Assessment of Genetic Diversity and Marker-Trait Associations Using Selected Early Maize Inbreds Derived From Local Landraces of Eastern Himalayan Regions

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Abstract

North- Eastern parts of India fall under Eastern Himalayan region and it is a diversity hotspot of many crops including maize. Evaluation of genetic diversity is required to tap the potentiality of genetic resources in any crop improvement programmes. In the present study, genetic diversity at fifty two microsatellite markers were conducted in 30 early maize inbreds developed from local landraces of NE India. Genetic diversity analysis revealed a total of 189 alleles with a mean of 3.63 alleles/ locus. The allele size ranged from 50 bp (*phi 036*) to 295 bp (*p 101049*) which revealed a high level of genetic diversity among the loci. The PIC among the 30 genotypes ranged from 0.17 (*umc 1622*) to 0.76 (*umc 1153*) with an average value of 0.49. The value of Expected Heterozygosity (H_{Exp}) ranged from 0.19 to 0.80 with an average of 0.57, whereas the Observed Heterozygosity (H_{Obs}) ranged from 0 to 0.89 with a mean of 0.14. The genetic dissimilarity between the genotype pairs ranged from 0.40 to 0.64 with a mean value of 0.57. Cluster analysis grouped the 30 inbreds into distinct three sub-clusters. Similarly, population structure and principal coordinate analysis) analysis also classified the 30 inbred lines into three-subpopulations. AMOVA revealed that 6% of total variance is due to differences among populations, while 94% of total molecular variance is accounted by within populations. Marker-trait associations showed a total of twelve SSR markers significantly associated with seven agronomic traits. From the present finding, these results show that the thirty maize inbreds have high genetic diversity which would be useful for choosing promising parents and for making cross combination based on genetic distance and clustering for genetic improvement programmes of maize.

Introduction

Maize (*Zea mays* L.) often referred to as “Queen of Cereals” due to its remarkable yield potential and economic value is the world’s third most grown cereal crop. It assumes global significance because of its versatility in utilization such as human food, livestock feed and as raw materials for several agro-based industries (Choudhary et al. 2015). Eastern Himalayan region mainly North- Eastern region of India is a diversity hub for many crops including maize. There are diverse maize germplasm found in this region. Maize in this region acquired different unique characters like coloured grain having different range from purple, blue, black, white, cream, yellow, orange, red and variegated; multi- cob bearing (3–6 cobs per plant) and waxiness having high amylopectin content. In addition to the above traits, this local maize has high potential as fodder due to its vigorous growth and tillering habit which makes them unique. Local maize has tremendous morphologically variability such as plant height, plant type, duration, kernel colour, texture, nutrient composition, appearance, etc. (Ratankumar et al. 2019). The plant type of local landraces ranged from wild primitive type to advanced landraces/ varieties maintained and selected by farmers from time immemorial. The weather conditions in North Eastern region (NER) of India are erratic; drought and floods are common phenomenon in the region. The local germplasm were evolved in balance with the prevalent agro-ecological conditions of the region and, therefore, they are expected to possess better adaptation to regional stresses.

Availability of wide genetic variability is a pre-requisite for undertaking a successful crop improvement programme. For undertaking breeding program of crop plants, characterization of genotypes, assessment of molecular diversity and studies of genetic relationship is a must (Bharadwaj 2018). Indigenous germplasm have been given preferential for the development of mapping populations, heterotic pools and inbreds (Yadav et al. 2015). Such inbreds can be used as parental lines in development of hybrids, which has potentially enhanced yield than those of local landraces. Characterization of inbreds and their information on genetic diversity and genotype relationships are of utmost importance in any crop improvement programme (Sserumaga et al. 2014; Zunjare et al. 2015; Devi et al. 2017b). Assessment of genetic diversity and inclusion of diverse parents in the breeding programme enhances the chance of achieving more heterotic F_1 s and in producing more variable segregants (Devi et al. 2015a). Further, genetic improvement of local germplasm and its utilization in various breeding programmes will be better option as compare to exotic lines due to its wide adaptability to the local ecologies. Therefore, the present study was undertaken to develop inbred lines from local germplasm and to assess genetic diversity, relationships and population structure of the early inbreds developed using SSR markers.

