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ASSESSMENT OF GENETIC DIVERSITY AND POPULATION STRUCTURE ANALYSIS IN ELITE MAIZE (*ZEA MAYS* L.) INBREDS ADAPTED TO NORTH WESTERN HIMALAYA

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ABSTRACT

This study aimed to evaluate the genetic diversity of thirty-six elite maize inbred lines using agro-morphological traits and SSR markers in α-RBD. Mahalanobis D^2 statistics grouped genotypes into six distinct clusters with Cluster I comprising the most genotypes (18), followed by Cluster III (9) and Cluster II (6). Principal component analysis revealed that first four principal components explained 87.81% of the overall variation. A set of 69 SSR maize primers was screened, and 25 showed polymorphism, resulting in 76 observed alleles. The Shannon index (I) had a mean value of 0.938 indicating a high level of genetic diversity in a set of maize inbreds. Using population structure analysis, these genotypes were categorized into three main groups. Based on combined approach, 13 genotypes were observed common showing the parallelism between morphological and SSR data. Moreover, the Mantel test revealed a significant positive moderate correlation (0.4007) between the two datasets. Eight genotypes *viz*., KI 46-1, CML 163, LM 16, LM 18, CML 140, KI 3-2, KI 45-2 and LM 24 were observed to be superior over the best check based on mean performance and diversity analysis. These genotypes may be further exploited for the development of high yielding single cross maize hybrids.

***Key words*:** Inbreds, diversity, cluster, SSR, Mantel's test.

Introduction

Maize (*Zea mays* L., 2n=20) known as queen of cereals is a highly versatile emerging crop that can be grown in a wide range of environmental conditions from 58°N to 40°S, sea level to elevations over 3000 m and in regions with annual rainfall ranging from 250 mm to over 5000 mm (Kumar *et al.*, 2014). The widespread cultivation and numerous applications of maize highlight its crucial role in maintaining global food security and its contribution to various industrial sectors. Furthermore, it is the most significant crop of the *kharif* season and possesses a diverse gene pool that can be further studied to enhance existing genotypes.

The aim of maize breeding programs is to create superior hybrids that outperform the existing ones in multiple traits. Studies have demonstrated that inbred lines from diverse stocks tend to exhibit higher productivity than crosses of inbred lines of the same cultivar (Vasal, 1998). Saxena *et al.*, (1998) also reported that heterosis is more likely to occur when the two parental lines have a high degree of genetic divergence. To determine the best breeding strategy, it is important to consider the diversity and relatedness of inbred lines within and between populations (Menkir, 2006). Genetic diversity involves analyzing genetic variations among individuals, groups or populations using a specific or a combination of methods such as multivariate analysis based on Mahalanobis D^2 statistics (Mohammadi and Prasanna 2003). This approach helps to group genotypes into different clusters to identify genotypically diverse genotypes. However, morphological markers are highly

affected by the environment, limited in number, have low polymorphism, late expression and low heritability (Smith 1332). Molecular markers that are not influenced by the environment are more reliable in characterizing genetic relationships (Reif *et al.*, 2005). The most common molecular markers used for assessing genetic diversity in maize are RFLP, RAPD, SSR, AFLP and SNP. Among these, SSR markers known for their codominant inheritance, locus specificity, extensive genome coverage and simple detection of loci using labeled primers have been successfully employed to genotype diverse maize germplasm collections (Xu *et al.*, 2013).

In view of the above, the study was conducted to assess the genetic diversity of elite maize inbreds using both morphological and SSR markers and to identify potential inbred(s) for yield and component traits. The findings will be useful for understanding available accessions and their potential for use in future maize hybridization programs.

Materials and Methods

Plant material and field experiment

The study was conducted during *kharif*, 2022 at Experimental farm of Shivalik Agricultural Research and Extension Centre (SAREC), Kangra which represents the subtropical conditions of North-Western Himalayas. A set of 36 maize inbreds including 3 checks (HKI 1105, LM 13 and LM 14) were evaluated in α-RBD design followed by phenotypic evaluation with 3 replications in a plot size of 3.0 x 1.2 m^2 (Supplementary Table S1). The row-to-row and plant-to-plant distance was maintained at 60 and 20 cm respectively, with two rows per plot and nine blocks per replication each containing four entries.

Agro-morphological data analysis

The mean values obtained for each agro-morphological trait were assessed using various statistical methods. Analysis of variance (ANOVA) was performed by using SPSS 29.0 software as described by Prasad (2007). The phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and broad sense heritability (h^2bs) were estimated with genetic advance (GA as % of mean) to determine additive and non-additive effects (Burton *et al.*, 1953; Johnson *et al.*, 1955). Mahalanobis D^2 statistic was employed to evaluate genetic diversity and grouped 36 maize genotypes into different clusters determined by Tocher's method (Rao, 1952) using Windostat software (version 8.0). Further, R software was used to determine Principal Component Analysis (PCA).

