant height (175.6 cm in pooled analysis), ear height (89.11cm); physiological traits viz; leaf relative water content (111.7%), chlorophyll content before flowering (55.26 SPAD units), chlorophyll content before maturity (17.62 SPAD units), canopy temperature before flowering (28.43°C), canopy temperature before maturity (16.46 °C) and stomatal count (64.04 mm^{-2}); yield traits viz., ears plant^{-1} (1.74), kernels row^{-1} (30.93), 100 grain weight (26.36 g), grain yield plot^{-1} (633.3 g); and protein content (10.01%) confirming their importance towards divergence (Table-5). Perusal of Table-5 revealed that protein content recorded highest contribution towards divergence in pooled analysis (4828.28%) followed by chlorophyll content before flowering (3646.46%) and 100 grain weight (1242.42 2%). Days to maturity, ear height, leaf relative water content, stomatal count, canopy temperature before flowering canopy temperature before maturity, ear plant^{-1}, kernels row^{-1} and grain yield plot^{-1} recorded negligible contribution towards divergence, whereas no contribution was recorded for days to 50% silking, anthesis-silking interval, plant height, chlorophyll content before maturity.

Table-1: ANOVA for dispersion (Pooled)

Sources of variation	d.f	Mean sum of squares for maturity, morphological, physiological, yield, and quality traits
Varieties	99	2.5965E20**
Error	962	1.9878E00
Total	791	1.6238E19
V statistics		66485.66

Table-2: Distribution of inbred maize (*Zea mays* L.) lines into clusters based on D^2 statistics for maturity, morphological, physiological, yield and quality traits (Individual &Pooled over years).

Year-1		year-2		Pooled over years	
Cluster No.	Number of lines	Cluster No.	Number of lines	Cluster No.	Number of lines
I	14	I	14	I	14
II	1	II	9	II	53
III	47	III	53	III	7
IV	16	IV	1	IV	1
V	1	V	1	V	1
VI	8	VI	1	VI	1
VII	9	VII	6	VII	1
VIII	2	VIII	10	VIII	11
IX	1	IX	1	IX	9
X	1	X	2	X	1
		XI	1	XI	1
		XII	1		

Table-3: Distribution of inbred maize (*Zea mays* L.) lines into clusters based on D² statistics for maturity, morphological, physiological, yield and quality traits (Pooled over years).

Cluster No.	Number of lines	Inbred line
I	14	KDM-463, KDM-912A, KDM-717, KDM-343A, KDM-961, KDM-932A, KDM-1051, KDM-402, KDM-918A, KDM-1156, KDM-1236, KDM-372, CM-129, KDM-331
II	53	KDM-420, KDM-1044, KDM-930, KDM-332A, KDM-917A, KDM-138, KDM-362A, KDM-445A, KDM-344, CML-139, KDM-415, KDM-1189, KDM-1106, KDM-370, KDM-456A, KDM-404, KDM-400, KDM-351, KDM-429, CML-414, KDM-1036, KDM-170, CML-72, KDM-911A, KDM-332B, KDM-1173, KDM-339, KDM-930A, KDM-724, KDM-895A, KDM-915, KDM-381B, CML-334, KDM-443, KDM-347, KDM-720, KDM-924A, KDM-716, KDM-382A, KDM-9114, KDM-1025, KDM-323A, CML-491, KDM-899A, KDM-381A, CML-502, KDM-969, KDM-1138, KDM-957, KDM-3001, KDM-1095, KDM-3006, HK-1040-4
III	7	KDM-131, KDM-362B, KDM-1196, KDM-940B, KDM-892A, KDM-958, KDM-1095B,
IV	1	KDM-361A
V	1	KDM-440
VI	1	KDM-741
VII	1	HK-1586
VIII	11	KDM-913A, KDM-921A, KDM-3007, KDM-1134, KDM-914A, KDM-1016, KDM-940A, CM-128, KDM-935A, KDM-1159, KDM-431
IX	9	KDM-926B, KDM-1124, KDM-909A, KDM895, KDM-356A, KDM-916A, KDM-340A, CM-135, KDM-3008
X	1	KDM-375
XI	1	KDM-439

Table -4: Average inter-cluster (above diagonal) and intra-cluster (diagonal) distances among inbred maize lines for maturity, morphological, physiological, yield and quality traits (Pooled over years)

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	7.72	246.90	108.86	27.45	289.56	290.09	236.48	195.15	280.38	396.44	351.73
II		21.98	82.22	385.54	34.00	39.94	41.28	54.61	82.86	46.58	113.09
III			17.00	193.41	122.45	113.10	98.36	71.13	109.21	194.65	180.52
IV				0.00	416.11	460.14	393.43	285.92	444.71	580.89	447.50
V					0.00	95.75	96.40	32.98	162.80	57.28	35.94
VI						0.00	5.47	128.06	35.43	35.83	236.94
VII							0.00	119.11	39.96	45.60	237.66
VIII								23.48	173.60	129.19	53.79
IX									45.00	105.33	305.44
X										0.00	174.09
XI											0.00

