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Priyanka Kumari
Ph.D. Research Scholar, Department of Genetics and Plant Breeding, Birsa Agricultural University, Kanke, Ranchi, Jharkhand, India

Manigopa Chakraborty
Chief Scientist, Department of Genetics and Plant Breeding, Birsa Agricultural University, Kanke, Ranchi, Jharkhand, India

Sudhir Kumar
Scientist, IIAB, Ranchi, Jharkhand, India

Anita Pande
Assistant Professor, College of Biotechnology, Ranchi, Jharkhand, India

Krishna Prasad
Assistant Professor, Department of Genetics and Plant Breeding, Birsa Agricultural University, Kanke, Ranchi, Jharkhand, India

Shashi Bhushan Choudhary
Scientist, Birsa Agricultural University, Kanke, Ranchi, Jharkhand, India

Corresponding Author:
Priyanka Kumari
Ph.D. Research Scholar, Department of Genetics and Plant Breeding, Birsa Agricultural University, Kanke, Ranchi, Jharkhand, India

Drought linked SSR markers used for the assessment of genetic diversity of different maize (*Zea mays* L.) germplasm and hybrids

Priyanka Kumari, Manigopa Chakraborty, Sudhir Kumar, Anita Pande, Krishna Prasad and Shashi Bhushan Choudhary

Abstract
The development of drought tolerant and diverse varieties in maize may improve the performance of the crop under water stressed conditions, and hence increase yield to meet the food requirements of the people of Jharkhand Genetic diversity is of prime importance for the successful adaptation to certain agro-climatic conditions and improvement of any crop species. For the purpose of this study the simple sequence repeat (SSR) markers (microsatellites) were used to screen for diversity and drought. In the present study forty five inbred lines of maize and twelve maize hybrids were evaluated through fourteen polymorphic SSR markers. Each detected band was considered to be an allele. Variations in alleles were recorded to generate the molecular data *viz*., number of alleles per locus (k), observed Polymorphic Information Content (PIC). A total number of 28 alleles were generated by 14 polymorphic SSR markers among the five inbred lines of maize. The number of alleles ranged from 1-2 with an average of 2 alleles per polymorphic primer pair. The highest number of alleles were observed in zmDi19-1 & gras20 (four alleles) and result showed moderately PIC value. Cluster analysis was carried out based on similarity index data derived from the ssr markers which grouped forty five inbreds and twelve hybrids into two major clusters, cluster A and cluster B. The jaccard's similarity coefficient ranged from 0.52 to 0.95. Further this 2 main cluster classified for six subcluster. Every genotypes name are mentioned that comes under different cluster and subcluster. Further Cluster A showed four subcluster and Cluster B showed two subcluster. Drought stress leads to reduction of genetic variance components and heritability, The results of this study will be helpful in giving basic information about indirect selection of traits through associated markers and hybrids are drought tolerant and diversified can be further used for the experimental studies. The research investigation was carried out in the experimental area of Birsa Agricultural university, Ranchi, kanke, during the Rabi season Oct 2019 to May 2020 to study the drought tolerance and genetic diversity with cluster analysis for the 45 maize genotypes as experimental materials that was laid out in Randomized Block Design (RBD) with two replications.
The 45 inbreds were screened under normal moisture (30kpa) and under controlled moisture stress (50kpa) condition at rainout shelter in crop season –I and after selection of 12 inbreds among forty five, single cross hybrids were obtained by crossing 8 lines and 4 tester L X T fashion. Thirty two hybrids were developed in crop season-II Screening of forty eight genotypes under normal moisture condition (30kpa) experiment in W section of research farm (*kharif* 2019-20) and controlled moisture condition under rainout shelter (50kpa) experiment was conducted.