Genomic DNA extraction and PCR analysis

The extraction of genomic DNA from fresh leaves at 3-4 leaf seedling stage was carried out using the CTAB (cetyl trimethyl ammonium bromide) method (Doyle and Doyle, 1987). The extracted DNA was evaluated for its integrity and quality on a 1% agarose gel using gel electrophoresis. A set of 69 SSR primers were screened for analysis, of which 25 were selected to be effective in detecting polymorphisms based on their strength of bands, smearing appearance and population discrimination potential. These primers have been utilized by several researchers and shown to be effective in previous studies (Kumar *et al.*, 2012; Salami *et al.*, 2016; Sharma *et al.*, 2017; Sathua *et al.*, 2018; Adhikari *et al.*, 2019; Malik *et al.*, (2020); Neelothpala *et al.*, 2022).

To perform polymerase chain reaction, a reaction mixture was prepared in 0.2 ml thin-walled flat-capped PCR tubes containing 25 µl of total volume, with 17 µl of sterilized distilled water, 1.0 µl of template DNA (20 ng/µl), 1.0 µl of forward and reverse primer each (50 ng), 2.5 µl of 10 X PCR buffer, 2.0 µl of dNTP mix (0.2 mM each of dNTP) and 0.5 µl of Taq polymerase (5 U/µl). The PCR tubes were then spun down in a minifuge and DNA amplification was performed in a thermal cycler (BIO-RAD) using the following thermal cycling conditions: an initial denaturation for 5 min at 94°C, followed by 35 cycles of 45 s at 58-67°C, and a final extension of 5 min at 72°C. The amplified PCR products were then subjected to separation on a 3% agarose gel (Bioworld™ Agarose) that was stained with ethidium bromide (3µl/100 ml agarose gel) and visualized under a gel documentation unit (UVITEC, Cambridge).

SSR analysis

The SSR profiles generated in the amplified DNA of 36 maize genotypes were scored manually to determine the presence or absence (1 or 0) of each SSR band of a particular molecular weight which was further used for various analyses. POPGENE software (version 1.32) was used to calculate several genetic parameters including the observed number of alleles (Na), effective number of alleles (Ne), Shannon's information index (I) and expected heterozygosity (He) for each locus.

The Polymorphic Information Content (PIC) for each SSR locus was computed using CERVUS software (version 3.0.3). The construction of a dendrogram involved using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm within the SAHN program of the NTSYS-pc package (version 2.02) (Rohlf, 1998). A genetic dissimilarity matrix was created using the Jaccard dissimilarity index and from this matrix, a Neighbor-Joining tree (UnWeighted Neighbor-Joining) was constructed using the DARwin software version 6.0

Table 1: Analysis of variance in 36 genotypes of maize for twelve agro-morphological traits.

Traits/Source	Mean sum of square			
	Repli-cation	Blocks within repli-cation	Geno-types	Error
d.f.	2	24	35	46
Days to 50% pollen shed	1.861	3.215	25.969*	2.636
Days to 50% silking	3.370	4.250	25.666*	2.750
Days to 75% brown husk	2.265	2.301	33.924*	2.985
Plant height (cm)	1.611	27.884	898.741*	11.188
Cob placement (cm)	7.520	11.417	364.231*	8.169
Cob length (cm)	0.117	1.887	17.681*	1.523
Cob girth (cm)	0.292	0.740	6.985*	0.706
No. of kernel rows/cob	0.398	2.943	7.224*	1.090
No. of kernels/row	0.843	1.576	41.136*	3.019
100 kernel-weight (g)	1.361	2.416	15.449*	1.931
Shelling (%)	0.498	0.097	4.840*	0.407
Grain yield/plant (g)	33.604	5.958	92.473*	10.701

(Perrier and Jacquemoud-Collet 2006). Branch robustness was tested using 10000 bootstraps. The software program STRUCTURE (Pritchard *et al.*, 2000) was employed to estimate the number of populations (K) and subgroups within each gene pool. The analysis involved a range of K values from 1 to 10, utilizing the admixture model along with 10,000 burning periods and 10,000 replicates. To determine the optimal K value the ÄK method was implemented with the assistance of the online web-based Structure Harvester tool (Evanno *et al.*, 2005). In addition, an analysis of molecular variance (AMOVA) and principal coordinates analysis (PCoA) was conducted using GenAlEx (version 6.5) in line with

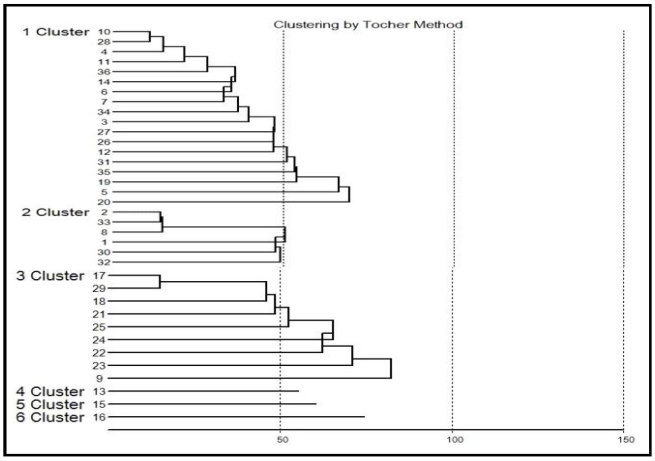

Fig. 1: Dendrogram showing grouping of 36 maize genotypes based on D^2 statistic using Tocher's method.

the protocol established by Peakall and Smouse, (2006). Furthermore, Mantel's test involving 99 permutations was also executed using GenAlEx (version 6.5) to determine the correlation between agro-morphological and SSR distance matrices (Mantel, 1967).

