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Study of genetic divergence in maize under drought condition

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Abstract

Genetic divergence in 42 Maize inbred lines and hybrids including two check were assessed based on some morphological traits and grain yield using Mahalanobis D^2 -statistics. The experiment was conducted during rabi 2023 in RBD design under managed drought condition. The genotypes were grouped into fourteen clusters. The cluster I contained the highest number of lines (12), while the cluster II, III, VII, VIII, IX, X, XI, XII and XIV contained only single genotype. The maximum inter-cluster distance was noticed between the cluster XIII and XIV and minimum between cluster II and III. The highest intra-cluster distance was observed in the cluster VI (13.08). The genotypes in the cluster XIV showed better performances for characters like good cob length, cob diameter, kernel rows per cob and reasonable yielding ability. It is expected that crossing of inbred lines belonging high to medium D^2 values may tend to produce for yield. Proline content had the greatest contribution to the genetic divergence. Days to pollen shedding and grain yield per plant were found to be responsible for primary differentiation.

Keywords: Maize, inbred, divergence, clusters, D^2 analysis.

Introduction

Maize among cereals ranked at third number after wheat and rice worldwide (Ali F. *et al.*, 2014) [2]. Over 80% of total global agricultural land is rain-fed (Berzsenyi *et al.*, 2006) thus the development of genotypes that survive better in water scarce condition is the need of hour. Phenotypic stability of the traits in maize hybrids is the best way to measure the genetic variability (Chavan *et al.*, 2015) [12] by overwhelming the two most common variables in field: soil heterogeneity and environment (Khorasani *et al.*, 2011; Ali *et al.*, 2014, 2015a) [2, 24]. Therefore, multivariate analysis (Ashmawy, 2003; El-Badawy and Mehasen, 2011) displays a better idea of the underlying latent factors and an interface between individual genotype and variable.

Drought is one of the most important abiotic stress factors (Bruce *et al.*, 2002) [10], which affects almost every aspects of plant growth (Aslam *et al.*, 2006). Drought is a permanent constraint to agricultural production in many developing countries, and an occasional cause of losses of agricultural production in developed ones (Ceccarelli and Grando, 1996) [11]. The best option for crop production, yield improvement and yield stability under drought stress conditions is to develop drought tolerant crop varieties. One of the main goals in breeding programs is selection of the best genotypes under drought stress conditions (Richards *et al.*, 2002) [34].

The analysis of genetic diversity aims at predicting combinations with higher heterozygosity so that there will be more possibilities of recovering higher genotypes in the segregant generations (Troyer, 2006) [40]. Morphological, physiological and productive differences are regarded in quantification of dissimilarity. Those genetic variations among maize cultivars are essential for predicting heredity and level of heterosis, which are essential for crop production (Duan *et al.*, 2006) [13]. Association degree among traits allows the use of indirect selection over another variable, especially when heritability of the principal trait is low, as is the case for yield (Iqbal *et al.*, 2003) [17].

Mahalanobis' D^2 statistic of multivariate analysis is recognized as a powerful tool in quantifying the degree of genetic divergence among the inbreds (Hemavathy *et al.*, 2008) [16]. 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. (Murty and Arunachalam, 1966; Panwar, 1970) ^[27, 28]. 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 (Fuzzato *et al.*, 2002). It also helps to identify the suitable inbreds for hybridization programme on the basis of their clustering pattern. The present investigation was undertaken with a view to estimate the genetic divergence in the 42 maize genotypes under drought stress and their response to water scarcity using Mahalanobis D2 statistic.

Material and methods

The experimental material for the variability studies comprised of 42 genotypes of maize. Field study was conducted at Maize Improvement Project, Kasba Bawda, Kolhapur during *rabi* 2023 under managed stress condition. The water stress was given to crop during its reproductive growth stage. The experiment was laid out in a randomized block design with three replications. The analysis of divergence was carried out by D2 statistics of Mahalanobis as described by Rao (1952) ^[32]. Cluster formation was done as per Tocher's method as described by Rao (1952) ^[32]. Diagrammatic representation of cluster divergence showing different genotypes was obtained with the help of D2 values between (Inter-cluster distances) and within (Intra-cluster distance) clusters.

