Morphological characterization and diversity analysis of maize inbred lines (*Zea Mays*L.)

ABSTRACT

To characterize Maize inbred lines in respect to morphological and physiological traits for its diversity and final utilization in the breeding programs, a study was conducted with twenty five inbred lines at Tirhut college of Agriculture, Dholi Muzaffarpur Bihar.Genetic diversity among inbred lines may be used for promising hybrid combinations development in maize breeding programme. In the present investigation,these twenty five inbred lines were sown in randomized block design with standard agronomical practices during *Kharif*, 2022.Analysis of variance revealed highly significant differences among the genotypes for all the parameters viz., Days to 50 percent Tasselingand Silking, 75% Brown husk, Plant Height (cn), Ear height (cm), Ear length (cm), Ear girth (cm), No. of Kernels per row, No. of kernel rows per ear, Iron and Zinc content (ppm), 100 grain weight (g), Harvest index and Grain yield (kg/ha.) showed presence of considerable variability among all the genotypes.Narrow deviation from genotypic and phenotypic variance evidenced that very less environmental influence on expression of almost all studied characters. According to the D^2 analysis, the 25 maize inbred lines were divided into eight clusters, with cluster I having the largest number of inbred lines, while clusters II, V, VI, VII, and VIII have only one inbred line. Cluster VI (21-213-CML-401) and cluster VIII (HKI-1105) was the most distant cluster among inbred lines within the clusters, indicating the greatest genetic diversity.

Key words: maize, inbred lines, morphological characters, genetic diversity.

Introduction:

Maize (*Zea mays* L.) is a cross pollinated crop, belong to the family of Gramineae (*Poaceae*).It is one of the most important crop in the world's agricultural sector. It is grown primarily for grain production, followed by its use as a source of foods and feed for both humans and animals,respectively. The use of maize as food, poultry feed, cattle feed, and other purposes, it occupied 34, 40, 12, 10 % respectively and 2% portionused as starch and seed. It is the third-most significant cereal the third-largest contribution to the national food basket after wheat and rice, with an 8% contribution with highest rate of increase among cereal (Bison *etal.* 2017).As a cross-pollinated species, maize has a wide range of morphological plasticity and geographic flexibility, making it one of the crop species with the most variability.Therefore, the fundamental issues facing maize curators and breeders are maintaining genetic variety and improving the management of genetic resources.Due to its cross-pollination in nature, maize has preserved heterozygous equilibrium in an open population.Inbred lines with genetically diverse origins with broad genetic base have been shown in research on maize to be more productive than crossings of inbred lines made from closely related sources having narrow genetic base and gene combinations as suggested by Vasal*et al*., (1998).Although morphological features represent the outward expression of an organism's underlying genetic structure, they serve as significant indicators for determining the genetic diversity.The morphological variability's characterization enables breeders to recognise accessions with desired traits and prevent duplication of accessions in the acquisition of accessible germplasm and their application in varietal programme for improvement mentioned by Magar *et al.*, (2021). Study of genetic variation in inbred maize populations will make it easier to determine the genotype's real potential utility. Further, statistical approaches to evaluating diversity that take into account the genetic distance between breeding purposes and the diversity-enhancing effects of individual characters (Singh, P. and Narayanan, et al., 2013). Using the information provided above, the current study was carried out to characterise 25 inbred lines of maize based on morphological and yield characteristics, as well as to determine their genetic diversity. The inbred lines having grater genetic distance with desirable traits may be taken for hybridization programme to develop promising hybrids in desired direction.

Materials and Methods:

This investigation was conducted at the Maize Research plot of Tirhut College of Agriculture, Dholi farm of Dr.Rajendra Prasad Central Agricultural University, Pusa, Samastipur (Bihar). The experiment was conducted during *Kharif* season 2022 in randomized complete block design under three replications. The plot was well drained and irrigated, and the topography was uniform.In each plot, two rows of 4m length are spaced 75 cm apart having plant distances at 20 cm each. A total of 25 maize inbred lines from a variety of maize populations were used in the experiment (Table-1).