Results

Agro-morphological diversity analysis

The 36 maize genotypes that were evaluated in á-RBD showed significant genetic variation for all yield and related traits indicated by the ANOVA results (Table 1). The mean performance of grain yield/plant (g) which is an important agronomic trait varied from 19.20 to 38.11 g with an average value of 29.14 g.

For all studied traits, the PCV values were higher than their GCV values. For Cob placement (cm), cob length (cm), number of kernels/row and grain yield/plant (g) moderate PCV and GCV (15-30%) were observed. High heritability accompanied by high genetic advance was observed only for cob placement (cm), whereas high heritability accompanied by moderate genetic advance

Table 2: Estimates of genetic parameters of variability for twelve agro-morphological traits among 36 genotypes of maize.

Traits	Mean±S.E (m)	Range	PCV (%)	GCV (%)	Heritability (h^2 bs) (%)	GA (as % of mean)
Days to 50% pollen shed	59.28±0.94	54.00-66.00	5.44	4.69	74.41	8.34
Days to 50% silking	62.47±0.97	57.00-69.33	5.15	4.39	72.92	7.73
Days to 75% brown husk	100.13±0.99	94.00-106.67	3.64	3.21	77.72	5.82
Plant height (cm)	142.43±1.99	101.00-175.00	12.37	12.13	96.16	24.49
Cob placement (cm)	70.72±1.65	34.00-85.00	15.86	15.34	93.51	30.55
Cob length (cm)	13.48±0.72	9.97-18.04	19.47	17.16	77.66	31.16
Cob girth (cm)	11.82±0.49	8.77-15.43	14.20	12.29	74.91	21.91
No. of kernel rows/cob	12.41±0.60	9.67-16.27	14.29	11.57	65.55	19.29
No. of kernels/row	22.58±1.00	15.00-29.00	17.68	15.89	80.91	29.46
100 kernel-weight (g)	21.61±0.81	17.33-26.33	11.73	9.79	69.64	16.83
Shelling (%)	80.26±0.36	77.94-82.94	1.71	1.52	79.06	2.78
Grain yield/plant (g)	29.14±1.91	19.20-38.11	21.27	17.99	71.52	31.34

Table 3: Average intra and inter-clusters values of D^2 among six cluster.

Clusters	I	II	III	IV	V	VI
I	**7.32**	11.53	10.29	10.04	11.76	16.01
II		**7.28**	16.63	12.07	12.25	12.57
III			**8.74**	13.62	13.05	20.65
IV				**0.00**	17.23	9.33
V					**0.00**	21.12
VI						**0.00**

Bold values are intra cluster distance

was observed for plant height (cm) and number of kernels/row. Moderate heritability with high genetic advance was observed for cob length (cm) and grain yield/plant (g) (Table 2).

Mahalanobis D^2 analysis grouped 36 genotypes into six distinct clusters (Fig. 1). Cluster I comprising the maximum number of genotypes (18) followed by Cluster III (9) and Cluster II (6). However, Clusters IV, V and VI each contain a single genotype. The intra-cluster D^2 values (Table 3) ranged from 0.00 to 8.74 with the highest intra-cluster distance (8.74) observed in cluster III. Conversely, inter-cluster distances ranged from 9.33 (between clusters IV and VI) to 21.12 (between clusters V and VI) with clusters V and VI (21.12) having highest inter-cluster distance followed by clusters III and VI (20.65). For cluster mean values (Table 4), Cluster V had maximum cluster mean values for cob length (17.48 cm), cob girth (15.43 cm), no. of kernel rows/cob (15.30), no. of kernels/row (28.33), 100 kernel weight (24.33 g), shelling (82.81%) and grain yield/plant (38.11 g). Additionally, Cluster V showed the desired minimum values for days to 50% pollen shed (56.33), days to 50% silking (59.67) and days to 75% brown husk (97.33). Among the traits studied, plant height contributed the most (54.92%) towards genetic divergence followed by cob placement (13.97%), shelling (%) (6.67%), cob length (6.19%) and days to 75% brown husk (3.97%).

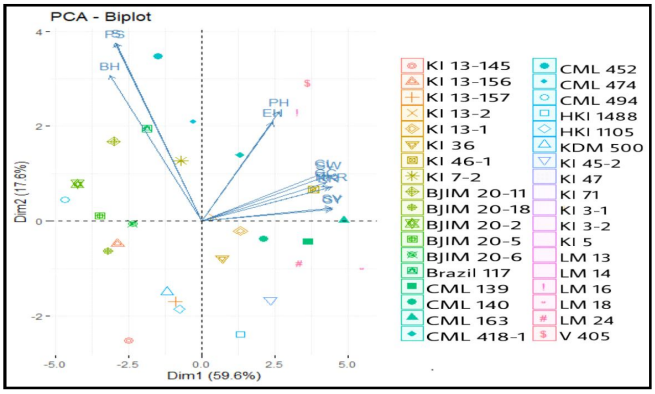

Fig. 2: Biplot of different traits and genotypes on PC1 and PC2.