Results and Discussion

A. Cluster formation

Understanding genetic diversity is crucial for effective breeding strategies in maize (*Zea mays* L.), particularly in the context of climate variability and the increasing prevalence of drought conditions. Genetic diversity within and among maize genotypes influences adaptability, resilience and yield potential under various environmental stresses. Cluster analysis serves as a valuable tool for categorizing genotypes based on their genetic similarities and differences, enabling the identification of diverse parental lines for hybridization. This method allows breeders to select genotypes that exhibit desirable traits, ultimately leading to improved maize varieties that can withstand adverse conditions.

In this study, Tocher's method, as described by Rao (1952) ^[32], was applied to classify 42 maize genotypes into 14 distinct clusters based on their genetic divergence. Cluster I was the largest, consisting of 12 genotypes, followed by cluster VI with 10 genotypes and cluster IV with 7 genotypes. Clusters V and XIII each contained 2 genotypes. The remaining clusters (II, III, VII, VIII, IX, X, XI, XII, and XIV) had only a single genotype, making them solitary and indicating that the genotypes in these clusters are highly divergent from others (Rao, 1952; Bhatt, 1970; Murthy and Arunachalam, 1966; Venkatesan and Subramanian, 2000) ^[32, 8, 27]. The formation of solitary clusters under drought conditions highlights the distinctiveness of certain genotypes that may possess unique traits enabling them to cope with drought stress (Singh *et al.*, 2015; Sharma *et al.*, 2013) ^[39, 37].

B. Intra and inter cluster distance

Assessing intra- and inter-cluster distances is crucial for analysing the genetic diversity among maize genotypes,

which plays a key role in shaping effective breeding strategies. Intra-cluster distance measures the genetic variation within a single cluster, whereas inter-cluster distance reveals the genetic divergence between separate clusters. Such insights are valuable for breeders seeking to improve targeted traits in maize through hybridization efforts.

Intra-cluster distance

In present investigation, the intra-cluster distances varied significantly, ranging from 5.61 to 13.08, indicating substantial variation within certain clusters. The highest intra-cluster distance was observed for Cluster VI ($D^2 = 13.08$), followed by Cluster XIII ($D^2 = 9.82$). This indicates a significant level of genetic variability among the genotypes within these clusters, suggesting that they may possess diverse adaptive traits beneficial for drought tolerance (Mather & Jinks, 1982; Rao, 1952; Singh *et al.*, 2015) ^[32, 39]. Conversely, the lowest intra-cluster distance was recorded for Cluster V ($D^2 = 5.61$), which may imply a narrower genetic base among the genotypes in this cluster. Notably, the solitary clusters II, V, VI, VII, VIII and IX exhibited no intra-cluster distances, reflecting their monogenotypic nature, which limits their potential for hybridization.

Inter Cluster Distance

The inter-cluster distance analysis revealed considerable variation, with the smallest distance observed between clusters II and III ($D^2 = 5.27$), indicating closer genetic relationships that may limit their effectiveness as diverse parents in breeding programs (Khan *et al.*, 2014; Mather & Jinks, 1982) ^[14]. The largest distance was observed between clusters III and XIV ($D^2 = 21.58$). Clusters that were further apart genetically exhibited more distinct differences, reinforcing the idea that greater distances between clusters indicate a wider genetic divergence between them (Islam *et al.*, 2020) ^[18]. This is in alignment with the findings of Bhusal *et al.* (2017) ^[9] and Bhadru *et al.* (2020) ^[7], who noted similar genetic separation across different clusters. Largest inter cluster distance suggesting that these genotypes can be utilized as parents for hybridization to develop drought-resistant varieties (Bhatt, 1970; Reddy *et al.*, 2004) ^[8, 33].