Materials And Methods

Plant materials

A set of 30 indigenous 3rd generation early inbred lines of maize were selected for molecular characterization and assessing the genetic diversity. These early lines were developed at ICAR Research Complex for North Eastern Hill Region, Manipur Centre using local germplasm collected from different places of Manipur and Mizoram states of India.

Agronomic performance of inbreds

Each of the 30 maize inbreds was grown in two rows of 3 m length each during *kharif* 2019 at ICAR-RC-NEH Region, Manipur Centre Research Farm under standard package of practices for maize cultivation. The row to row and plant to plant distance was maintained at 60 cm and 20 cm, respectively. The individual plants were carefully selfed and harvested to avoid contamination of inbreds. These early inbreds were evaluated for plant height (PH), ear height (EH), number of kernel rows per ear (KRPE), number of kernels per row (KPR), cob length (CL), cob diameter (CD) and 100 kernel weight (KW).

Genomic DNA isolation and PCR amplification

Genomic DNA was extracted from the leaf of the selected genotypes using modified CTAB extraction protocol (Porebski et al. 1997). DNA concentration was measured on Genova Nano Analyzer UV/VIS (Mumbai, India). Final concentration of extracted genomic DNA was made to 10 ng/ μ L and used for the PCR amplification. A set of 52 Simple Sequence Repeats' (SSR) markers covering the whole genome were used for the present study and their primer sequences were collected from the maize genomic database (<http://www.maizegdb.org/>). The oligonucleotide primers were synthesized in purified and lyophilized form from GCC Biotech India Pvt Ltd. (Kolkata, India).

Polymerase chain reactions were carried out on 96-well thermal cycler, Eppendorf- Mastercycler Nexus Gradient (Hamburg, Germany). All PCRs were performed in a final reaction volume of 20 μ L with the following reagent components: 2 μ L of template DNA (20 ng/ μ L), 10 μ L 2X Hi-Primer Taq PCR Master Mix (GCC Biotech India Pvt Ltd., India), 1 μ L of each forward and reverse primers and 6 μ L RNA grade nuclease free water (GCC Biotech India Pvt Ltd., India). The amplification conditions were set as follows: initial denaturation at 95°C for 5 min, 4 cycles consisting of denaturation at 95°C for 45 sec, primer annealing ranged between 57 to 60°C for 45 sec, primer extension at 72°C for 45 sec and then 35 cycles consisting of denaturation at 95°C for 45 sec, primer annealing 60°C for 45 sec, primer extension at 72°C for 45 sec and final extension at 72°C for 8 min. The amplified PCR products were resolved using 3.0% ISSR Agarose (GCC Biotech India Pvt Ltd., India) stained with Ethidium Bromide, documented under gel doc system (Gel DocTM XR+, Biorad, India).

Statistical analyses

The genotypic data of 52 SSR markers were used for estimation of genetic diversity parameters such as number of alleles per locus (K), Observed Heterozygosity (H_{Obs}), Expected Heterozygosity (H_{Exp}) and Polymorphic Information Content (PIC) using the Cervus 3.0 program (Field Genetics Ltd., London, England). GenAEx 6.502 software (Peakall and Smouse 2012) was used to compute the PCoA (Principal Coordinate Analysis) by plotting the eigenvector values in a scatter graph taking the first principal component and the second principal component as the axes. A hierarchical analysis of molecular variance (AMOVA) of 30 genotypes between the populations was also conducted using GenAEx 6.502 software (Peakall and Smouse 2012). To determine the presence of the genetic structure, STRUCTURE version 2.3.4 (Pritchard et al. 2000) was run with the number of clusters from K = 1 to K = 5, with 5 independent replications per K using the admixture model and correlated allele frequencies, a 50,000 burn-in period and 50,000 MCMC to determine approximations of posterior distributions. The optimum K value was determined through the STRUCTURE HARVESTER programme (Earl and Vonholdt, 2012) by estimating the peak value of ΔK using Evanno et al. (2005) method. The STRUCTURE analyses give a Q-matrix (QST) for each value of K, which lists the estimated membership coefficients for each genotype in each subgroup. An individual having more than 70% of its genome fraction value under a particular K subgroup was assigned to that subgroup. The allele size was evaluated by comparing with 100 bp DNA ladder (GCC Biotech India Pvt Ltd., India) and a phylogenetic tree was constructed using Neighbour-Joining method implemented in DARwin-6.0 (Perrier and Jacquemoud-Collet (2006)).