Principal component analysis identified three significant principal components that collectively accounted for 87.81% of the overall variation (Table 5). The PC1 accounted for 59.64% of the overall variation, primarily attributed to days to 75% brown husk followed by days to 50% pollen shed and days to 50% silking. PC2 and PC3 accounted for 17.61% and 10.56% of total variation, respectively. The loading plot for PC1 and PC2 also emphasized the importance of various agro-morphological traits and genotypes in elucidating the variation among accessions and understanding the relationships between genotypes. (Fig. 2). The scatter plot revealed that most genotypes were unique and occupied different areas in the biplot. It was clearly visible that genotypes KI 13-156, CML 163 and CML 494 which belonged to three different solitary clusters were situated in different quadrants of the biplot and were in agreement with the D^2 clustering pattern.

SSR diversity analysis

A set of 69 SSR maize primers were screened for

Table 4: Cluster means of six clusters for various agro-morphological traits among 36 genotypes of maize.

Trait	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Mean	Max	Min
Days to 50% pollen shed	59.89	57.83	58.63	60.33**	56.33*	64.67	59.61	64.67	56.33
Days to 50% silking	63.07	61.17	61.81	63.00**	59.67*	67.67	62.73	67.67	59.67
Days to 75% brown husk	100.59	99.00	99.22	103.00	97.33*	106.67**	100.97	106.67	97.33
Plant height (cm)	142.96**	117.33	163.06	135.33	140.00	107.33*	134.34	163.06	107.33
Cob placement (cm)	72.62	58.92	79.39	54.67	82.00**	34.00*	63.60	82	34
Cob length (cm)	12.51	13.19	15.30	13.46	17.48**	12.11*	14.01	17.48	12.11
Cob girth (cm)	11.31	11.46	13.00	10.48	15.43**	10.28*	11.99	15.43	10.28
No. of kernel rows/cob	11.89	12.27	13.48	10.76*	15.30**	11.78	12.58	15.3	10.76
No. of kernels/row	21.68	22.01	24.96	19.67	28.33**	17.83*	22.41	28.33	17.83
100 kernel-weight (g)	20.93	21.56	23.44	18.33*	24.33**	18.33*	21.15	24.33	18.33
Shelling (%)	79.87	79.94	81.30	78.98	82.81**	78.70*	80.27	82.81	78.7
Grain yield/plant (g)	27.47	29.11	33.14	21.18*	38.11**	22.43	28.57	38.11	21.18

*Minimum; **Maximum

Table S1: List of inbred lines of maize evaluated under the study (***Supplementary***).

Sr. No.	Inbred line	Source
1	KI 3-1	AICRP, SAREC, Kangra
2	KI 3-2	-do-
3	KI 5	-do-
4	KI 7- 2	-do-
5	KI 13-1	-do-
6	KI 13-2	-do-
7	KI 36	-do-
8	KI 45-2	-do-
9	KI 46-1	-do-
10	KI 47	-do-
11	KI 71	-do-
12	KI 13-145	-do-
13	KI 13-156	-do-
14	KI 13-157	-do-
15	CML 163	ICAR, IIMR, New Delhi
16	CML 494	-do-
17	Brazil 117	-do-
18	CML 140	-do-
19	CML 418-1	-do-
20	CML 452	-do-
21	LM 16	AICRP, PAU, Ludhiana
22	LM 18	-do-
23	LM 24	-do-
24	CML 139	ICAR, IIMR, New Delhi
25	V 405	VPKAS, Almora
26	BJIM 20-2	AICRP, HAREC, Bajaura
27	BJIM 20-5	-do-
28	BJIM 20-6	-do-
29	BJIM 20-11	-do-
30	BJIM 20-18	-do-
31	CML 474	ICAR, IIMR, New Delhi
32	KDM 500	AICRP, SKUAS&T, Srinagar
33	HKI 488	AICRP, CCS HAU, Karnal
34	HKI 1105	-do-
35	LM 13	AICRP, PAU, Ludhiana
36	LM 14	-do-

genetic diversity in 36 maize inbreds. Of these, 25 primers showed polymorphism (Supplementary Table S2, Supplementary Fig. S3) and were subsequently used in the final analysis. The SSR profiles of these genotypes generated 76 observed alleles, ranging from 2 to 5, with an average of 3.04 alleles per primer. The number of effective alleles varied from 1.246 (umc1339) to 3.624 (umc1029) with an average of 2.454 fragments or alleles per primer. The PIC value ranged from 0.183 (umc1339) to 0.680 (umc1029) with an average of 0.439 per primer. The Shannon index ranged from 0.390 to 1.419 with an average value of 0.938. Additionally, expected heterozygosity (He) ranged from 0.201 to 0.735 with a mean value of 0.526.

Table 5: Eigenvectors for the first three components of various agro-morphological traits among 36 genotypes of maize.