The notable differences observed in both intra- and inter-cluster distances highlight the importance of choosing genetically diverse parents for hybridization. Genotypes that exhibit greater inter-cluster distances have a higher likelihood of generating progeny with superior traits, such as increased drought tolerance and enhanced yield performance.

C. Cluster means

The analysis of cluster means for the 16 characters evaluated in maize genotypes demonstrated considerable variability, emphasizing their potential for selection in breeding programs under both drought and normal conditions. For days to 50 per cent tasselling, cluster XI exhibited the earliest flowering, taking only 60 days, while cluster XIV required significantly longer, at 73 days (Sahu *et al.*, 2020) ^[35]. Similar trends were observed for days to 50 per cent silking, where cluster XI showed the earliest silking at 61 days, whereas cluster XIV needed the most time, at 77 days (Mishra *et al.*, 2019) ^[26].

The trait days to 75 per cent dry husk, cluster XI required the least time under drought (102.00 days), while cluster XIV needed the most (119 days) (Jha *et al.*, 2019) ^[20]. In terms of plant height, solitary cluster XIV recorded the tallest plants at 176.30 cm, whereas cluster III was the shortest at 125.60 cm. (Patel *et al.*, 2021) ^[29]. Cob height followed a similar trend; monogenic cluster VIII had the shortest height at 70.50 cm, while cluster XIV reached a maximum of 98 cm (Sharma *et al.*, 2020) ^[36]. Cob length results indicated that cluster XIV had the longest cob at 21.50 cm, while cluster X was the shortest at 12.50 cm (Jaiswal *et al.*, 2020) ^[19].

For cob diameter, cluster XIV displayed the maximum width of 6.85 cm, while cluster V recorded the minimum at 3.98 cm (Ali *et al.*, 2017) ^[1]. Furthermore, solitary cluster XIV exhibited the highest number of kernel rows per cob at 17.50, while clusters X had the lowest at 12.50. (Patel *et al.*, 2022) ^[30]. Regarding the number of kernels per row, cluster XIV achieved the highest at 38.00, while cluster XIII showed the lowest.

Cob weight was highest in solitary cluster XIV at 225.10 g, while cluster XIII had the lowest at 100 g (Singh *et al.*, 2020) ^[38]. For 100 seed weight, solitary cluster XIV recorded the highest at 36 g, while cluster X and II was the lowest at 28 g. The genotype lying in the cluster IX (22.00) had less initial plant count, while genotype in solitary cluster V (28.25) had more initial plant count. The genotype lying in the cluster IX (22.00) had less final plant count while, genotype in solitary cluster X (27.00) had more final plant count. The genotype lying in the cluster IX (22.00) had less number of ears per plot, while genotype in solitary cluster XII (28.00) had more. The proline content was highest in cluster XIV at 0.71 mg/g, while cluster V and XIII had the lowest at 0.40 mg/g. (Khan *et al.*, 2025) ^[15].

This drought analysis of cluster means reveals the varied performance of maize genotypes across stress conditions,

offering important guidance for breeders in identifying appropriate parent lines for hybridization programs.

D. Percent contribution of various characters for divergence

The percentage contribution of different traits to overall genetic divergence in maize is essential for breeding efforts, especially when developing drought-tolerant varieties adapted to diverse environmental conditions. This analysis helps pinpoint critical traits for selection, supporting enhanced yield and improved resilience under water-limited situations.