All the statistical analysis of agronomic data was carried out using XLSTAT v2020.4.1 software (www.xlstat.com). The association analysis of seven agronomic traits along with the 52 SSR markers was calculated using GGT 2.0 software (Van Berloo 2008). The association analysis was calculated based on the squared correlation coefficients between the marker data and trait values (R^2 values) and the associated probabilities of the correlation values. The threshold to declare a significant marker-trait associations was set to $-\log_{10}(p) = 3$.

Results And Discussion

Genetic diversity of maize inbreds

In the present study, all the 52 markers were successfully amplified in 30 maize inbred lines (Fig. 1). A total of 189 alleles were generated across the 30 genotypes with a mean of 3.63 alleles per locus (Table 1). The allele size of the 52 SSR markers ranged from 50 bp (*phi 036*) to 295 bp (*p 101049*) which revealed a high level of genetic diversity among the loci. Several researchers, viz., Hung et al. (2012) [117 alleles, 3.26 average alleles/ locus], Yu et al. (2012) [60 alleles, 2.73 average alleles/ locus], Devi et al. (2017b) [203 alleles, 2.69 average alleles/ locus] observed similar result while working on genetic diversity of maize genotypes. The high number of alleles per locus is most likely attributable to the higher genetic diversity of the lines. The PIC of the markers refers to the relative value of each marker regarding the amount of polymorphism exhibited, ranged from 0.17 (*umc 1622*) to 0.76 (*umc 1153*) with an average value of 0.49 demonstrating the high discriminatory power of these markers among the 30 genotypes (Table 1). Out of the 52 SSR markers, 24 SSR loci (46.15%) were found to be more informative by having of PIC value of ≥ 0.50 , depicting the higher capability of these loci to differentiate between the genotypes, determine genetic diversity, gene introgression, genetic conservation, etc. Genetically diverse genotypes will have higher value of PIC, whereas closely related lines will have lower value (Muthusamy et al. 2015; Devi et al. 2017b). The PIC value of 0.46, 0.31 and 0.40 was also observed by Hung et al. (2012), Hao et al. (2015) and Devi et al. (2017b), respectively. The number of alleles per locus (K) ranged from 2 to 8 with an average of 3.634 ± 1.428 . The *bnlg1031* marker mapped at 8.06 bin showed the highest number of alleles (8). The value of Expected Heterozygosity (H_{Exp}) ranged from 0.19 to 0.80, whereas the Observed Heterozygosity (H_{Obs}) ranged from 0 to 0.89, indicating that further generations of inbreeding are required to stabilize the inbreds. The presence of high heterozygosity in some of the loci like *umc 1354* (0.89), *bnlg 2162* (0.79), *bnlg 1031* (0.73), *umc 2064* (0.57), *bnlg 565* (0.39), *bnlg 2291* (0.34), *umc 2252* (0.33), *bnlg 1496* (0.29), *bnlg 1601* (0.27), etc. indicated the needs for further inbreeding in order to stabilise the lines. Hung et al. (2012) also reported high heterozygosity in his study among waxy maize inbreds.