Keywords: SSR markers, *Zea mays* L., hybrids

Introduction
Maize (*Zea maize* L.) is one of the important economic and staple food crops and an energy plant among cereals that is cultivated globally for fulfilling the requirements of human beings (Banda CM 2020) [3]. It is a vital source of the income-overwhelming population (Stanley A 2020) [21]. With a high yield potential, it has become a model crop among cereals and is therefore called the queen of cereal crops (Kumar P, 2019) [15]. In addition, it is utilized as an industrial resource for the production of starch, pharmaceuticals, alcoholic beverages, oil, cosmetics, and textiles (Wada, N., et. al., 2008) [23]. Due to the diverse uses of maize and its products, its demand has been increasing continuously globally (Prasanna, BM., and Sharma, L., 2005) [17]. In ancient times, landraces were more popular, and maize was highly resistant to biotic and abiotic factors due to its heterogeneous nature, although the yield was low (Rebourg, C., 2003) [18]. Now, the landraces are being replaced with hybrid maize, which has a higher yield as compared to landraces (Doebley, J., 2004) [7]. The present cultivated form of maize is originated from its wild relative teosinte (*Zea mays* ssp. parviglumis), but cultivated maize is considerably different from teosinte in terms of morphology and several other

characteristics (Wani, SH., *et al.*, 2022) [24]. The production of high-yielding maize cultivars has always been the primary objective of breeding. For increasing the production of maize, several varieties, including sweet corn, popcorn, and high-quality protein corn, are being developed globally (Antony, BJ., *et al.*, 2021) [1]. In spite of huge work on developed varieties of maize, its yields are below their potential because of abiotic and biotic stresses, indicating the need to assess the genetic diversity. Knowledge of genetic diversity in maize crop, especially of germplasm and inbred lines, have significantly impacted crop improvement (Esquinas-Alcázar, J., 2005) [10]. Maize is characterized by tremendous genetic diversity (Wada, N., *et al*. 2008) [23].

Genetic diversity is defined as the total variability present in an individual or organism/population (Platt, A., 2010) [16]. Genetic diversity is an important aspect of breeding programs to develop high-yielding varieties (Dubreuil, P., and Charcosset, A., 2008) [9]. The potential of genetic diversity of maize has reduced drastically due to the continuous use of homogeneous varieties. To conserve the diversity, breeders need to study its huge germplasm (Dubreuil, and P, Charcosset A.1999) [8] and inbreds (Govindaraj, M., 2015) [13]. To obtain the knowledge of genetic diversity in maize, that is, which lines have more diversity than others, several studies have been conducted to study the morphological, biochemical, and molecular characteristics of maize (Franco, MM., 2005) [11]. Although morphological and biochemical methods are being extensively used (Beyene, A., 2006) [4], these approaches are highly sensitive to environmental effects (Smith, OS., 1992) [20]. To address the challenges of morphological and biochemical methods, a new approach based on molecular markers is being utilized to study the relationship among lines and varieties (Franco, MM., 2005) [11]. The expression of DNA markers/molecular markers is rarely influenced by the environment and avoid genotypic × environmental interactions; hence, these markers could reveal the actual level of different population analysis. Thus, these markers are being utilized in populations (table 4.), resulting in huge progress (Senior, ML., 1998) [19]. In this review, we focused on the potential role of genetic diversity for starting breeding programs and the kinds of markers being used to assess genetic diversity.

Among the various types of markers, microsatellites or SSRs, which are short sequences containing tandemly repeated copies of one to six nucleotide fragments (Rafalski et. al., 1996) [29], are currently considered as the molecular markers of choice. They are rapidly being adapted by plant researchers because of their simplicity, high levels of polymorphism (Fufa *et al*., 2005) [12], high reproducibility and co-dominant inheritance patterns. Therefore, this study was conducted to investigate the genetic polymorphism and relationships among forty five inbred lines of maize and twelve hybrids.

Material and Methods

The experimental material comprised of a total forty five inbred lines and twelve hybrid (table1 &2) procured from the department of genetics and plant breeding and from NBPGR, Ranchi present investigation was conducted for screening of forty five inbreds under normal moisture (30kpa) and under controlled moisture stress (50kpa) condition at rainout shelter (*Rabi* 2019-20) in crop season –I and after selection of 12 inbreds among forty five, single cross hybrids were obtained by crossing 8 lines and 4 tester L X T fashion and thirty two hybrids were developed whereas in crop season-II Screening of forty eight genotypes under normal moisture condition (30kpa) experiment in W section of research farm (*Kharif* 2020) but in molecular work forty five inbred lines were used and only twelve hybrid were screened with ssr markers. Drought tolerant parents were used as tester and high yielding genotypes under normal water level were used as female lines.

