Diversity Analysis in Biofortified Inbred Lines of Maize (*Zea mays* L.)

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Authors' contributions

This work was carried out in collaboration among all the authors. Authors PJ and DSJ designed and executed the experiments. Authors PJ and B prepared the original draft of the manuscript and performed the statistical analysis. Author RS prepared the final draft. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Thirty biofortified inbred lines of maize were evaluated for 11 parameters to study the genetic diversity by using D^2 statistics during kharif 2017in Randomized Block Design (RBD) with three replications at Agricultural Research farm, Institute of Agricultural Sciences, BHU Varanasi. In present investigation all genotypes were grouped into ten cluster. Among the different clusters of inbred lines, the cluster II with 8 inbreds emerged as the largest cluster. The intra cluster D^2 value ranged from 10.82 to 44.89. The maximum intra cluster distance was observed for cluster X (D^2 = 44.89). The maximum inter cluster distance was observed between cluster V and VI (D^2 = 180.90) followed by cluster V and VII (D^2 = 166.10), cluster IV and V (D^2 = 155.60), cluster V and VIII (D^2 = 135.02) and cluster I and VI (D^2 = 127.66). The maximum contribution towards divergence was due to 100 seed weight (52.18%), thus, estimates of variation in seed weight could be used as a basis for selection of distantly related parents. Highest mean value for grain yield per plant (80.8) and Zn concentration (39.53) were observed in cluster IV, while the highest mean value for 100 seed weight was found in cluster V. Therefore, these clusters prove to be of prime importance for selection of parents in hybridization programme aimed at higher yield along with enhanced grain Zn concentration.

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1. INTRODUCTION

Maize (*Zea mays* L.) is the principal cereal crop in world and third most important staple crop harvested after rice and wheat in India [1]. It assumes to play a significant role in Indian agriculture. In India it is cultivated over an area of 8.69 million hectares, with a production of 21.81 million tons and average productivity of 2509 kg /ha [2]. A major section of Indian societies especially poorer people is dependent on maize to meet their daily calorie as well as nutritional requirements. Hence, maize shines as a better alternative for biofortification to overcome the hidden hunger of underprivileged societies. For improving nutrient composition of food grain (biofortification) either by increasing micronutrient concentration or its bioavailability, selection of prospective parent is an essential prerequisite. D^2 statistics is a useful statistical tool for assessment of genetic divergence among various genotype for identification of potential parent to be used in hybridization programme [3].The divergence study in maize may be used in the potential selection of parents for allowing specific combinations amongst hybrids [4]. Based on genetic divergence the genotypes are clustered into different groups. The clustering process specifies overall distribution patterns of genetic diversity among the genotypes and grouped them in such a way that the genotypes in the same cluster are more similar to each other than to those in other clusters. The genetic divergence among genotypes of same cluster is defined by the intra cluster distances while those among genotypes of different clusters is defined by inter cluster distances.In present investigation genetic divergence in 30 genotype of maize was studied based on Mahalanobis [5] D^2 statistics. The D² values for all the possible pair of comparison between the 30 genotypes were calculated and analysed which revealed presence of considerably diversity in the genotype studied.

2. MATERIALS AND METHODS

The experimental material consists of thirty biofortified inbred lines of maize(listed in supplementary Table 1 received from Dept. of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi.The experiment was conducted in Randomized Block Design (RBD) with three replicationswith a spacingof 60cm× 20cm. The border effect was avoided by planting two border rows. The crop was raised following recommended packages of agronomic practices.

Observations on pre and post-harvest parameter(one biochemical and ten morphological traits) viz., Zn concentration, days to 50 % tasseling, days to 50 % silking, width of leaf (cm),tassel length (cm) ear height(cm), plant height(cm), cob length without husk (cm), cob diameter(cm) ,100 seed weight(g) and grain yield per plant (g) were recorded on five plants selected at random from each genotype for each replication and average value is used for biometrical analysis. Analysis of genetic divergence among inbred lines were carried out by employing Mahalanobis D^2 analysis based on quantitative traits and grouped as Tocher's clustering pattern by Rao [6]. Biochemical analysis for kernel Zn concentration was carried out with diaciddigestion (HNO_3 and $HClO_4$) of seeds followed by atomic absorption spectrometry (AAS) method as per the protocol described by Zarcinas et al. [7] with some modifications suggested by Singh *et al.* [8] Grain Zinc concentration was estimated following AAS based on the Beer - Lambert law using the relation Element concentration (mg/kg) =AAS reading × dilution factor.