The study indicates that proline content makes the larger contribution to divergence, with a 51.57 per cent share, indicating its importance in osmotic adjustment and stress tolerance (Jinks, 1986; Ranjan *et al.*, 2022). Other notable contributors included days to 50 per cent tasselling (21.84%) and yield per plant (8.71%), both critical for yield stability. The traits like cob length contributes 4.30 per cent, number of kernel rows per cob contributes 3.72 per cent, 100 seed weight 3.48 per cent and the number of kernels per row 3.14 per cent, which emphasizes the importance of these traits in promoting genetic diversity and adaptability. However, traits such as number of ears per plot (1.97%), final plant count (0.70%), plant height (0.35%), days to 50 per cent silking and initial plant count (each 0.12%) showed negligible contributions, indicating less relevance in normal growth conditions.

These findings highlight the importance of including these traits such as proline content and days to 50 per cent tasseling in breeding strategies to enhance both yield and resilience in maize. The varying contributions of these traits under drought and normal conditions suggest that breeding efforts should prioritize their selection to ensure strong performance across different environmental conditions.

Table 1: Distribution of 42 genotypes of maize into 14 different clusters in drought condition

Clusters	No. of genotypes included	Name of genotypes	Specific Character
I	12	ZH23645, ZH23646, ZH23630, ZH23647, ZH23645, ZH221150, ZH23620, ZH221143, ZH221145, ZH221086, ZH221136, ZH23619	Kernel rows per cob, cob diameter
II	1	ZH23621	Moderate cob height
III	1	ZH221116	Low plant height, Cob Height
IV	7	ZH23622, ZH23627, ZH23621, ZH23626, ZH23633, ZH23624, ZH23639	Moderate plant height, Cob diameter
V	2	ZH23634, ZH221087	Initial Plant Count
VI	10	26DKC9144, 27P3302, ZH221130, ZH23625, ZH221143, ZH221133, ZH23641, ZH23648, ZH221086, ZH23629	100 seed weight, Grain yield
VII	1	ZH221143	Cob length
VIII	1	ZH221105	Moderate cob height
IX	1	ZH23647	Low Plant Population
X	1	ZH23637	Low kernel row per cob
XI	1	ZH23644	Early Anthesis and Silking
XII	1	ZH23638	Plant height
XIII	2	ZH23516, ZH23644	Low yield, Low proline
XIV	1	ZH23642	High Proline

Table 2: Average intra and inter cluster D2 and D values (in parenthesis) of maize in drought condition

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
I	8.45 (2.91)	10.31 (3.21)	10.65 (3.26)	14.21 (3.77)	17.50 (4.18)	14.86 (3.85)	10.73 (3.28)	11.00 (3.32)	15.20 (3.90)	11.06 (3.33)	10.47 (3.24)	11.89 (3.45)	20.97 (4.58)	20.56 (4.53)
II		0.00	5.27 (2.30)	9.23 (3.04)	18.23 (4.27)	12.07 (3.47)	10.66 (3.26)	14.48 (3.81)	14.63 (3.82)	10.11 (3.18)	15.55 (3.94)	11.40 (3.38)	22.85 (4.78)	18.91 (4.35)
III			0.00	8.25 (2.87)	15.20 (3.90)	14.49 (3.81)	9.27 (3.04)	13.13 (3.62)	11.97 (3.46)	11.80 (3.43)	15.60 (3.95)	10.25 (3.20)	21.24 (4.61)	21.58 (4.65)
IV				9.34 (3.06)	15.73 (3.97)	16.61 (4.08)	11.47 (3.39)	16.80 (4.10)	12.71 (3.56)	15.80 (3.97)	20.37 (4.51)	11.64 (3.41)	22.47 (4.74)	23.24 (4.82)
V					5.61 (2.37)	25.19 (5.02)	14.02 (3.74)	13.74 (3.71)	15.34 (3.92)	19.79 (4.45)	21.45 (4.63)	13.68 (3.70)	14.40 (3.79)	33.20 (5.76)
VI						13.08 (3.62)	17.41 (4.17)	20.79 (4.56)	19.68 (4.44)	15.54 (3.94)	18.06 (4.25)	16.60 (4.07)	30.01 (5.48)	14.37 (3.79)
VII							0.00	12.75 (3.57)	7.74 (2.78)	16.39 (4.05)	15.89 (3.99)	9.92 (3.15)	18.37 (4.29)	23.49 (4.58)
VIII								0.00	17.54 (4.19)	11.60 (3.41)	10.62 (3.26)	14.02 (3.74)	14.40 (3.79)	28.45 (5.33)
IX									0.00	21.54 (4.64)	20.34 (4.51)	12.39 (3.52)	21.86 (4.68)	24.76 (4.98)
X										0.00	11.83 (3.44)	14.25 (3.77)	22.36 (4.73)	22.31 (4.72)
XI											0.00	16.96 (4.12)	22.41 (4.73)	22.88 (4.78)
XII												0.00	21.49 (4.64)	21.75 (4.66)
XIII													9.82 (3.13)	38.69 (6.22)
XIV														0.00