DNA extraction

Healthy leaves were collected from young plants and the genomic DNA extraction was carried using Cetyl trimethyl ammonium bromide (CTAB) method by Murray and Thompson (1980) [13]. The concentration of genomic DNA was determined by Nanodrop spectrophotometer and agarose gel electrophoresis. The final concentration of the samples was adjusted to 100 ng/µl for polymerase chain reaction (PCR).

PCR and Agarose gel electrophoresis

Polymerase Chain Reaction was carried out in a 10µl reaction mixture consisting of 2 µl of 100 ng/ µl template DNA, 4 µl of TAKARA master mix (PCR buffer, Taq polymerase, MgCl2 and dNTP's), each of 0.5 µl of forward and reverse primers and 3 µl of molecular grade water. The amplification profile was maintained at 94 °C for 5 min followed by 35 cycles of 94 °C for 45 sec, 55 °C for 1 min and 72 °C for 1 min with a final extension of 10 min at 72 °C. The amplified PCR products were electrophoretically resolved on a 3% agarose gel using 1×TAE buffer. DNA banding patterns were visualized using BIO-RAD Imaging gel documentation system. SSR Analysis Genetic diversity of the inbred lines was estimated through fourteen polymorphic markers (Table 4). To estimate the discriminatory power of a marker, the Polymorphic Information Content (PIC) for each SSR marker along observed.

Results and Discussion

The analysis of variance according to line × tester method revealed significant difference among the lines, testers and line × tester interaction for grain yield in both conditions. Genetic diversity is of prime importance for the successful adaptation to certain agro-climatic conditions and improvement of any crop species. In the present study forty five inbred lines of maize and twelve maize hybrids were evaluated through fourteen polymorphic markers. Each detected band was considered to be an allele. Variations in alleles were recorded to generate the molecular data *viz*., number of alleles per locus (k), observed Polymorphic Information Content (PIC). A total number of 28 alleles were generated by fourteen polymorphic SSR markers among the five inbred lines of maize (Table 3). The number of alleles ranged from 1-2 with an average of 2 alleles per polymorphic primer pair. The highest number of alleles were observed in zmDi19-1 & gras 20 (two alleles) and result showed moderately PIC value similarly Warburton *et al*., (2002) reported an average of 4.9 alleles with 85 SSR markers in 57 maize inbreds while Patto *et al*., (2004) [27] reported 5.3 alleles using 80 SSR markers. However, the current results are close to the findings of Gupta and Singh (2010) [14] who recorded an average value of 2.5 alleles using nine polymorphic SSR primers in twenty maize inbreds. Polymorphic information content demonstrates the informativeness of SSR markers to

detect the differences among the inbred lines based on their genetic differences. Usually, markers with PIC value more than 0.5 are considered to be highly informative while markers with PIC value 0.25 to 0.50 are considered to be moderately informative in measuring the polymorphism for a marker locus (DeWoody *et al*., 1995) [6]. A set of 14 SSR marker used for the characterization of total sixty genotypes in which forty five inbreds (Table 1 &2), twelve hybrids, 2 check and 1 random genotype of maize based on polymorphism 28 alleles recorded all bands of maize showed different bands for different markers. Polymorphic information content demonstrates the informativeness of SSR markers to detect the differences among the inbred lines based on their genetic differences. Maximum PIC (Polymormic Information Content) value observed for the marker For marker ZmDi19-1 PIC value found 0.59 whereas lowest PIC observed for the marker bnlg1063 i.e. 0.41. The average PIC value determined in the present study was similar to the findings of Legesse *et al*., (2007) [25] who recorded an average PIC of 0.58 in 56 inbred lines of maize while Babu *et al*., (2012) [2] reported average PIC value of 0.49 in 22 maize inbred lines. Drought stress leads to reduction of genetic variance components and heritability, Banding pattern also mentioned in there in table 5. The results of this study will be helpful in giving basic information about indirect selection of traits through associated markers. Hybrid cultivars have played a vital role in increasing the productivity of maize in this findings.