Table 1: List of inbred lines and their source

No.	Inbred line	Abbreviation	Source
1	BML-6	G1	TCA, Dholi Center
2	POP-65	G2	TCA, Dholi Center
3	2006-6-CML-471	G3	TCA, Dholi Center
4	HKI-323-B	G4	TCA, Dholi Center
5	21-113-CML-411	G5	TCA, Dholi Center
6	CM-202	G6	TCA, Dholi Center
7	CM-142	G7	TCA, Dholi Center
8	CM-210	G8	TCA, Dholi Center
9	DTPYC$_9$-F$_{46}$-3-4-1-1-B*-8	G9	TCA, Dholi Center

10	G18seqC$_5$F$_{105}$-1-1-1-2-BB-B2-B4	G10	TCA, Dholi Center
11	CML-224	G11	TCA, Dholi Center
12	8-12-38-IEC-618960	G12	TCA, Dholi Center
13	HKI-1105	G13	TCA, Dholi Center
14	LM-13	G14	TCA, Dholi Center
15	IC296599	G15	TCA, Dholi Center
16	HKI-163	G16	TCA, Dholi Center
17	CML-41	G17	TCA, Dholi Center
18	EC-618219	G18	TCA, Dholi Center
19	CML-117-3-4-1-1-4-1	G19	TCA, Dholi Center
20	P-3396-51	G20	TCA, Dholi Center
21	P-3404-57	G21	TCA, Dholi Center
22	(CA145021CA14509) F2-32	G22	TCA, Dholi Center
23	(CML-165xK145)-B-11-3-BB-1-B*7	G23	TCA, Dholi Center
24	DTPWC$_9$-F$_{24}$-2-3-1-3-2-1-2-B*9	G24	TCA, Dholi Center
25	Temp x Trop (HO)QPM-BBB-23-BBB	G25	TCA, Dholi Center

Altogether, 14 morphological and quality traits on five randomly selected plants were recorded among the 25 inbred lines at appropriate (Table 2).

Table 2: Morphological and quality traits taken for study

SI. No	Name of the character	Abbreviation
1	Days to 50 percent Tasseling	DTT
2	Days to 50 percent silking	DTS
3	Days to 75 percent Brown husk	DBH
4	Plant height(cm)	PH

5	Ear height(cm)	EH
6	Ear length(cm)	EL
7	Ear girth(cm)	EG
8	Number of kernels per row	NKR
9	Number of kernel rows per ear	NKRE
10	Iron content (ppm)	Fe
11	Zinc content (ppm)	Zn
12	100 grain weight (g)	100- GW
13	Harvest index	HI
14	Grain yield (kg/ha)	GY

Standard statistical approaches were used to draw appropriate interpretations after computations for Analysis of Variance, Genotypic and Phenotypic Co-efficient of Variability and Genetic divergence studies (OPSTAT of HAU, Hisar). Zinc and Iron content in kernel is analyzed biochemically after digestion with di acid followed by AAS method after harvesting Graham,*et al*., (1999).

Results and discussion:

Genetic potential of 25 maize inbred lines for yield and component traits exhibited sufficient variation as per analysis of variance in the inbred lines which revealed that these lines may be used further for hybrid combinations.Treatment mean sum of squares were highly significant for all the fourteen traits as mentioned in table 3. Similar results of significant mean sum of squares due to genotypes was observed by Nzuve*et al*., (2014), Sharma *et al*., (2014), Begum *et al*., (2016),Jilo*et al*., (2019), Rai *et al*., (2021), Magar *et al*., (2021). The mean performance is known to show quantitative inheritance pattern and provides a reflection of the breeding value of inbred lines high-mean-performance inbred lines in various situations. According to the data on mean performances of 25 inbred lines (Table 3), the best inbred line in terms of grain yield (Kg/ha) was (CML-165 x K145)-B-11-3-BB (3933.91kg/ha). It was the highest yielder among the inbred lines with superior ear lengths, ear length, and minimal kernel rows per ear followed by HKI-1105 and 21-113-