3. RESULTS AND DISCUSSION

In present investigation analysis of genetic divergence among thirty biofortified inbred lines were carried out by using Mahalanobis D^2statistics and Tocher's clustering pattern [9]. The ANOVA for eleven traits under study is presented is Table 1 and the grouping of genotypes into ten clusters is presented in Fig. 1. Thirty genotypes were grouped into ten clusters. Among the different clusters of inbred lines, the cluster II with 8 inbredsemerged as the largest cluster. The cluster Iand cluster Xhad 5 inbreds, cluster VII had 4 inbreds, cluster Vand VIII with 2 inbreds while cluster III, IV, IX were monogenotypic.The intra cluster D^2 value ranged from 10.84 to 44.85 Table 2. The maximum intra cluster distance was observed for cluster X (D^2 = 44.85) followed by cluster VII (D^2 = 22.94) and minimum intra cluster distance was observed in cluster V (D^2 = 10.84) followed by cluster VIII (D^2 = 14.68). The maximum inter cluster distance was observed between cluster V and VI (D^2 = 180.85) followed by cluster V and VII (D^2 = 166.10), cluster IV and V (D^2 = 155.60), cluster V and VIII (D^2 = 135.02) and cluster I and VI (D^2 =

127.66). The minimum inter cluster distance was observed between cluster II and IV ($D^2 =$ 25.40).The nearest and farthest clusters for individual cluster based on D^2 values was determined and presented in Table 3. Cluster I was nearest to cluster III (27.50) and farthest from cluster VI (127.66). Cluster II exhibited closest proximity with cluster IV (25.40) and maximum divergence with cluster V (108.98). Cluster III was nearest to cluster I (27.50), while it was farthest from cluster VI (121.82). Cluster IV showed closest proximity with cluster II (25.40) and maximum divergence with cluster V (155.60). Cluster V exhibited closest proximity with cluster I (41.96) and widest diversity with cluster VI (180.85). Nearest and farthest clusters for cluster VI are II (29.70) and V (180.85) clusters, respectively. Cluster VII was nearest to cluster IV (32.65) and maximum divergence with cluster V (166.10). Nearest and farthest clusters for cluster VIII are II (35.86) and V (135.02) clusters, respectively. Cluster IX was nearest to cluster III (31.08) and farthest to VIII (77.95).Cluster X exhibited closest proximity with cluster II (39.63) and wide diversity with cluster V

(79.29). Rao [7] reported that theoretical concept behind such grouping is that the genotypes grouped into the same cluster presumably are less diverse from each other than those belonging to different clusters and thus crossing between the genotypes belonging to same cluster will not give expected desired heterotic response and desiredsegregants in further generations. Consequently, breeding programme should be from diverse parents selected from different clusters. Greater is the distance between two clusters, wider is the genetic diversity in the genotypes. The crosses involving the parents with extreme divergence have also been reported to exhibit decrease in heterosis [10]. Hence, for effective exploitation of heterosis selection of parents with optimum genetic divergence depend on clear understanding of cluster distance. However, in improvement programmes centered around some specific traits desired heterotic response, cluster distance alone is not fully informative in selection of parents, this is further resolved with information on mean value for concerned traits in respective clusters and presented in Table 4.
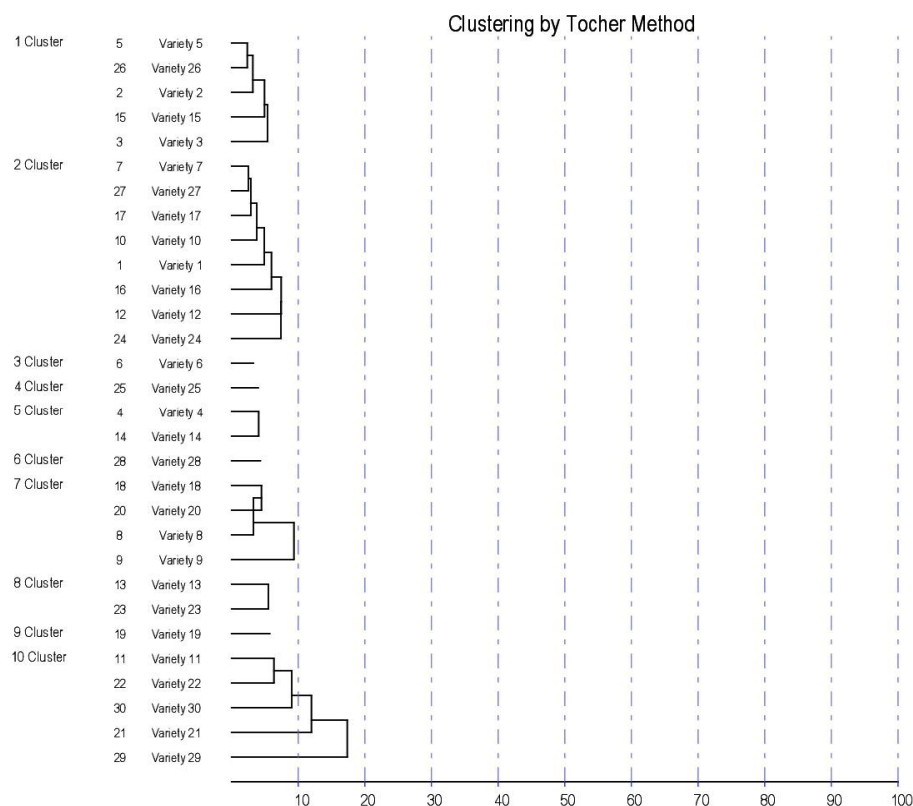
Fig. 1. Dendogram showing genetic diversity of thirty genotypes of maize and Grouping of thirty maize genotypes into ten clusters by Tocher's method