Clustering through dendrogram of forty five maize (*Zea mays* L.) inbreds and twelve hybrids

Cluster analysis was carried out based on similarity index data derived from the SSR markers which grouped forty five inbreds and twelve hybrids into two major clusters, cluster A and cluster B (fig.1). The jaccard's similarity coefficient ranged from 0.52 to 0.95. Further this 2 main cluster classified for six subcluster. Every genotypes name are mentioned that comes under different cluster and subcluster. Further Cluster A showed four subcluster and Cluster B showed two subcluster.

(Table 6) In cluster A under subcluster-I 14 inbred lined recorded, i.e.- BAUIM-1, IC624180, BAUIM-1, IC624161, IC624151, BAUIM-5, IC624175, IC624180, BAUIM-2, CM600, IC624178, Suwan composite, IC622968,, IC624173 whereas under subcluster-II Six genotypes occurred i.e. BAUIM-5, IC622968, IC624142, IC624147, BAUIM-2, IC624150, while under subcluster-III only two inbred lines recorded i.e., IC622968, IC624176 which showing have different genetic architecture at genetic level and under subcluster-IV total twenty seven genotypes found in which twenty five inbred lines and two hybrids recorded i.e., IC624157, IC624148, IC624141, IC624148, IC624160, IC624145, IC624158, IC624166, IC624159, IC624146, IC624174, IC624180, IC624154, IC624164, BAUIM-5, IC624181, IC624165, IC624177,, IC624151,, IC624159, IC624160, IC622968, IC624154, BAUIM-1 X IC624160, BAUIM-2 X IC624151.

In cluster B there only two subcluster1 recorded in subcluster-I two inbred and five hybrid i.e. - IC624148, IC622968, BAUIM-1 X IC624157, BAUIM-5 X IC624154, BAUIM-1 X IC624160 found whereas subcluster-II seven hybrid recorded BAUIM-2X IC624148, BAUIM-2 XIC624159, IC622968 X IC624161, IC624151 X BAUIM-5, BAUIM-2 X IC624174, BAUIM-5 X IC624180, IC622968 X IC624159 hence the results revealed that the maize inbred lines in the same cluster were genetically similar to each other than the inbred lines in the other cluster. High yielding maize hybrids could be developed by combining the inbred lines from different clusters. This is in agreement with the other investigators Senior *et al*., (1998) [19] and Reif *et al*., (2003) who demonstrated the correspondence of SSR markers with cluster analysis in maize.

Table 1: The seeds of Maize (Zea Mays) accession procured from NBPGR (National Bureau of Plant Genetic Resources) Ranchi

S. No.	Collector No.	I.C No.	S. No.	Collector No.	I.C No.
1.	SKB/PM-5	IC624140	9.	SKB/PM-17	IC624151
2.	SKB/PM-6	IC624141	10.	SKB/PM-19	IC624153
3.	SKB/PM-7	IC624142	11.	SKB/PM-21	IC624154
4.	SKB/PM-10	IC624145	12.	SKB/PM-28	IC624157
5.	SKB/PM-11	IC624146	13.	SKB/PM-31	IC624158
6.	SKB/PM-12	IC624147	14.	SKB/PM-35	IC624159
7.	SKB/PM-14	IC624148	15.	SKB/PM-36	IC624160
8.	SKB/PM-16	IC624150	16.	SKB/PM-39	IC624161
17.	SKB/PM-46	IC624164	24.	SKB/PM-73	IC624175
18.	SKB/PM-47	IC624165	25.	SKB/PM-75	IC624176
19.	SKB/PM-48	IC624166	26.	SKB/PM-76	IC624177
20.	SKB/PM-56	IC624169	27.	SKB/PM-77	IC624178
21.	SKB/PM-58	IC624170	28.	SKB/PM-78	IC624179
22.	SKB/PM-66	IC624173	29.	SKB/PM-79	IC624180
23.	SKB/PM-71	IC624174	30.	SKB/PM-83	IC624181