CML-411. The best inbred line for grain yield (kg/ha) was (CML-165 x K145)-B-11-3-BB followed by HKI-1105 (3535.66) and BML-6 (3318.50). Yield should be given under bracket

The findings demonstrated that the phenotypic variance was slightly greater than the genotypic variance for nearly all of the fourteen traits indicated little role of environment in expression of traits. There was a higher phenotypic variance for all fourteen characters in comparison to their corresponding genotypic variance. For grain yield, there was a high genotypic coefficient of variation and a high phenotypic coefficient of variation showing greater mean range of variation for grain yield. Thecharacters having moderate genotypic coefficient of variation was recorded for iron content, zinc content and moderate Phenotypic of variation for plant height, ear length, ear girth, number of kernel rows per ear, iron content, zinc content. However, low genotypic coefficient of variation was recorded for plant height, ear height, days to 50 % tasseling, days to 50% silking, days to 75 % brown husk, ear length, ear girth, number of kernels per row, number of kernel rows per ear, 100 grain weight, harvest index and low Phenotypic coefficient variation were recorded for ear height, days to 50 % tasseling, days to 50% silking, days to 75 % brown husk, number of kernels per row, 100 grain weight, harvest index. Similar conformity with Hepziba *et al.*, (2013), Vashistha*et al*., (2013), Nzuve*et al*., (2014) Sandeep *et al*., (2015), Reddy *et al*., *(*2012);Bartaula*et al*., (2019),Magar *et al*., (2021).A broad sense estimation of heritability (Table 3) showed that the majority of characters have higher heritability, such as Zn (ppm) (97.95), Fe (ppm) (97.59), DTT (86.34), DBH (85.72), DTS (85.31), 100-GW (58.82), EL (58.58), HI(53.85). Characters having moderate heritability were recorded for EG (39.15), PH (34.32), NKRE (32.63), NKR (31.55).According to genetic advance estimates, GY (kg/ha) (46.54), Zn (ppm) (32.28), and Fe (ppm) (23.12) were also having high genetic advance. The following traits have moderate genetic advance as percent of mean namely EL (15.49), 100-GW (12.04), DTT (11.22) HI (11.05), DTS (11.03), DBH (10.42). Despite this, genetic advance was low with respect to EH (9.99), EG (8.25), PH (7.12), NKRE (6.88), NKR (6.42) as mentioned in Table 3. Therefore, characters having higher heritability in broad sense withhigh genetic advance werefound for iron and zinc contentalong withgrain yield suggested that variation might be primarily caused by additive gene effect and a genotype may be created that is widely used if these traits are selected for utilizing fixable genetic variance. High GCV, PCV, heritability, and GA as% of mean values were observed for grain yield from variability studies, which is attributable to additive gene action and demonstrates the significance of these variables in evaluation and selection for future hybridization programmers. The findings of Reddy *et al*., (2012), Begum *etal*., (2016),Matin *et a*l., (2017),Hosamani*et al*., (2018), Sravani*et al*., (2021),Rai *etal*., (2021) also showed similar trends.

Table 3: Mean, Variances, Heritability and Genetic advance as percent of mean.