Table 1
Genetic diversity analysis for 52 SSR markers in 30 genotypes

SI No.	Locus	Bin location	K	H _{Obs}	H _{Exp}	PIC
1	umc 1354	1.00	6	0.89	0.71	0.66
2	umc 1685	1.01	2	0.03	0.50	0.37
3	umc 2225	1.02	4	0.07	0.59	0.50
4	bnlg 2238	1.04	5	0.07	0.66	0.59
5	umc 2064	1.07	4	0.57	0.75	0.69
6	umc 1622	1.12	3	0	0.19	0.17
7	bnlg 1064	2.03	3	0.2	0.56	0.47
8	umc 2007	2.04	5	0.03	0.68	0.61
9	umc 2252	2.05	3	0.33	0.44	0.37
10	bnlg 2077	2.07	4	0.15	0.75	0.69
11	umc 2085	2.08	2	0	0.51	0.37
12	P 101049	2.10	5	0.07	0.68	0.61
13	phi 036	3.04	5	0.21	0.74	0.67
14	bnlg 1601	3.05	4	0.27	0.72	0.66
15	umc 2050	3.07	2	0.14	0.46	0.35
16	umc2174	3.08	2	0.05	0.34	0.28
17	bnlg 1496	3.09	6	0.29	0.74	0.68
18	umc 1008	4.00	2	0	0.51	0.37
19	umc 2410	4.02	3	0.03	0.62	0.54
20	umc 1117	4.04	3	0	0.67	0.58
21	bnlg 2291	4.06	3	0.34	0.52	0.45
22	bnlg2162	4.08	5	0.79	0.77	0.71
23	umc 2289	4.10	5	0.13	0.64	0.56
24	umc 1305	5.00	4	0.07	0.35	0.32
25	bnlg 565	5.02	5	0.39	0.76	0.71
26	umc 2304	5.05	4	0.18	0.47	0.41

SI No.	Locus	Bin location	K	H _{Obs}	H _{Exp}	PIC
27	umc 1225	5.08	5	0.1	0.79	0.74
28	umc 1686	5.03	2	0	0.50	0.37
29	umc 1153	5.09	6	0	0.80	0.76
30	umc 1002	6.00	2	0	0.49	0.36
31	umc 2318	6.05	2	0	0.44	0.34
32	umc 1257	6.02	3	0	0.55	0.45
33	umc 1912	6.06	5	0	0.70	0.64
34	bnlg 1740	6.07	5	0.22	0.63	0.58
35	umc 1642	7.00	4	0	0.61	0.51
36	umc 2325	7.01	2	0	0.48	0.36
37	mmc 0411	7.03	3	0.22	0.56	0.45
38	umc 1154	7.05	2	0.04	0.40	0.31
39	umc 1543	7.04	2	0	0.34	0.27
40	umc 1932	7.02	2	0	0.46	0.35
41	umc 2042	8.01	2	0	0.50	0.37
42	bnlg 1194	8.02	4	0.36	0.72	0.65
43	bnlg 1031	8.06	8	0.30	0.73	0.68
44	umc 1607	8.07	2	0	0.41	0.32
45	bnlg 1012	9.04	3	0	0.58	0.49
46	bnlg 1091	9.05	4	0.12	0.58	0.49
47	umc 1380	10.00	3	0.13	0.56	0.46
48	umc 1576	10.02	2	0	0.47	0.36
49	umc 1962	10.03	5	0.23	0.44	0.40
50	umc 1506	10.05	4	0	0.62	0.54
51	bnlg2190	10.06	5	0.07	0.65	0.58
52	umc 2021	10.07	3	0.17	0.55	0.46

Sl No.	Locus	Bin location	K	H _{Obs}	H _{Exp}	PIC
Mean ± SD			3.634 ± 1.428	0.139 ± 0.193	0.574 ± 0.137	0.493 ± 0.146

The heterozygosity percentage of the inbred lines at 52 SSR markers was studied which revealed the range of heterozygosity from 0 (SCM 15/9LC) to 27.45 (GP H 6) (Table 2). The graphical representations of heterozygosity in 30 maize inbreds are depicted in Fig. 2. Out of 30 inbred lines, 13 lines showed heterozygosity $\leq 10\%$, which means 43% of the lines attained more than 90% homozygosity. Another 3 inbred lines *viz.* GP (H) 38, MN 1 and SCM15/9LC showed heterozygosity less than 5%, which revealed a high level of homozygosity in these lines. These three inbred maize lines may be considered as pure lines which will be useful for development of mapping populations, genetic mapping, etc. Analysis of molecular variance (AMOVA) is an efficient way to assess overall distribution of diversity within and among populations (Belalia et al. 2019). In the present study, the AMOVA result revealed a higher molecular variance within individuals of population than among individuals of population. Similar findings were reported by Prasanna (2012), Silva et al. (2015) and Belalia et al. (2019).