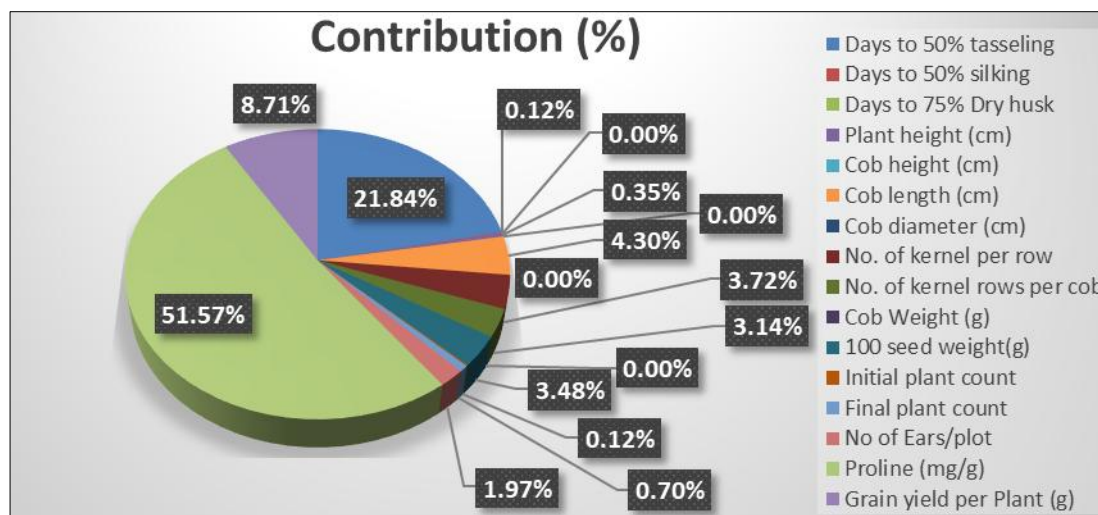
Table 3: Mean performance of 14 clusters for 16 characters in 42 genotypes of maize in drought condition