Table 1. Analysis of Variance (ANOVA) for eleven traits in thirty maize genotypes

Source of variation	D.F.	Mean sum of square (MSS)										
		DTT (50%)	DTS (50%)	LW (cm)	TL (cm)	EH (cm)	PH(cm)	CLWOH (cm)	CD (cm)	100SW (gm)	Y/P (gm)	Zn(mg/kg)
Rep.	2	8.18	0.63	0.04	0.85	26.86	34.17	7.01	0.07	0.74	20.65	0.57
Trt.	29	11.90**	11.93**	1.73**	55.72**	117.59**	276.96**	3.50**	0.90**	58.10**	80.94**	54.42**
Error	58	7.14	6.62	0.06	2.02	6.76	49.96	2.02	0.20	0.59	12.68	3.94
S.E ±		1.54	1.48	0.14	0.82	1.50	4.08	0.82	0.25	0.44	2.05	1.14
C.V. (%)		5.02	4.51	3.10	5.06	5.67	5.14	6.89	9.02	3.85	4.84	6.17
C.D. (5%)		4.37	4.20	0.39	2.32	4.25	11.55	2.32	0.73	1.25	5.82	3.24
C.D. (1%)		5.81	5.59	0.53	3.09	5.65	15.37	3.09	0.98	1.67	7.74	4.32

**and*Significant at 1% and 5% level of significance, respectively. Where PH (cm) = plant height, DTT (50%) = days to 50% tasseling, DTS (50%) = days to 50% silking, LW(cm)=leaf width, EH(cm) = ear height, TL(cm) = tassel length, CLWOH(cm) =cob length without husk, CD(cm) = cob diameter, 100SW(g) =100 seed weight, Y/P = yield per plant, Zn (mg/kg)=Zinc concentration.

Table 2. Average intra and inter-cluster D^2 and D values among ten clusters of thirty maize genotypes

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X
I	15.63 (3.95)	59.61 (7.72)	27.50 (5.24)	74.23 (8.62)	41.96 (6.48)	127.66 (11.30)	77.07 (8.78)	85.87 (9.27)	55.02 (7.42)	49.15 (7.01)
II		18.87 (4.34)	58.34 (7.64)	25.40 (5.04)	108.98 (10.44)	29.70 (5.45)	36.30 (6.02)	35.86 (5.99)	37.09 (6.09)	39.63 (6.30)
III			0.00	81.56 (9.03)	58.89 (7.67)	121.82 (11.04)	55.57 (7.45)	88.25 (9.39)	31.08 (5.57)	49.06 (7.00)
IV				0.00	155.60 (12.47)	41.79 (6.46)	32.65 (5.71)	43.91 (6.63)	71.90 (8.48)	56.52 (7.52)
V					10.84 (3.29)	180.85 (13.45)	166.10 (12.89)	135.02 (11.62)	73.69 (8.58)	79.29 (8.90)
VI						0.00	58.40 (7.64)	57.71 (7.60)	57.45 (7.58)	72.17 (8.50)
VII							22.94 (4.79)	60.35 (7.77)	56.70 (7.53)	64.31 (8.02)
VIII								14.68 (3.83)	77.95 (8.83)	71.44 (8.45)
IX									0.00	50.61 (7.11)
X										44.85 (6.70)