Trait	Eigenvectors		
	PC1	PC2	PC3
Eigen Value	7.16	2.11	1.27
Variation (%)	59.64	17.61	10.56
Cumulative (%)	59.64	77.25	87.82
Days to 50% pollen shed	0.23	0.52	0.14
Days to 50% silking	0.22	0.52	0.15
Days to 75% brown husk	0.24	0.43	0.24
Plant height (cm)	-0.19	0.32	-0.56
Cob placement (cm)	-0.18	0.29	-0.62
Cob length (cm)	-0.31	0.14	0.16
Cob girth(cm)	-0.32	0.11	0.13
No. of kernel rows/cob	-0.33	0.09	0.26
No. of kernels/row	-0.34	0.10	0.17
100 kernel-weight (g)	-0.33	0.14	0.12
Shelling (%)	-0.34	0.03	0.16
Grain yield/plant (g)	-0.34	0.04	0.03

A dendogram was created using the UPGMA method in the NTSYS-PC package (version 2.02) based on the polymorphism of SSR markers. The genotypes were grouped into two main clusters (Fig 3), with 10 genotypes in Cluster A and 26 genotypes in Cluster B. Cluster A was further divided into A1 (8 genotypes) and A2 (2 genotypes), whereas Cluster B was subdivided into four sub-clusters: B1 (6 genotypes), B2 (5 genotypes), B3 (4

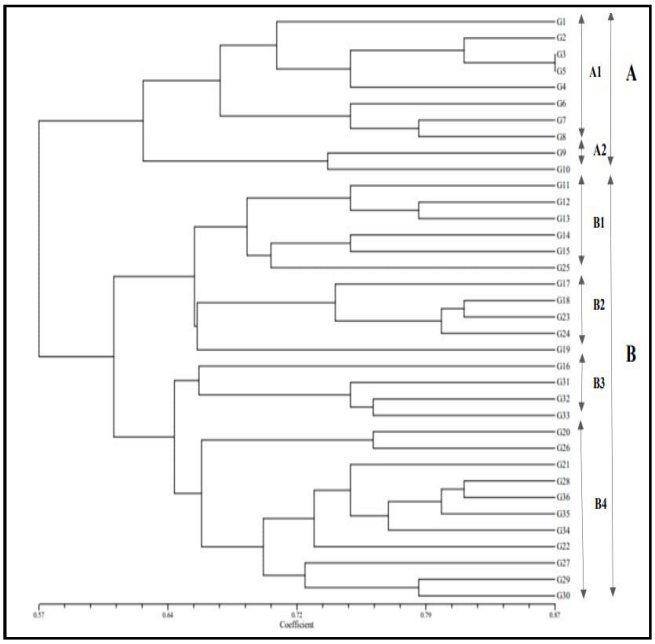

Fig. 3: Dendrogram created by NTSYS-PC (version 2.02) utilizing the UPGMA method to determine genetic relationships among 36 maize genotypes.

Table S2: Polymorphic and scorable SSR bands along with their fragment size produced by 25 primers (***Supplementary***).

S. No.	Primers	No. of observed alleles	No. of effective alleles	Expected heterozygosity	PIC Value	Shannon Index (I)	Fragment Size (bp)
1.	bnlg2191	3	1.839	0.464	0.395	0.774	150-300
2.	bnlg1808	3	2.078	0.526	0.439	0.852	120-190
3.	umc1710	3	2.370	0.587	0.486	0.934	160-200
4.	umc1456	3	2.922	0.669	0.584	1.085	110-150
5.	umc2197	4	2.688	0.638	0.574	1.150	170-210
6.	umc1339	3	1.246	0.201	0.183	0.390	150-220
7.	umc1812	3	2.943	0.668	0.583	1.089	130-180
8.	umc1060	3	2.710	0.639	0.557	1.048	110-150
9.	umc2038	3	2.059	0.522	0.444	0.860	120-250
10.	umc1690	2	1.993	0.506	0.374	0.691	160-200
11.	umc1808	3	2.847	0.659	0.574	1.070	250-290
12.	umc1029	5	3.624	0.735	0.680	1.419	110-210
13.	umc1136	4	3.408	0.717	0.651	1.287	130-170
14.	umc2139	2	1.753	0.437	0.337	0.621	140-180
15.	umc2226	3	2.583	0.622	0.532	1.007	130-190
16.	umc2358	3	2.814	0.655	0.569	1.063	120-170
17.	umc1178	3	1.342	0.260	0.240	0.509	150-200
18.	umc2334	3	2.390	0.602	0.508	0.947	120-170
19.	phi087	3	2.941	0.671	0.586	1.089	130-190
20.	umc1353	4	3.556	0.730	0.667	1.321	250-350
21.	umc1761	2	1.985	0.504	0.373	0.689	190-210
22.	umc2373	3	2.626	0.629	0.539	1.018	120-190
23.	phi034	3	2.454	0.601	0.505	0.965	130-190
24.	bnlg657	3	2.332	0.580	0.482	0.927	290-330
25.	umc2189	2	1.849	0.468	0.354	0.652	130-170
	Mean	3.04	2.454	0.526	0.439	0.938	