Table 2
Population structure group and heterozygosity (%) of 30 early inbred lines

Sl No.	Genotypes	Heterozygosity (%)	Q1	Q2	Q3	Structure groups
1	MZ 44 (c)	14.28	0.006 0	0.042 0	0.952	G3
2	MZ 13(a)	8.0	0.144	0.011	0.845	G3
3	MZ 34 (a)	13.04	0.196	0.006	0.798	G3
4	GP(H) 1	14.58	0.024	0.017	0.959	G3
5	GP(H) 6	27.45	0.112	0.036	0.852	G3
6	MZ 45 (A)	10.20	0.947	0.018	0.035	G1
7	MZ 21 (a)	8.51	0.077	0.009	0.914	G3
8	MN 17	8.69	0.609	0.344	0.046	AD
9	GP(H)-2	5.12	0.036	0.009	0.956	G3
10	MZ 41 (A)	6.60	0.986	0.006	0.008	G1
11	MZ 46 (B)	16.67	0.905	0.011	0.084	G1
12	GP (H)38	4.25	0.988	0.004	0.008	G1
13	Kolasib1(2)	20.41	0.961	0.029	0.01	G1
14	MN 30	6.12	0.022	0.003	0.975	G3
15	MN 12	6.52	0.988	0.008	0.004	G1
16	MZ 42	8.33	0.956	0.007	0.037	G1
17	MZ 40	14.00	0.956	0.006	0.038	G1
18	MZ 70	20.41	0.983	0.004	0.013	G1
19	MZ 24	10.41	0.965	0.028	0.007	G1
20	MZ 2	11.53	0.991	0.005	0.004	G1
21	MN 29	10.0	0.005	0.902	0.092	G2
22	MN 1	3.92	0.005	0.992	0.003	G2
23	SCM 15/9LC	0	0.012	0.948	0.039	G2
24	MZ 56 (a)	6.38	0.022	0.972	0.006	G2
25	MZ 48	14.89	0.006	0.986	0.007	G2
26	MZ 29	8.33	0.003	0.017	0.981	G3
AD: Admixture						

Sl No.	Genotypes	Heterozygosity (%)	Q1	Q2	Q3	Structure groups
27	MN 13	12.76	0.01	0.951	0.038	G2
28	MN 3	14.0	0.003	0.994	0.003	G2
29	GP(H) 28	7.84	0.008	0.931	0.061	G2
30	MZ 22	6.38	0.207	0.759	0.034	G2
AD: Admixture						

Genetic Relationships Analysis Among Early Maize Inbreds

The genotypic data of 52 SSR markers were used to estimate genetic distance matrix and a corresponding dendrogram was developed. Cluster analysis of 30 early maize inbred lines developed from local landraces was conducted based on genetic dissimilarities from SSR data using neighbour-joining method. The genetic dissimilarity between the genotype pairs ranged from 0.40 to 0.64 with a mean value of 0.57. Cluster diagram grouped the 30 maize genotypes into three distinct major clusters *viz.*, A, B and C (Fig. 3). Cluster A comprised of 3 genotypes *viz.*, GP(H) 2, GP(H) 28 and SCM15/9LC and was further sub divided into 2 sub- groups (A1; GP(H) 2 and A2; GP(H) 28 and SCM15/9LC). Another cluster, B holds the highest number of genotypes *i.e.* 24 with three sub- groups *viz.* B1, B2 and B3. B1, B2 and B3 comprised of 13, 3 and 8 genotypes, respectively. There were only 3 genotypes under cluster C which comprised of inbreds MZ 21(A), MN 30 and MZ 29.

To established genetic relationships among the 30 maize inbred lines based on 52 SSR markers, the PCoA (Principal Coordinate Analysis) using GenAlEx 6.502 software was constructed. The genotypic PCoA revealed that the lines were distributed in all the four quadrangles, showing high degree of genetic variability. A scatter plot generated from the PCoA analysis showed that the first two components accounted for 9.82% and 8.23% of the genetic variation which resulted in a total genetic variation of 18.05% (Fig. 4). Similar works on genetic relationship analysis in maize were also reported by Choudhary et al. 2015, Muthusamy et al. 2015 and Devi et al. 2017b.