S. No.	Characters	Mean	Mean sum of squares Treatment (df=24)	σ^2_g	σ^2_p	GCV	PCV	h^2(Broad sense) %	GA as % of Mean
1	Plant height (cm)	149.22 ± 7.03	381.57**	77.64	226.27	5.90	10.08	34.32	7.12
2	Ear height (cm)	73.20 ± 2.70	94.39 **	24.12	46.13	6.70	9.27	52.3	9.99
3	Days to 50 % tasseling	93.10 ± 1.29	100.92**	31.95	37.01	5.86	6.31	86.34	11.22
4	Days to 50% silking	98.00 ± 1.34	102.95 **	32.50	37.94	5.78	6.25	85.31	11.03
5	Days to 75% brown husk	122.54 ± 1.62	150.15**	47.41	55.31	5.46	5.90	85.72	10.42
6	Ear length (cm)	17.53 ± 0 .83	11.008**	9.82	12.83	9.82	12.83	58.58	15.49
7	Ear girth (cm)	11.62 ± 0.53	2.52**	6.40	10.23	6.40	10.23	39.15	8.25
8	Number of kernels per row	27.69 ± 1.30	12.21**	2.36	7.49	5.55	9.88	31.55	6.42
9	Number of kernel rows per ear	12.94 ± 0.62	2.90**	0.57	1.75	5.85	10.24	32.63	6.88
10	Iron content (ppm)	43.52 ± 0.44	73.29**	24.23	24.83	11.31	11.45	97.59	23.02
11	Zinc content (ppm)	36.90 ± 0.48	103.13**	34.13	34.85	15.83	15.99	97.95	32.28
12	100 grain weight (g)	31.22 ± 1.14	20.95**	5.66	9.63	7.62	9.93	58.82	12.04
13	Harvest index	0.36 ±0 .01	0.0026**	0.0007	0.0013	7.31	9.96	53.85	11.05
14	Grain yield (kg/ha)	2610.30 ± 97.55	1151609**	28552.75	374352	23.43	24.31	92.91	46.54

Table 4. Analysis of variance (ANOVA)

S. No	Character	Mean sum of squares				
		Replication (df=2)	**Treatment (df=24)**	**Error (df=48)**	**CV**	**CD at 5%**
1	Plant height	7.65	381.57**	148.63	8.64	20.07
2	Ear height	2.69	94.39 **	22.00	6.42	7.72
3	Days to 50 % tasseling	0.009	100.92**	5.05	2.25	3.69
4	Days to 50% silking	0.007	102.95 **	5.44	2.67	3.83
5	Days to 75% brown husk	1.99	150.15**	7.90	2.23	4.61
6	Ear length	0.16	11.008**	100.80	8.65	2.38
7	Ear girth	0.06	2.52**	0.86	7.9	3.73
8	Number of kernels per row	0.35	12.21**	5.12	8.17	1.79
9	Number of kernel rows per ear	3.21	2.90**	1.18	8.69	1.27
10	Iron content	0.22	73.29**	0.59	1.72	1.39
11	Zinc content	0.05	103.13**	0.71	2.29	3.28
12	100 grain weight	1.02	20.95**	3.96	6.38	3.2
13	Harvest index	0.00014	0.0026**	0.0005	7.69	0.04
14	Grain yield	2561	1151609**	28553	6.37	278.26

** Significant at *P* =0.01

All the 25 inbred lines of maize were studied for their genetic diversity based on the Mahalanobis D^2statistic (1936) based on fourteen important quantitative characteristics. A hierarchical clustering divided these inbred lines into eight clusters according to their genetic distance (table 5&6). Those genotypes belonging to the same cluster are likely to be less diverse from each other than those belonging to the different clusters, so crossing between genotypes belonging to the same cluster may not result in the desired heterotic response and desired segregants in subsequent generations. Amongeight clusters using Tochers' method, Cluster I possessed the largest number of inbred lines (12), while clusters II, V, VI, VII, and VIII had only one inbred line each. There was a maximum inter cluster distance (110.86) between clusters VI (21-113-CML-411) and cluster VIII (HKI-1105), indicating that these clusters have a high degree of genotype diversity among themselves.The results of breeding programs can therefore be achieved by selecting diverse parents from different clusters. Genotypic diversity is higher in clusters with a greater distance between them. Earlier workers such as Kage *et al*. (2013), Antony *et al*. (2021), Singh *etal*. (2005), and Hassan *etal*. (2018) adopted similar approaches.