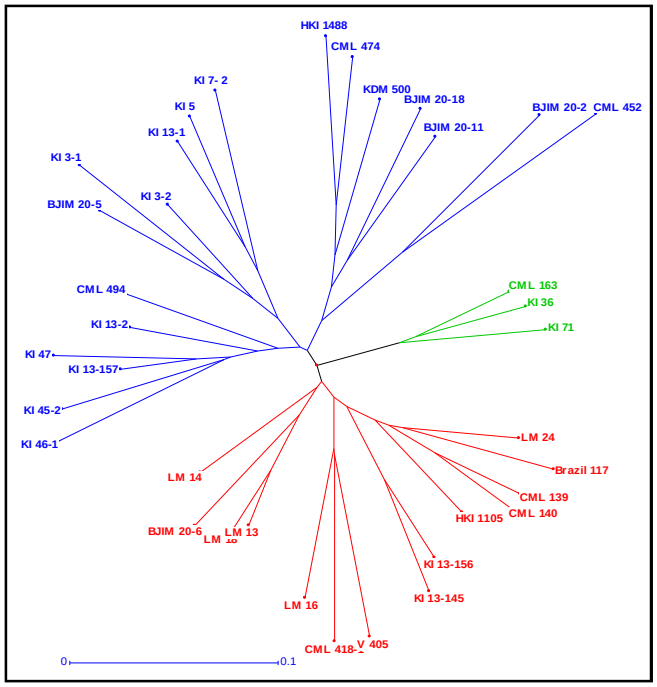

Fig. 4: Neighbor-joining tree of 36 maize genotypes created by DARwin software using SSR markers.

genotypes) and B4 (11 genotypes). Genetic similarity coefficients varied from 0.57 to 0.87 with an average of 0.72 indicating significant genetic diversity.

Genetic identity was further verified using the DARwin software (v.6.0.21) for a neighbor-joining tree. Three major groups were identified in the cluster tree (Fig 4). Cluster I was further divided into IA (12 genotypes) and IB (7 genotypes). Cluster II contained 3 genotypes, and Cluster III was further divided into IIIA (10 genotypes) and IIIB (4 genotypes). Molecular analysis

Table 6: Analysis of Molecular Variance of 36 maize genotypes using SSR markers.

Marker type	Source	df	SS	MSS	Vari-ation	% of total vari-ation
SSR data	Among population	5	132.98	26.59	2.59	17
	Within population	30	374.87	12.49	12.49	83
	Total	35	507.86		15.09	100

Table 7: Percentage of variation explained by PCoA among 36 maize genotypes using SSR markers.

Axis	1	2	3
Variation (%)	15.58	10.53	7.92
Cumulative (%)	15.58	26.11	34.03

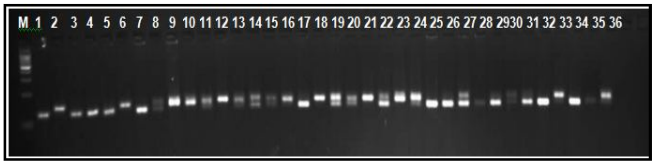

Fig. S3a: SSR profiles of 36 maize genotypes using primers phi034 and lane M 100 bp ladder. (***Supplementary***)

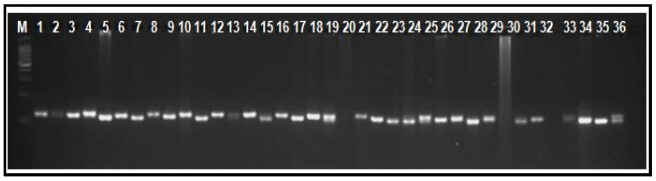

Fig. S3b: SSR profiles of 36 maize genotypes using primer umc1060 and lane M 100 bp ladder. (**Supplementary**)

of variance (AMOVA) revealed that 83% of the genetic variation was partitioned between individuals within populations, whereas 17% was partitioned among populations (Table 6). PCoA confirmed the genetic relationships among the 36 maize genotypes, classifying them into six populations (Fig. 5). The first three axes of PCoA explained 34.03% of the overall variation with PC axis 1, 2 and 3 explaining 15.58%, 10.53% and 7.92% respectively (Table 7). Little introgression was observed between the gene pools in dimensions 1 and 2.

PCA and UPGMA clustering of the 36 maize genotypes were validated using STRUCTURE analysis.

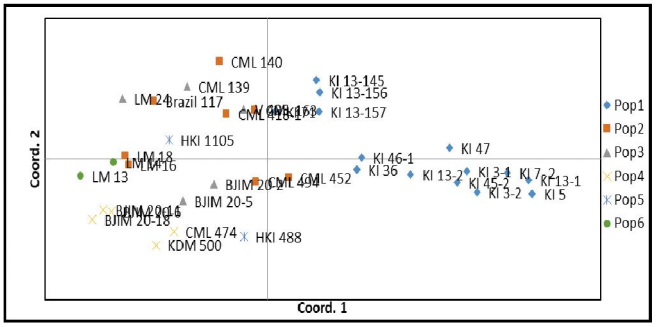

Fig. 5: Principal Coordinate Analysis (PCoA) using twenty-five SSR Markers among 36 genotypes of maize.