Table 5: Cluster means and percent contribution of characters towards divergence in maize inbred lines (Pooled over years)

Clusters	DT	DS	ASI	DM	EH	LRWC	CTF	SC	CCF	EPP	KPR	GYP	PC
I	69.24	72.24	3.00	137.58	88.76	107.06	28.92	69.72	52.78	1.67	30.33	551.55	9.16
II	80.70	85.59	4.89	152.06	78.61	89.01	31.98	82.90	37.98	1.04	25.19	386.02	7.19
III	78.77	82.82	4.05	147.76	80.09	93.94	31.75	82.57	46.83	1.05	24.77	397.16	8.11
IV	69.81	72.81	3.00	137.94	89.11	111.71	28.43	64.04	55.26	1.74	30.93	633.30	10.03
V	82.88	87.88	5.00	152.94	81.63	98.98	31.84	79.69	34.94	1.14	27.23	395.21	7.64
VI	80.63	85.38	4.75	152.31	77.08	91.78	31.53	81.51	37.64	0.95	21.40	377.14	6.33
VII	81.13	86.63	5.50	151.88	81.75	104.81	32.17	84.65	41.56	1.14	25.64	383.11	6.65
VIII	79.53	84.42	4.89	150.43	79.24	84.90	31.80	79.87	37.56	0.99	24.25	382.19	8.21
IX	80.38	84.81	4.43	150.68	78.20	87.88	32.00	84.79	43.46	0.98	23.80	391.01	6.44
X	79.88	85.13	5.25	150.75	78.43	91.78	32.14	96.77	33.90	1.09	26.87	392.02	6.36
XI	79.63	84.25	4.63	148.69	83.48	94.88	32.22	80.95	33.90	1.15	28.05	398.53	8.47
Number of times ranked 1st	1	-	-	5	10	-	26	4	1805	3	79	2	2390
Contribution %	2.02	-	-	10.1	20.2	-	52.53	8.08	3646.46	6.06	159.6	4.04	4828.28

Days to 50% tasseling=DT; Days to 50% silking=DS; Anthesis-silking interval=ASI; Days to maturity=DM; Plant height (cm)=PH; Leaf relative water content (%)=LRWC; Canopy temperature before flowering (˚C)= CTF; Stomatal count (mm⁻²)= SC; Chlorophyll content before flowering=CCF; Ears plant⁻¹=EPP; Kernels row⁻¹=KPR; Grain yield plot⁻¹ = GYP; Protein content (%)=PC

DISCUSSION AND CONCLUSIONS

Cluster analysis on pooled analysis for morphological, maturity, yield, physiological and quality attributes grouped 100 maize lines into 11 clusters with maximum number of lines (53) in cluster-II followed by cluster-I (14 lines), cluster VIII (11 lines), cluster IX (9 lines) and cluster-III (7 lines), whereas clusters V, VI, VII, X, XI were monogenotypic. Distribution of inbred lines (at S6 stage of selfing) for morphological, maturity, yield, physiological and quality attributes into different clusters revealed maximum grouping of these lines to the tune of 59% in cluster-II followed by 13.6% in cluster-I, 7% in cluster-VIII, 6% in cluster-III and cluster-IX and 1% each of cluster-V, VI, VII and cluster X, whereas maximum per cent distribution of S8 lines was observed in cluster-II (39.39%), followed by cluster-VIII (18.18%), cluster-I and cluster-IX (15.15%), cluster-III (9%) and cluster- IV (3%). KDM-347 (S9 lines) was accommodated in cluster-II. Four white lines of S6 origin were grouped in cluster-II, cluster-VIII and cluster-IX, whereas purple lines of S6 origin was grouped in cluster-II. Grouping of lines into different clusters was random and was irrespective of colour, and/or number of selfed generations.

Considering the grouping of lines into different clusters in the present study, both in the individual year and in the pooled year suggested that the geographical diversity was not an essential factor to group the lines from a particular source or origin into one particular cluster. Although the maize lines under study mostly originated from one place but were scattered over different clusters. Such diversity among lines of common geographic origin could be attributed to factors like heterogeneity, genetic architecture of the populations, natural and artificial selection, past history of selection, developmental traits, degree of general combining ability, genetic drift, exchange of genetic material which might have played an important role in the diversity of genotypes [4], geographical diversity, though important, was not the only factor in determining the genetic divergence. The various lines shift their position from one cluster to another cluster across the years because changes in years alter the clustering pattern due to G × E interaction. Formation of more clusters in one year indicates that more diversity is expressed by lines in a particular environment and this happens because of genetic drift and selection in different environments. This diversity observed in a particular environment might also have arisen from modifying factors like temperature difference than change in major genes. These findings were in accordance with that of De and Rao [9], Sinha *et al*. [10], Teixeira *et al*. [11], Hemavathy *et al*. [12], Reddy *et al*. [13] and Seshu *et al*. [14].