It was found that the mean intra cluster distance varied from 29.96 to 44.66. The maximum intra cluster distance exhibited in cluster III (44.66), followed by cluster I (39.14), cluster IV (29.96), and cluster VIII (0.00). However, there was no intra cluster distance observed in cluster II, cluster V, cluster VI, cluster VII, and cluster VIII. Based on D^2values, cluster I is (44.22) having distance from cluster II, and cluster VIII is (75.76) distant from cluster I. Cluster II has the least divergence (32.11) with cluster VI, and cluster II has the most distant with cluster VIII (71.39). Cluster III was close to cluster VI (55.48), while cluster IV (83.43) was far away. There was the greatest divergence with cluster IV and cluster VIII (95.62) and the greatest closeness between cluster IV and cluster VII (65.29). There was closeness between cluster V and cluster VI (34.91), and a high degree of diversity between cluster VIII and cluster V (110.86). Cluster VI showed closeness with cluster VI (79.72) and wide diversity with cluster VIII (96.83). The most distant cluster from cluster VII is cluster VIII (65.37) (Table 5).

Table 5: Mean inter and intra cluster distances among eight cluster in maize for 25 inbred lines

Cluster	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
I	39.14	44.22	54.76	53.56	58.82	55.09	48.68	75.76
II		0.00	39.15	70.05	45.06	32.11	67.24	71.39
III			44.66	83.43	69.17	55.48	59.68	61.30
IV				29.96	74.36	83.92	65.29	95.62
V					0.00	34.91	86.00	110.86
VI						0.00	79.72	96.83
VII							0.00	65.37
VIII								0.00

Table 6: Clustering pattern of 25 maize inbred lines on the basis of D^2 statistics

Cluster	No. of inbred lines within cluster	Inbred lines in cluster
I	12	CM-142, P-3404-57, (CA145021CA14509) F2-32, DTPYC$_9$-F$_{46}$-3-4-1-1-B*-8, CM-210, LM-13, HKI-323-B, IC296599 CML-117-3-4-1-1-4-1, P-3396-51, CML224, EC-618219.
II	1	POP-65
III	6	BML-6, CM-202, Temp x Trop (HO)QPM-BBB-23-BBB CML-41, HKI 163, DTPWC$_9$-F$_{24}$-2-3-1-3-2-1-2-B*9
IV	2	2006-6-CML-471, 8-12-38-IEC-618960
V	1	G18seqC$_5$F$_{105}$-1-1-1-2-BB-B2-B4
VI	1	21-113-CML-411
VII	1	(CML-165xK145)-B-11-3-BB-1-B*7
VIII	1	HKI-1105

Table 7: Cluster mean for fourteen traits in maize inbred lines

Cluster	PH (cm)	EH (cm)	DTT	DTS	DBH	EL (cm)	EG (cm)	NKR	NKRE	Fe (ppm)	Zn (ppm)	100-GW	HI	GY
I	152.8	74.2	93.5	101.5	128.2	17.6	11.5	27.4	13.1	44.3	36.8	31.0	0.36	2643.5
II	147.5	72.8	93.9	95.9	122.2	19.1	11.7	22.8	11.7	45.0	36.2	29.9	0.33	1867.5
III	145.1	75.5	90.0	92.0	118.8	17.0	11.4	27.6	12.8	44.8	38.9	31.8	0.37	2676.0
IV	146.8	70.2	101.4	105.4	131.0	17.6	12.0	29.2	13.1	32.5	24.5	27.4	0.38	2672.1
V	136.0	62.3	102.1	105.1	128.7	14.6	10.1	25.8	10.2	42.0	35.4	32.7	0.36	1320.1
VI	133.0	65.5	95.7	97.7	124.7	15.1	12.3	29.5	14.0	49.3	43.3	32.5	0.28	1477.5
VII	168.6	72.6	97.0	99.0	125.9	20.1	13.5	31.0	13.7	38.0	42.0	34.1	0.40	3933.9
VIII	148.5	72.9	87.1	89.1	115.9	20.7	12.7	30.6	13.5	48.6	42.0	27.5	0.41	3535.7