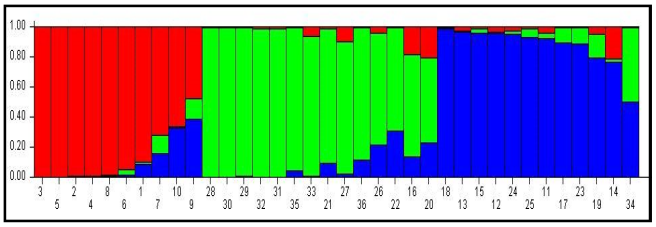

Fig. 6: Gene pool introgression based on the population structure analysis at k=3 using SSR marker.

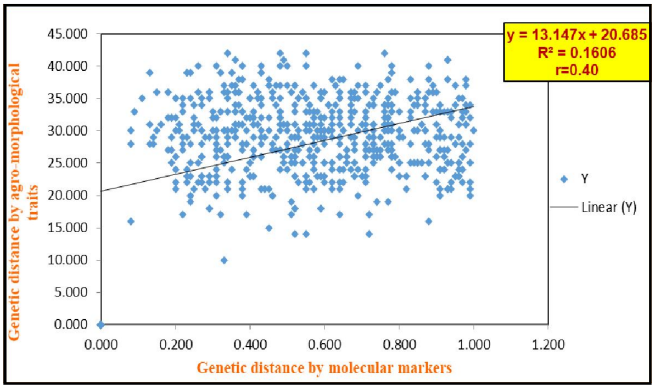

Fig. 7: Mantel test to determine correlation between morphological and molecular distance matrices.

Evano test showed that the optimal number of inferred clusters was at K = 3, revealing that the genotypes could be classified into three populations, P1, P2 and P3 (Fig. 6) with 10, 14 and 12 genotypes, respectively. Membership in P1 revealed a maximum of 100% and a minimum of 26%, P2 revealed a maximum of 100% and a minimum of 37% and P3 revealed a maximum of 100% and a minimum of 35% indicating the genotype distribution within each population and introgression. This provided a more effective grouping than a dendrogram.

Comparison of agro-morphological and SSR diversity

Thirteen genotypes viz., KI 3-1, KI 3-2, KI 45-2, LM 24, Brazil 117, CML 139, CML 140, CML 163, KDM 500, HKI 488, LM 13, LM 14 and BJIM 20-6 were observed to common showing the parallelism between morphological and SSR data. Furthermore, a significant moderate positive correlation of 0.4007 (Fig. 7) was revealed by Mantel test between the morphological and SSR datasets indicated phenotype can be attributed to genetic factors upto a certain extent but the effect of the environment cannot be ignored.

Discussion

Maize is of immense agricultural importance and plays a vital role in food security and in several industries. The increasing demand for high-yield and adaptable crop varieties highlights the need to exploit their genetic diversity.

Our study showed a wide range of genetic variability and scope for the selection of superior genotypes for various agro-morphological traits, which is consistent with previous studies conducted byRajwade *et al.*, (2017); Kandel *et al.*, (2018); Wali, (2019); Rai *et al.*, (2021); Pradhan *et al.*, (2022); Yadav *et al.*, (2023). The PCV estimate was higher than their GCV values for all studied traits. Therefore, relying solely on phenotypes for the selection of these traits may not be reliable as

environmental variation is unpredictable. Similar studies were observed by Jilo *et al.,* (2018); Belay (2018); Bisen *et al.,* (2018); Bartaula *et al.,* (2019); Wedwessen and Zeleke, (2020); Pranay *et al.,* (2022) for various traits. The high heritability and high genetic advance observed for cob placement (cm) suggest that additive gene action is the predominant factor influencing the inheritance of this trait. However, moderate heritability with high genetic advance indicates that both additive and non-additive gene action are present providing opportunities for improving this trait through hybridization and selection.

Using Tocher's method, the Mahalanobis D^2 statistic categorized the 36 genotypes into six distinct clusters. These clusters revealed the presence of divergent genotypes within different clusters. Different clustering patterns have also been reported earlier by (Maruthiand Rani, 2015) (six clusters), (Rafique *et al.,* 2018) (ten clusters), (Chandel and Guleria 2019) (nine clusters), (Suman *et al.,* 2020) (three clusters), (Rashmi *et al.,* 2021) (seven clusters) and (Patel *et al.,* 2023) (twenty-two clusters). Cluster III exhibited the highest intra-cluster distance (8.74) signifies the presence of genetic diversity within the inbreds indicated selection of parents within the cluster would be effective. The largest inter-cluster distance (21.12) was observed between clusters V and VI, suggesting wide genetic diversity between these clusters. Therefore, crosses between the genotypes of these clusters would be desirable for producing heterotic hybrids and likely to yield transgressive segregants as suggested by Falconer, (1996). Earlier studies by Chandel and Guleria, (2019) and Rashmi *et al.,* (2021) also reported sufficient genetic diversity as revealed by a wide range of D^2 values. The cluster mean values indicate the overall performance of the genotypes in each group suggesting the selection of genotypes from clusters V and VI for the improvement of the respective traits. Based on mean values, a wide range of variation in various agro-morphological traits was previously observed by Sood and Lata, (2020). Earlier, Chandel and Guleria, (2019) observed that plant height and 1000-grain weight contributed the most to genetic divergence. Rashmi *et al.,* (2021) reported that grain yield/plant contributed the most towards genetic divergence followed by cob girth and cob length. Therefore, these traits are effective in selecting parents to generate variations in the population.