Inter-cluster distances for maturity, morphological, physiological, yield and quality traits revealed divergence among 82 lines grouped in cluster-I, II, IV, V, VI, VII, IX, X and XI. Maximum inter-cluster distance exhibited between cluster- IV and cluster- X indicating maximum genetic divergence for lines KDM-361A and KDM-375 followed by cluster-IV (KDM-361A) and cluster-VI (KDM-741), cluster-IV and cluster-XI (KDM-439), cluster-IV and cluster-IX (KDM-926B, KDM-1124, KDM-909A, KDM895, KDM-356A, KDM-916A, KDM-340A, CM-135 and KDM-3008), cluster IV and cluster V (KDM-440); and cluster I (KDM-463, KDM-912A, KDM-717, KDM-343A, KDM-961, KDM-932A, KDM-1051, KDM-402, KDM-918A, KDM-1156, KDM-1236, KDM-372, CM-129 and KDM-331) and cluster X (KDM-375). Measures of intra cluster distance also recorded similar response of lines towards genetic divergence with maximum intra cluster distance exhibited by lines viz; KDM-926B, KDM-1124, KDM-909A, KDM895, KDM-356A, KDM-916A, KDM-340A, CM-135 and KDM-3008 (grouped in cluster-IX). The genotypes belonging to the clusters separated by high statistical distance could be used in hybridization programme for obtaining a wide spectrum of variation among the segregates. These findings were in conformity with the findings of Khumkar and Singh [15]; Miranda *et al*. [16]; Yin *et al*. [17]; Datta and Mukherjee [18]; Singh *et al*. [19]; Hemavathy *et al*. [12]; Singh *et al*. [20]; Alam and Alam, [21]; Ganesan *et al*. [22]; Azad *et al*. [23] and Seshu *et al*. [14]. The maximum intra cluster distance (D^2) indicated high heterogenity in genetic constitution of genotypes in that cluster while minimum intra cluster distance (D^2) indicated homogenity in genetic constitution of genotypes in that cluster. Also as the highest value of intercluster distance indicated more heterogeneous genetic constitution of genotypes included in both the clusters. In contrast, minimum intercluster distance indicated closer relationship among the genotypes included [24, 25]. Intra-group distances appeared much smaller than the inter-groups, suggesting a lower genetic diversity among the lines of the same group than those from different groups. This is collaborated with the results of Hemavathy *et al*. [12]; Ivy *et al*. [26]; Nataraj *et al*. [27]; Seshu *et al*. [14]..

Cluster means revealed that substantial variability existed for all the maturity, morphological, physiological, yield and quality traits. Lines viz; KDM-463, KDM-912A, KDM-717, KDM-343A, KDM-961, KDM-932A, KDM-1051, KDM-402, KDM-918A, KDM-1156, KDM-1236, KDM-372, CM-129, KDM-331 and KDM-361A grouped in cluster-I and cluster-IV, recorded maximum cluster means confirming their maximum contribution towards divergence. Similar pattern of contribution of the lines was also observed for seedling and root related traits showing maximum cluster means for cluster-I and cluster-VII.

Characters contributing to divergence are reported to vary from crop to crop [4]. Protein content recorded highest contribution towards divergence in pooled analysis followed by chlorophyll content at flowering and 100 grain weight. Days to maturity, ear height, leaf relative water content, stomatal count, canopy temperature before flowering, canopy temperature before maturity, ear plant^{-1}, kernels row^{-1} and grain yield plot^{-1} recorded negligible contribution towards divergence whereas no contribution was recorded for days to 50% silking, anthesis-silking interval, plant height, chlorophyll content at maturity and ears plant^{-1}. The present results were in agreement with those of Kumar and Singh [28]; Teixeira *et al.* 11]; Alom *et al.* [29]; Singh *et al.* [19] and Marker and Krupakar [30] who also identified above said characters as the principle components contributing maximum to the total variation in maize. Higher contribution by traits like plant height, number of kernels per row, 1000 grain weight and grain yield per plant to the total divergence was reported by Azad *et al.* [23]. Zaman and Alam [31] reported days to 50% tasseling, silking, days to maturity, plant height and ear height to contribute maximum towards total divergence. Similarly, highest contribution towards divergence in this regard was put forth by grain yield per plant followed by plant height, number of kernels per row and 100 grain weight. The above results imply that the traits contributing maximum towards the divergence should be given great emphasis for deciding the clusters to be chosen for hybridization and the subsequent selection of the parents from the clusters be based on their *per se* performance. In maize, maximum contribution from traits towards divergence has been reported to be different for different sets of materials used in experimentation depending upon the genotypes under study. Also, the traits showing high contribution towards genetic divergence could be improved upon by selecting the genotypes from those clusters having maximum cluster means for the respective traits, which in turn depends upon the objective of the breeding programme.

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