Population Structure Analysis And Allelic Patterns Across Populations

The genotypic data of 52 SSR markers were also used to determine the population structure in 30 maize inbred lines using STRUCTURE software. Using STRUCTURE HARVESTER program, the peak plateau of ad hoc measure ΔK was detected at $K = 3$, which indicated that the entire population can be grouped into three subgroups (Fig. 5).

Population structures of 30 genotypes based on 52 SSR markers are shown in Fig. 5. Different colour within group indicates the proportion of shared ancestry with other group which has the same colour with the admixture. The population structure group of 30 genotypes based on inferred ancestry values were also estimated (Table 2). The percentage of individuals in each population belonging to each group were estimated based on Q-matrix (QST) for each value of K and the cut off probability for assigning to a

group was assumed to be 70%. A set of 29 genotypes out of 30 showed the estimated membership coefficients of more than 70% of its genome fraction value and were therefore assigned to a specific group. Whereas, only one genotype was classified as admixture since it showed the estimated membership coefficients of less than 70%. This revealed that almost all the genotypes under study are not admixture. In the present study, only one inbred was under the category of admixture, indicating that it is of mixed pedigree, possibly because of introgression of gene(s) while developing the inbred line (Yin et al., 2019). Group 1 consisted of 11 genotypes out of which 9 (MZ 45 (A), MZ 41 (A), MZ 46 (B), Kolasib 1 (2), MZ 42, MZ 40, MZ 70, MZ 24 and MZ 2) genotypes have originated from Mizoram and only 2 (GP(H) 38 and MN 12) genotypes from Manipur. Group 2 gathered 9 genotypes out of which 6 genotypes (MN 29, MN 1, SCM15/9LC, MN 13, MN 3 and GP (H) 28) originated from Manipur and only 3 genotypes (MZ 56 (A), MZ 48 and MZ 22) have origin in Mizoram. Group 3 has 9 genotypes out of which 5 are originated in Mizoram (MZ 44 (C), MZ 13 (A), MZ 34 (A), MZ 21 (A) and MZ 29) and 4 from Manipur (GP (H) 1, GP (H) 6, GP (H) 2 and MN 30).

In depth knowledge of genetic diversity and population structure of maize would have a big impact on maize improvement program (Liu et al., 2003). The clustering pattern of all the genotypes based on marker information was found to have a close correlation with their pedigree information. The 30 early inbreds were developed from several populations collected from two north- eastern States of India *i.e.*, Manipur and Mizoram. The lines developed from same source population from Manipur fall under same cluster/ sub- cluster while those developed from Mizoram also fall under same cluster/ sub- cluster. For instance, lines developed from populations collected from Manipur *viz.* GP (H) 2, GP (H) 28 and SCM 15/9LC were together in same cluster *i.e.*, A. Likewise, lines like MZ 2, MZ 70, MZ 24, MZ 42, MZ 40, Kolasib 1(2), MZ 46(B), etc. developed from populations collected from Mizoram clustered together in same sub- group *viz.*, B1. Lines derived from the populations from Manipur were found to be closely placed in genotypic PCoA, while that from Mizoram occupied the nearby axes in the PCoA. To conduct the analysis of molecular variance (AMOVA), the 30 maize inbred lines were grouped into two: Manipur (developed from Manipur collection) and Mizoram (developed lines using Mizoram collection). AMOVA revealed highly significant variance among populations and within individuals of population (Table 3). The overall genetic differentiation indicated that 6% of total variance is due to differences among populations, while 94% of total molecular variance is accounted by within population molecular variance (Fig. 5).

Table 3
Results of analysis of molecular variance (AMOVA) between the two populations

Source	df	SS	MS	Est. Var.	Percentage of variation
Among Pops	1	0.316	0.316	0.010	6
Within Pops	28	4.751	0.170	0.170	94
Total	29	5.067		0.180	100
df: degree of freedom; SS: sum of squares; MS: mean squares; Est. Var. = estimated variance (<i>P</i> value > 0.001)					