Principal component analysis (PCA) identifies important traits that contribute maximum to yield so that they can be considered during hybridization programs. The first three significant principal components explained 87.81% of the overall variation with days to 75% brown husk having the highest positive value followed by days to 50% pollen shed and days to 50% silking. Hence, these traits were identified as important contributors to genetic divergence, corroborating the studies of Avinash and Mishra, (2016); Pandit *et al.,* (2016); Shrestha, (2016); Sinana *et al.,* (2023).

The D^2 statistic which is commonly used to identify divergent genotypes based on morphometric traits can vary across environments and locations resulting in inconsistent clustering of some genotypes. However, molecular markers such as SSRs provide more precise information on genetic diversity. SSR analysis of 36 maize genotypes revealed 76 observed alleles, with no. of effective alleles ranging from 1.246 (umc1339) to 3.624 (umc1029). Similar results were previously reported by Awasthi *et al.,* (2021) and observed 61 fragments with an average of 3.28 polymorphic fragments per primer. The highest PIC value was obtained for umc1029 (0.680), suggesting that it was considered to be the most appropriate marker. The average PIC value was comparable to the study of Neelothpala *et al.,* (2022) who recorded an average PIC of 0.345 using 50 polymorphic SSR markers. Adhikari *et al.,* (2022) also revealed a wide range of PIC values (0.29 to 0.86) indicating significant allelic variation and distribution in the population.

The UPGMA algorithm in NTSYS-pc software grouped the genotypes into two distinct clusters, reflecting the diversity between populations. Similar results using SSR markers in maize germplasms were reported by Awasthi *et al.,* (2021); Chaudhary *et al.,* (2018); Rana *et al.,* (2020); Abdulazeez *et al.,* (2021). To further validate the genetic identity, DARwin software was used to construct a neighbor-joining tree, revealing three main groups. The discrepancies between the results obtained from NTSYS-pc v.2.02 and DARwin v.6.0 may be attributed to differences in the clustering methods, including the computation of similarity and dissimilarity coefficient.

AMOVA analysis based on molecular markers revealed that 83% of the allelic diversity was within populations with only 17% among populations. Abebe *et al.,* (2020) reported a higher level of genetic variability (77%) among the inbred lines indicated that microsatellite markers used were effective in differentiating inbred lines. Mathiang *et al.,* (2022) also reported 93.0% of the variation within populations and 7.0% among populations. PCoA confirmed genetic relationships among 36 maize genotypes, with the first three axes explaining 34.03% of the total variation. Sathua *et al.,* (2018) also analyzed a set of 25 maize inbreds, with the first three axes of PCoA

explaining 38.07% of the total variation. The clustering of maize genotypes through PCoA and UPGMA was validated using STRUCTURE analysis which divided the population into three main groups.

To determine the degree of similarity between the two, it is essential to equate molecular characterization with morphological markers. This combined approach not only allows for a more comprehensive understanding of the genetic variation among different genotypes but also enables the identification of valuable genetic resources for use in hybridization programs aimed at improving desirable traits. Thirteen genotypes viz., KI 3-1, KI 3-2, KI 45-2, LM 24, Brazil 117, CML 139, CML 140, CML 163, KDM 500, HKI 488, LM 13, LM 14 and BJIM 20-6 were observed common showing the parallelism between morphological and SSR data. Furthermore, Mantel test revealed a significant positive moderate correlation of 0.4007 between the morphological and SSR-based distance matrices. Joshi *et al.*, (2021) also observed a significant moderate positive correlation of 0.499 among maize germplasm suggesting that they both accurately reflect the same pattern of genetic diversity. Thus, both data sets can be used simultaneously to effectively utilize the genetic diversity.

Conclusion

In this study, we combined agro-morphological and molecular markers to select diverse parental lines. The results demonstrated significant variation in economically important agro-morphological traits among the maize accessions. Furthermore, SSR markers used for molecular characterization revealed substantial genetic diversity at the molecular level. Among the genotypes evaluated, eight *viz.*, KI 46-1, CML 163, LM 16, LM 18, CML 140, KI 3-2, KI 45-2 and LM 24 were identified as potential genotypes based on study and can be utilized for the development of high yielding single cross maize hybrids under ecology of North Western Himalaya.

Statement and Declaration

Competing interests: The authors confirm that there are no financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

Authors contribution: UC and KM conducted the experiment, collected the data and developed the methodology. UC and NK carried out validation, while KM and NK performed formal statistical analysis. Resources were provided by UC and data curation was handled by KM, SG and IG. The original draft of the manuscript was prepared by KM and underwent review and editing by UC, IG and SG. The final draft was written by KM and all authors commented on previous versions of the manuscript. All authors have read and agreed to the published version of the manuscript.

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