Table 2: The seeds of the Maize (Zea Mays) inbred line procured from the Maize Research Scheme of Plant Breeding and Genetics department of BAU Kanke, Ranchi

S. No.	Inbred lines	Accession number	S. No.	Inbred lines	Accession number
1.	96 Rohyo	BAUIM-1	10.	HKI-1532	IC563958
2.	Suwan	BAUIM-2	11.	HKI-335	IC405279
3.	55Dholi	BAUIM-3	12.	P1M1PV1	IC622967
4.	B1105TE	BAUIM-4	13.	P1M1PV2	IC622968
5.	95IOWA	BAUIM-5	14.	LM13	IC527290
6.	BQPM-2	IC45673	15.	LM14	IC527291
7.	CM425	IC67543	16.	CM600 (Check1)	
8.	CML169	IC643215	17.	Suwan composite (Check2)	
9.	HKI-193-1	IC470149	18.		

Table 3: List of hybrids used for the SSR molecular analysis

S. No.	I.C No.	S. No.	I.C No.
1.	BAUIM-1 X IC624160	7.	BAUIM-2X IC624148
2.	BAUIM-2 X IC624151	8.	BAUIM-2 XIC624159
3.	BAUIM-1 X IC624157	9.	IC622968 X IC624161
4.	BAUIM-5 X IC624154	10.	IC624151 X BAUIM-5
5.	BAUIM-1 X IC624160	11.	BAUIM-2 X IC624174
6.	IC622968 X IC624159	12.	BAUIM-5 X IC624180

Table 4: List of polymorphic SSR markers used in the genetic diversity assessment of inbred lines in maize (*Zea mays* L.)

Marker /gene name	Trait linked	Chromo some no.	Farward requence	Reverse sequence	Tm
ZCN8	Tasseling initiation time	8,1	TAAGAGCAACGGCCAATACC	CCGATATCGACTCGTGGTTT	60.10 / 59.96
gras20,	Chlorophyll content	8	CACTCGGTGGGTATCGACTT	TGATCTGCCTCATGCTCAAC	59.99 /59.95
acs7, ZmAcs7	Leaf angle	10	GTGTGCTCCCTGCTAAGCTC	CAGGTCGAAAGGGTTCATGT	60.16/59.97
qLA4-1 QTL, ZmCLA4	Leaf drooping	4	ACCGCCAACGAATACTTCAC	ACCTGGTCTTCGTCATCACC	60.0/59.97
GRMZM2G163251, nac7,	STAY GREEN	1	CGGGTACTACTGGGACGAGA	TGGTCACAACAACGTCCTGT	60.13 /60.02
Bnlg1346	ASI	9	CATCATGAAGCAATGAAGCC	CCGCGCCATTATCTAGTTGT	60.56/60.06
umc1962	Female flowering time	8	ATAAGTGGGGGAGGCGAGCTA	GAGAACCAACCACCAAAGAA GTCC	50.89/60.22
ZmDi19-1	Drought tolerant indices	3	ATGGAAGGCTCACTGTGCTC	TGTAGCAAGCCTCTACCTCCA	60.42 /60.02
bnlg1063	Flowering time	9	GGAGACAACCCCGACGAC	GGTACCAGAGCCACAGATCC	60.02/59.6
bnlg2190	DTI	4	TCCTCCTTCATCCCCTTCTT	CCCAGTATCATTGCCCAATC	60.06/59.03
dupssr13	GY	1	CAAATATCTCTCATCTTTGCTGAC	TCGTTCGGTCCATGAAAT	60.6/59.08
phi022	GY	1	TGCGCACCAGCGACTGACC	GCGGGCGACGCTTCCAAAC	59.0/57.01
bnlg2248	ASI	4	CCACCACATCCGTTACATCA	ACTTTGACACCGGCGAATAC	59.0/60.02
phi081	Flowering time	2	AAGGAACTGGTGAGAGGGTCCTT	AGCCCGATGCTCGCCATCTC	59.0/56.07

Table 5: Number of alleles, PIC values, %P type band, %M type band, %H type band of % NA type band of polymorphic SSR markers among the maize inbred lines and hybrids