Expression of different characters on the basis of cluster mean values of used inbred lines provided in table 7. Based on desirable nature of studied characters like fordwarfness the inbred lines from the clusterIII may be used as parent for further cross breeding programme to reduce the maturity duration. In the same way inbred line inclusterVIII, revealed long ear length and inbred line in cluster VII had highest ear girth may be exploited as parent for further cross breeding programme for using as yield contributing trait. For developing hybrid with a greater number of kernel rows the genotype in cluster I may be used as a parent in further crossing programme in the same way for developing hybrid with a greater number of kernels per row cluster VII may be used as parent. Likewise, the trait 100 grain weight, harvest index and grain yield, inbred lines in cluster III, VIII, VII viz G1, G13, G23 with 35.67, 0.41, 3933.91 per se performance may be used as parent for further crossing programme.In case of quality parameter such as iron and zinc content contents, the inbred lines in cluster VI may be used as a parent for further quality improvement.

In terms of percent contribution of fourteen character to total divergence were observed maximum for days to 75% brown husk, followed by zinc content, 100 grain weight, ear length, iron content, days to 50% silking, ear girth,number of kernel rows per ear, days to 50% tasseling, harvest index, grain yield, plant height, number of kernels per row. Similar approach was opted by Anderson *et al.*,(1957) and Rao*et al.*, (1952) regarding contribution of days to husking and silking, however, Ganesan *et al.*, (2010) and Rigon *et al.*,(2015), Antony *et al.*,(2021) exhibited higher percent contribution of yield contributing traits for divergence in maize

Table 8: Contribution percentage of 14 characters towards genetic divergence

S.no	Source	Times ranked 1st	Contribution %
1	Plant height (cm)	1	0.65
2	Ear height (cm)	10	6.35
3	Days to 50 % tasseling	8	5.22
4	Days to 50 silking	11	7.18
5	Days to 75% brown husk	35	22.87
6	Ear length(cm)	14	9.15
7	Ear girth (cm)	10	6.53
8	Number of kernels per row	1	0.65
9	Number of kernel rows per ear	10	6.53
10	Iron content (ppm)	12	7.84
11	Zinc content (ppm)	18	11.76
12	100 grain weight (g)	14	9.15
13	Harvest index	5	3.26
14	Grain yield (kg/ha)	4	2.61

Conclusion

The 25 inbred lines were divided into 8 clusters using Tochers' method. There was a maximum inter cluster distance (110.86) between clusters VI (21-113-CML-411) and cluster VIII (HKI-1105), indicating that these clusters have a high degree of genotype diversity among themselves.Hence, the crosses 21-113-CML× HKI-1105 and (CML-165×K145)-B-11-3-BBare likely to produce heterotopic hybrids or transgressive sergeants. Further, these Genotypes should be tested for their combining ability and gene action using different mating designs to produce hybrids based on the type of gene action involved. Among all combination, number of inbred lines combination ranked highest for days to 75% brown husk followed by zinc content, ear girth, 100 grain weight, number of kernel rows / ear. Maximum contribution of all traits toward genetic divergence showed by, days to 75% brown husk, zinc content, ear length, and grain yield suggesting scope for improvement and assessment of genetic diversity in maize inbred lines.On the basis of cluster mean performance, the inbred line (CML-165xK145)-B-11-3-BB-1-B*$_7$ from cluster VII may be selected for trait ear girth and grain yield (kg/ha) and inbred lines P-3396-51 in cluster I for the trait iron content and inbred cluster VI 21-113-CML-411 for zinc content were identified as promising parents for their further utilization in maize breeding programme.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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