The highest mean value for days to 50% tasseling (54.96), days to 50% silking (58.32), plant height (142.69) and cob diameter (5.59) were recorded in cluster X. The higher mean value for tassel length (36.67), ear height (55.20) and leaf width (9.08) were found in cluster VIII. In cluster IV, higher mean value for grain yield per plant (80.80), Zn concentration (39.53) were observed. The highest mean value for 100 seed weight (29.13) were found in cluster V. Lowest mean value for cob diameter (4.23), plant height(122.57) and leaf width (6.30) were observed in cluster IX, while that for grain yield per plant (68.04) and 100 seed weight (14.37) were recorded in cluster VI. Contrary to effective exploitation of heterosis, when the aim of a breeding programme is to select the desired segregant for a specific trait, per cent contribution of traits studied towards total divergence is important for selection of potential parents

Table 3. The nearest and farthest cluster from each cluster based on D^2 values in thirty maize genotypes

Cluster No.	Nearest cluster with D^2 value	Farthest cluster with D^2 value
I	III (27.50)	VI(127.66)
II	IV (74.23)	V (108.98)
III	I (27.50)	VI (121.82)
IV	II(25.40)	V(155.60)
V	I(41.96)	VI (180.85)
VI	II(29.70)	V(180.85)
VII	IV(32.65)	V(166.10)
VIII	II(35.86)	V(135.02)
IX	III(31.08)	VIII(77.95)
X	II(39.63)	V(79.29)

Table 4. Cluster Mean values for 11 traits

Cluster	DTT	DTS	LW	TL	EH	PH	CLWOH	CD	100 SW	Y/P	Zn
I	53.88	57.72	8.53	24.75	43.83	140.91	20.43	4.68	24.41	69.39	32.69
II	53.02	57.1	7.66	29.43	45.06	138.04	20.99	4.75	17.66	73.89	33.35
III	51.53	56.03	7.37	25.87	53.87	125.07	19.57	5.90	24.07	77.19	30.87
IV	49.93	53.8	8.20	25.10	38.07	140.97	20.47	5.03	15.03	80.8	39.53
V	52.50	57.07	8.12	32.88	40.87	134.88	20.32	5.05	29.13	74.71	28.38
VI	51.63	55.64	6.77	32.13	41.30	139.70	21.87	4.57	14.37	68.04	34.53
VII	52.44	56.29	7.50	23.43	53.27	136.39	20.08	4.93	16.37	71.28	34.60
VIII	52.90	56.00	9.08	36.67	55.20	129.38	20.35	5.35	15.62	78.46	32.60
IX	53.77	57.77	6.30	29.73	47.63	122.57	21.83	4.23	22.07	73.41	36.07
X	54.96	58.32	7.56	27.64	41.99	142.69	20.59	5.59	20.85	75.88	26.77

Table 5. Percentage contribution of characters towards diversity in thirtymaize genotypes

S.No.	Characters	Times Ranked 1st	Percentage Contribution
1	Days to 50% tasseling	0	0
2	Days to 50% silking	0	0
3	Leaf width	66	15.17
4	Tassel length	49	11.26
5	Ear height	43	9.89
6	Plant height	5	1.15
7	Cob length without husk	2	0.46
8	Cob diameter	3	0.69
9	Seed weight	227	52.18
10	Grain yield	9	2.07
11	Zn	31	7.13
	Total	435	100

The per cent contribution of 11 characters studied towards total divergence was presented in Table 5. The maximum contribution towards divergence was due to 100 seed weight (52.18%) [11], followed by leaf width (15.17%) tassel length (11.26%), ear height (9.89%) [12] and Zn concentration (7.13%). Grain yield, cob diameter and plant height has comparatively low contributions towards divergence. The above results are in similitude with that of Kumar and Singh [13].

3. CONCLUSION

In present investigation genetic diversity was assessed by using Mahalanobis's D^2 statistic for eleven characters. From D^2 analysis cluster II is reported to be the biggest cluster consisting of 8 inbred lines, grouping of inbred lines in different cluster on the basis of diversity analysis may prove to be quite useful in heterotic grouping of maize inbreds. The maximum intra cluster distance was observed for cluster X (D^2 = 44.85) while the maximum inter cluster distance was observed between cluster V and VI (D^2 = 180.85) with marked genetic differences to exploit maximum heterosis. 100 seed weight is largest contributor (52.18%) toward divergence followed by leaf width, tassel length, ear height Zn concentration and grain yield per plant. The presence of tremendous diversity among the inbred lines depicts their suitability for utilization in hybridization programme to develop potential hybrid varieties.

SUPPLEMENTARY MATERIALS

Supplementary material is available in the following link:
https://www.journalijpss.com/index.php/IJPSS/libraryFiles/downloadPublic/16

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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