SL. No.	Marker Name	PIC	%P type band	%M type band	%H type band	% NA type band
1	Bnlg1346	0.42	38.33	36.67	25.00	1.00
2	dupssr13	0.45	35.00	36.67	26.67	1.67
3	nac7, nactf7	0.45	35.00	35.00	25.00	5.00
4	bnlg1063	0.41	60.00	10.00	25.00	5.00
5	ZCN8	0.43	10.00	61.67	26.67	1.67
6	bnlg2248	0.46	43.33	31.67	25.00	0.00
7	umc1962	0.45	31.67	46.67	21.67	0.00
8	gras20	0.40	18.33	16.67	65.00	0.00
9	zmDi19-1	0.59	65.00	20.00	13.33	1.67
10	Acs7	0.55	28.33	41.67	30.00	0.00
11	phi081	0.54	36.67	55.00	6.67	1.67
12	phi022	0.39	45.00	33.33	21.67	0.00
13	bnlg2190	0.54	45.00	41.67	13.33	0.00
14	ZmCLA4	0.46	60.00	15.00	25.00	0.00
	Mean	0.47				

Here band M represents allele from recipient parent, P represents allele from donor parents, H= Heterozygous type and NA= not amplified

Table 6: Groupism of maize (*Zea mays* L.) inbreds and hybrids based on cluster

Cluster	Subcluster	Inbreds	Hybrids
Cluster A	Subcluster 1	BAUIM-1, IC624180, CM600, IC624161, IC624151, BAUIM-5, IC624175, IC624180, BAUIM-2, Suwan composite, IC624178, IC622968, BAUIM-1, IC624173	
	Subcluster 2	BAUIM-5, IC622968, IC624142, IC624147, Random Genotype of maize, IC624150	
	Subcluster 3	IC622968, IC624176	
	Subcluster 4	IC624157, IC624148, IC624141, IC624148, IC624160, IC624145, IC624158, IC624166, IC624159, IC624146, IC624174, IC624180, IC624154, IC624164, BAUIM-5, IC624181, IC624165, IC624177, IC624151,, IC624159, IC624160, IC622968, IC624154,	BAUIM-1 X IC624160 BAUIM-2 X IC624151
Cluster B	Subcluster 1	IC624148, IC622968,	BAUIM-1 X IC624157 BAUIM-5 X IC624154 BAUIM-1 X IC624160
	Subcluster 2	,	BAUIM-2X IC624148, BAUIM-2 XIC624159, IC622968 X IC624161, IC624151 X BAUIM-5, BAUIM-2 X IC624174, BAUIM-5 X IC624180, IC622968 X IC624159