Cluster	DFT	DFS	DDH	PH	CH	CL	CD	NKR	NKRC	CW	HSW	IPC	FPC	NEPP	PL	GY
I	64.63	67.08	113.08	146.12	81.21	17.21	5.48	32.13	14.42	170.37	32.83	25.96	25.67	25.67	0.56	142.21
II	69.50	71.50	114.50	141.50	78.50	16.50	5.26	31.00	13.50	152.50	28.00	25.00	25.00	25.00	0.55	124.76
III	69.50	71.50	115.50	125.60	69.50	15.50	4.94	33.00	13.50	169.70	28.50	24.50	24.50	24.50	0.51	138.41
IV	72.14	75.36	114.36	137.69	76.50	16.43	5.23	30.57	14.21	151.30	30.71	26.64	26.29	26.29	0.48	125.75
V	65.00	70.25	116.25	150.68	83.75	13.75	4.38	28.00	14.25	145.90	30.75	28.25	26.25	26.25	0.40	121.72
VI	70.05	73.50	117.50	155.54	86.50	18.65	5.94	33.90	15.40	183.71	32.60	25.75	24.95	25.05	0.62	145.89
VII	68.00	69.00	115.00	154.00	85.50	19.00	6.05	33.00	16.50	185.50	29.00	24.50	24.50	24.50	0.47	155.89
VIII	61.00	62.50	109.00	126.25	70.50	14.00	4.46	28.50	14.00	144.40	33.00	23.00	23.00	23.00	0.52	120.15
IX	72.00	74.50	118.00	144.25	80.00	19.50	6.21	37.50	17.00	188.90	34.00	22.00	22.00	22.00	0.42	156.03
X	63.50	66.50	112.50	130.20	72.50	12.50	3.98	27.50	12.50	134.50	28.00	27.00	27.00	27.00	0.62	111.70
XI	60.00	61.00	102.00	136.05	75.50	15.50	4.94	35.50	13.50	157.30	33.00	24.50	24.50	24.50	0.61	129.40
XII	69.50	75.00	117.00	164.85	92.00	14.50	4.62	30.50	16.50	163.50	30.00	26.50	26.50	28.00	0.51	143.01
XIII	62.25	64.00	110.00	145.48	80.75	12.75	4.06	25.25	13.75	100.80	28.25	26.00	25.25	22.25	0.40	84.21
XIV	73.00	77.00	113.00	176.30	98.00	21.50	6.85	38.00	17.50	225.10	36.00	26.00	26.00	27.50	0.71	184.39

DFT= Days to 50 per cent tasselling, DFS= Days to 50 per cent silking, PH= Plant height, CH= Cob height, CL= Cob length, CD= Cob diameter, CW= Cob weight, IPC= Initial Plant Count, FPC= Final Plant Count, NEPP= Number of ears per plot, NKR= Number of kernel per row, NKRC= Number of kernel rows per cob, HSW= 100 seed weight, DDH= Days to 75 per cent dry husk, PL= Proline and YPP= Yield per plant.

Table 4: Per cent contribution of 16 characters for divergence of maize in drought condition

Sr. No.	Source	Times ranked 1 st	Contribution (%)
1	Days to 50 per cent tasseling	188	21.84%
2	Days to 50 per cent silking	1	0.12%
3	Days to 75 per cent dry husk	0	0.00%
4	Plant height (cm)	3	0.35%
5	Cob height (cm)	0	0.00%
6	Cob length (cm)	37	4.30%
7	Cob diameter (cm)	0	0.00%
8	No. of kernel per row	32	3.72%
9	No. of kernel rows per cob	27	3.14%
10	Cob weight (g)	0	0.00%
11	100 seed weight(g)	30	3.48%
12	Initial plant count	1	0.12%
13	Final plant count	6	0.70%
14	No of ears/plot	17	1.97%
15	Proline (mg/g)	444	51.57%
16	Grain yield per plant (g)	75	8.71%
	Total		100



Conclusion

The investigation of genetic diversity among maize genotypes under drought conditions has unveiled critical insights for breeding programs. The distinct cluster formations indicate that certain genotypes exhibit more effective adaptation to drought stress. The evaluation of intra and inter-cluster distances reinforces the importance of selecting genetically diverse parents to enhance desirable traits such as drought resistance and yield potential. The analysis of cluster means illustrates significant variability in agronomic traits, emphasizing the potential for selecting superior parents for hybridization. Additionally, Proline content, tasseling time, and grain yield per plant were major contributors to genetic divergence, emphasizing their importance in selecting drought-resilient genotypes.

This research underscores the necessity of integrating genetic diversity assessments into maize breeding strategies, paving the way for the development of varieties that are not only high-yielding but also resilient to the challenges posed by climate variability. Future breeding efforts should prioritize these traits to ensure sustainable maize production and food security in the face of ongoing environmental changes.

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