The mean effective numbers of allele (N_e) were estimated to be 2.31 and 3.99 for Manipur (MAN) and Mizoram (MIZ) population, respectively (Table 4). The number of alleles (allelic richness) in a population is an important and fundamental measure of genetic variation in a population, and is a useful statistic for identifying populations for conservation (Kalinowski 2004). The Shannon's information index (I) was calculated as 0.96 and 1.58 for MAN and MIZ population, respectively. Among 189 alleles generated across the 30 genotypes, 3 private alleles (number of alleles unique to a single population) were detected in SSR marker *umc 1354*. These private alleles were revealed in 3 genotypes originated from Mizoram. The mean values of population parameters such as allelic richness/ number of different alleles (N_a), number of different alleles with a frequency $\geq 5\%$ ($N_a \text{ FrEq. } \geq 5\%$), expected Heterozygosity (H_e) and Unbiased expected Heterozygosity (uH_e) were also estimated (Table 4). The results of this study provides useful information to maize breeders for selection of inbred lines and for making promising cross combinations based on genetic distance and clustering.

Table 4
Mean Allelic Patterns Across Populations

Mean values		
Population	MAN	MIZ
Na	3.000	6.000
Na FrEq. $\geq 5\%$	3.000	6.000
Ne	2.305	3.986
I	0.958	1.575
No. Private Alleles	0.000	3.000
He	0.566	0.749
uHe	0.593	0.772
Na = No. of Different Alleles		
Na (Freq $\geq 5\%$) = No. of Different Alleles with a Frequency $\geq 5\%$		
Ne = No. of Effective Alleles = $1 / (\sum p_i^2)$		
I = Shannon's Information Index = $-1 * \sum (p_i * \ln(p_i))$		
No. Private Alleles = No. of Alleles Unique to a Single Population		
No. LComm Alleles ($\leq 25\%$) = No. of Locally Common Alleles (FrEq. $\geq 5\%$) Found in 25% or Fewer Populations		
No. LComm Alleles ($\leq 50\%$) = No. of Locally Common Alleles (FrEq. $\geq 5\%$) Found in 50% or Fewer Populations		
He = Expected Heterozygosity = $1 - \sum p_i^2$		
uHe = Unbiased Expected Heterozygosity = $(2N / (2N-1)) * He$		

Agro- Morphological Diversity And Marker- Trait Association

The statistical parameters (mean, standard deviation and correlation coefficients) related to agro-morphological traits of the lines are presented in Tables 5 and 6. The plant height (cm) ranged from 172.00- 286.67 with a mean and standard deviation of 225.88 and 29.68, respectively. The ear height (cm) was recorded between 44.50– 170.00 with a mean and standard deviation of 104.18 and 27.70, respectively. For number of kernel rows per ear, the minimum range was 8.83 and maximum was 16.67 with a mean and standard deviation of 12.45 and 1.73, respectively. The number of kernel per row was estimated between 9.50- 54.72 with a mean and standard deviation of 20.78 and 8.16, respectively. The cob length (cm) ranged from 8.75- 17.00 with a mean value and standard deviation of 12.99 and 2.25, respectively. The cob diameter (cm) and 100 kernel weights (g) had recorded minimum value of 1.30 and

14.62 and maximum value of 3.32 and 32.42, respectively with a mean and standard deviation of 2.46 and 22.12 and 0.47 and 4.50, respectively. A positive correlation was found between some important agronomic traits (Table 6). The highest and positive significant correlation was observed between PH and EH ($r = 0.76$), followed by CD and KW ($r = 0.46$), PH and KW ($r = 0.38$), CL and KW ($r = 0.37$) and EH and KRPE ($r = 0.37$).

Table 5
Descriptive statistics of seven traits in the 30 early maize inbreds used in the study

Statistic	PH	EH	KRPE	KPR	CL	CD	KW
Minimum	172.000	44.500	8.833	9.500	8.750	1.300	14.623
Maximum	286.667	170.000	16.667	54.720	17.000	3.320	32.423
Mean	225.876	104.177	12.455	20.782	12.993	2.465	22.119
Standard deviation	29.683	27.695	1.726	8.158	2.247	0.472	4.498

Table 6
Estimation of correlation coefficients at seven agronomic traits in the 30 early maize inbred lines used.