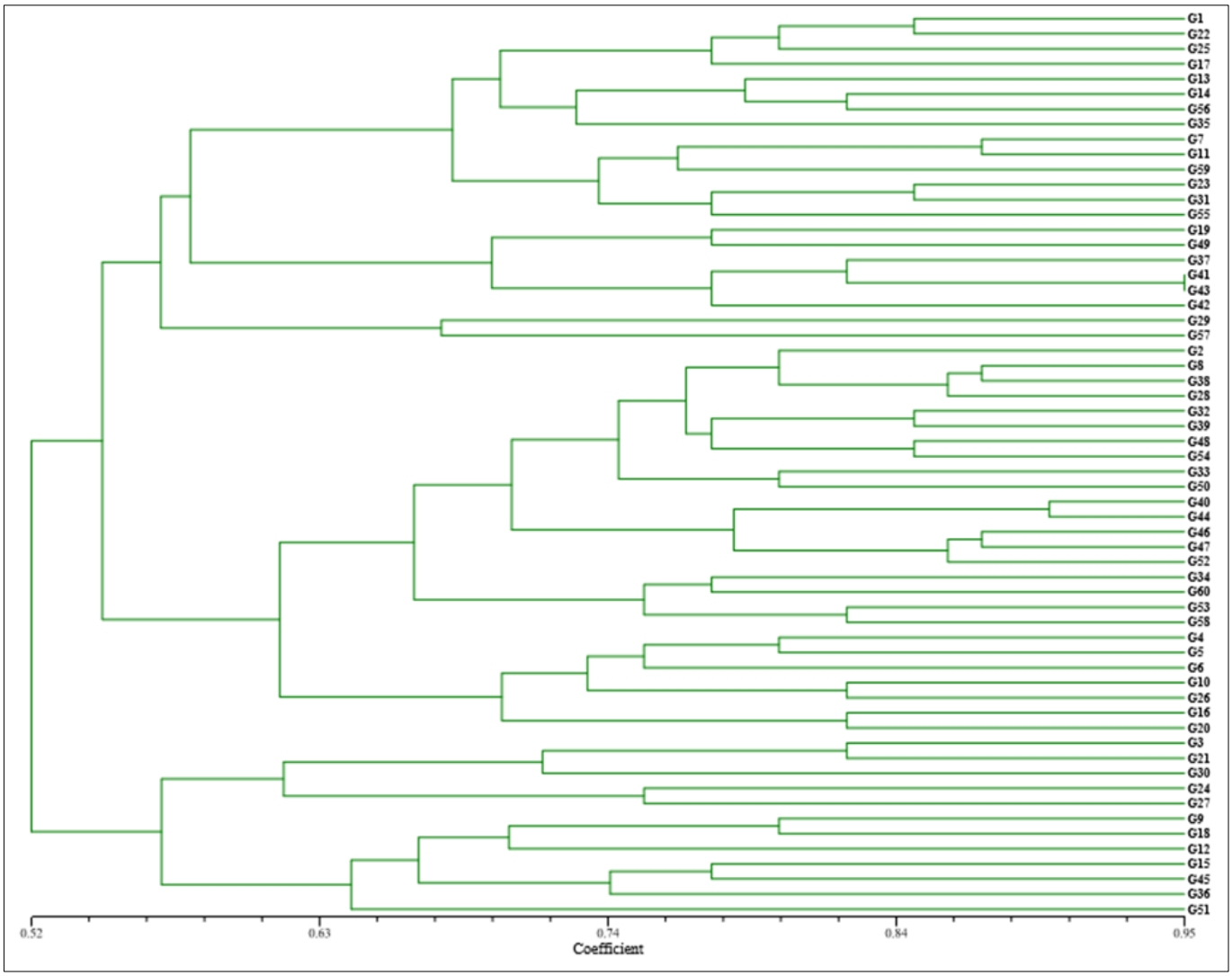

Fig 1: Cluster Dendrogram of Maize (*Zea mays* L.) inbred and hybrid using Jaccard's similarity coefficient between genotypes

References

1. Antony BJ, Kachapur RM, Naidu GK, Harlapur SI. Genetic diversity study among maize (*Zea mays* L.) inbred lines. J Farm Sci. 2021;34:352-356.
2. Babu BK, Agrawal PK, Gupta HS, Kumar A, Bhatt JC. Identification of candidate gene–based SSR markers for lysine and tryptophan metabolic pathways in maize (*Zea mays*). Plant breeding. 2012;131(1):20-27.
3. Banda CM. Genetic analysis for heat tolerance in tropical maize (*Zea mays*) (Doctoral dissertation). Zambia: University of Zambia; c2020.
4. Beyene, A., Gibbon D, Haile M. Heterogeneity in land resources and diversity in farming practices in Tigray, Ethiopia. Agric Syst. 2006;88:61-74.
5. De Faria SV, Zuffo LT, Rezende WM, Caixeta DG, Pereira HD, Azevedo CF, *et al*. Phenotypic and molecular characterization of a set of tropical maize inbred lines from a public breeding program in Brazil. BMC Genomics. 2022;23:54.
6. DeWoody JA, Honeycutt RL, Skow LC. Microsatellite markers in white-tailed deer. The Journal of heredity. 1995;86(4):317-319.
7. Doebley J. The genetics of maize evolution. Annu Rev Genet. 2004;38:37-59.
8. Dubreuil P, Charcosset A. Relationships among maize inbred lines and populations from European and North-American origins as estimated using RFLP markers. Theor Appl Genet. 1999;99:473-480.
9. Dubreuil P, Charcosset A. Genetic diversity within and among maize populations: A comparison between isozyme and nuclear RFLP loci. Theor Appl Genet. 1998;96:577-587.
10. Esquinas-Alcázar J. Protecting crop genetic diversity for food security: Political, ethical and technical challenges. Nat Rev Genet. 2005;6:946-953.
11. Franco MM, Antunes RC, Silva HD, Goulart LR. Association of a PIT1, GH and GHRH polymorphism with performance and carcass traits in Landrace pigs. J Appl Genet. 2005;46:195-200.
12. Fufa Hundera, Baenziger PS, Beecher BS, Dweikat I, Graybosch RA, Eskridge KM. Comparison of phenotypic and molecular marker-based classifications of hard red winter wheat cultivars. Euphytica. 2005;145(1):133-146.
13. Govindaraj M, Vetriventhan M, Srinivasan M, Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. Genet Res Inter. 2015;2015:14.
14. Gupta ASTHA, Singh AK. Studies on molecular diversity in maize inbreds using SSR markers. Trends in Biosciences. 2010;3(2):106-109.
15. Kumar P, Hossain F, Singh NK, Choudhary P, Gupta M, Singh V, *et al*. Nutritional quality improvement in maize