Traits	EH	KRPE	KPR	CL	CD	KW
PH	0.758***	0.093	-0.023	0.342	0.263	0.379*
EH		0.371*	0.074	0.075	0.188	0.175
KRPE			0.233	0.004	0.290	-0.186
KPR				0.292	0.183	0.005
CL					0.265	0.374*
CD						0.463**
*, ** & *** Significance at P value 0.001, 0.01 & 0.05 respectively						

In the present study, significant marker- trait associations were conducted between the SSR markers and the 7 agro- morphological traits (Table 7). The lowest R^2 value (0.06) was detected in *umc 1257*, *mmc 0411*, *umc 1607* and *umc 1002*, which was associated with CL, KRPE, CD and KW and the highest (0.21) was in *umc 2325*, which was associated with KW. The PH was associated with 5 markers viz., *umc 1275*, *umc 1912*, *mmc 0411*, *umc 1932* and *umc 1962* with R^2 value ranged from 0.08–0.13. For EH, the R^2 value was ranged from 0.08–0.14 with 3 markers associated with the trait viz., *umc 1912*, *mmc 0411* and *umc 1962*. The KPR was associated with only 2 markers *umc 1153* (0.08) and *umc 2318* (0.08). The CL was found to be associated with 4 markers viz., *umc 1257*, *mmc 0411*, *umc 1031* and *umc 1506* with R^2 value ranged from 0.06- 0.12. In case of KRPE, 4 markers were associated with the trait with R^2 value that ranged from 0.06–0.20. Likewise, in CD and KW, 3 markers were linked with these traits with R^2 value

ranging from 0.06–0.07 and 0.06–0.21, respectively. The SSR markers identified in the present study may serve as useful molecular markers for selecting important agronomic traits in maize breeding.

Table 7
Association analysis of seven agronomic traits with 52 SSR markers in 30
early maize inbred lines

SI No.	Traits	Markers	R^2 values
1	Plant Height (PH)	Umc1275	0.08
		Umc1912	0.13
		Mmc0411	0.11
		Umc1932	0.08
		Umc1962	0.12
2	Ear Height (EH)	Umc1912	0.14
		Mmc0411	0.08
		Umc1962	0.10
3	Number of kernel per row (KPR)	Umc1153	0.08
		Umc2318	0.08
4	Cob Length (CL)	Umc1257	0.06
		Mmc0411	0.06
		Umc1031	0.12
		Umc1506	0.10
5	Number of kernel rows per ear (KRPE)	Mmc0411	0.06
		Umc2042	0.09
		Umc1962	0.20
		Umc201	0.10
6	Cob Diameter (CD)	Umc1607	0.06
		Umc1543	0.07
		Umc2325	0.07
7	Kernel Weight (KW)	Umc1002	0.06
		Umc1257	0.07
		Umc2325	0.21

A high range of variation was found for all the seven agronomic traits which revealed a high level of variation in these lines. The marker- trait association study showed that many markers are associated with the agronomic traits studied and these markers will be useful for selecting lines having good agronomic characteristics. Moreover, these results will be useful in selection of promising inbred lines for use as parents in hybrid breeding program and in marker assisted selection of maize.

Declarations

Ethical statement: We consciously assure that this manuscript is the authors' own original work, which has not been previously published or is not currently being considered for publication elsewhere.

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Code availability: NA

Ethics approval (include appropriate approvals or waivers): NA

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Consent for publication (include appropriate statements): We, the undersigned, give our consent for the publication of the research paper in the above Journal.

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Figures

Figure 1

Representative of gel image showing SSR profiles of 30 early maize inbreds using SSR marker bnlg 1064, p 101049 and umc 1380, respectively. A 100 bp standard DNA ladder was used for size estimation. Lane 1-20 denote maize inbreds)

Figure 2

Depiction of allelic patterns of the 52 SSR markers among the 30 early maize inbreds lines through GGT 2.0 software (Van Berloo 2008). The colour legends such as red, purple, yellow and gray denotes A, B, H (heterozygotic) and missing alleles, respectively.

Figure 5

Population structure of 30 genotypes based on 52 SSR markers and graph of estimated membership fraction for $K = 3$.

Figure 6

Analysis of molecular variance (AMOVA) of 30 genotypes based on 52 SSR markers

Figure 7

Graph showing allelic patterns across populations