(*Zea mays*): Progress and challenges. Indian J Agric Sci. 2019;89:895-911.
16. Platt A, Horton M, Huang YS, Li Y, Anastasio AE, Mulyati NW, *et al*. The scale of population structure in Arabidopsis thaliana. PLoS Genet. 2010;6:e1000843.
17. Prasanna BM, Sharma L. The landraces of maize (Zea mays L.): Diversity and utility. Indian J Plant Genet Resour. 2005;18:155-168.
18. Rebourg C, Chastanet M, Gouesnard B, Welcker C, Dubreuil P, Charcosset A. Maize introduction into Europe: The history reviewed in the light of molecular data. Theor Appl Genet. 2003;106:895-903.
19. Senior ML, Murphy JP, Goodman MM, Stuber CW. Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. Crop Sci. 1998;38:1088-1098.
20. Smith OS. Measurement of genetic diversity among maize hybrids: A comparison of isozymic, RFLP, pedigree and heterosis data. Maydica. 1992;37:53-60.
21. Stanley A, Menkir A, Paterne A, Ifie B, Tongoona P, Unachukwu N, *et al*. Genetic diversity and population structure of maize inbred lines with varying levels of resistance to striga hermonthica using agronomic trait-based and SNP markers. Plants. 2020;9:1223.
22. Szalma SJ, Hostert BM, LeDeaux JR, Stuber CW, Holland JB. QTL mapping with near-isogenic lines in maize. Theor Appl Genet. 2007;114:1211-1228.
23. Wada N, Feng C, Gulati A. Introduction, and overview. In: Maize in Asia: Changing markets and incentives. New Delhi: Academic Foundation; c2008.
24. Wani SH, Samantara K, Razzaq A, Kakani G, Kumar P. Back to the wild: Mining maize (*Zea mays* L.) disease resistance using advanced breeding tools. Mol Biol Rep. 2022. DOI: 10.1007/s11033-021-06815-x.
25. Legesse BW, Myburg AA, Pixley KV, Bohta AM. Genetic diversity of African maize inbred lines revealed by SSR markers. Hereditas. 2007;144:10-17.
26. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research. 1980;8(19):4321-4325.
27. Patto MC, Satovic Z, Pego S, Fevereiro P. Assessing the genetic diversity of Portuguese maize germplasm using microsatellite markers. Euphytica. 2004;137(1):63-72.
28. Perrier X, Flori A, Bonnot F, Hamon P, Seguin M, Glaszmann JC. Genetic diversity of cultivated tropical plants. Data analysis methods. Edited by P. Hamon, M. Seguin, X. Perrier, and JC Glaszmann. Enfield, Montpellier; c2003. p. 43-76.
29. Rafalski A, Morgante M, Powell W, Vogel JM, Tingey SV. Generating and Using DNA Markers in Plants. In: Birren B., Lai E. (Eds.): Analysis of Non-Mammalian Genomes-A Practical Guide. Academic Press; c1996.