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RESEARCH ARTICLE
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Diversity Analysis of Convergent-Derived Breeding Maize Inbred Lines using SSR Markers

Muh Farid[1], Muhammad Azrai[1], Amin Nur[2], Karlina Syahruddin[3], Roy Efendi[3], Willy Bayuardi Suwarno[4], Nining Nurini Andayani[3], Slamet Bambang Priyanto[3], Mukminati Mukminati[5] and Muhammad Fuad Anshori[1]*

[1]Hasanuddin University, Department of Agronomy, Faculty of Agriculture, Jl. Perintis Kemerdekaan Km 10, Makassar 90245, South Sulawesi, Indonesia
[2]Agency for Agricultural Assemblies and Modernization, Ministry of Agriculture, Jl. Ratulangi No.74, Maros, South Sulawesi, Indonesia
[3]Research Center for Food, Research Organization for Agriculture and Food, National Research and Innovation Agency, Kawasan Sains dan Teknologi (KST) Dr. (H.C) Ir. H. SoekarnoJl. Raya Jakarta-Bogor KM. 46, Cibinong, Bogor, 16915, Indonesia
[4]Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Bogor, Indonesia
[5]Agrotechnology Master Study Program, Faculty of Agriculture, Hasanuddin University, Makassar, 90245, Indonesia
*Corresponding author: fuad.anshori@unhas.ac.id

ABSTRACT

This study investigates the genetic diversity of maize (Zea mays L.) inbred lines derived from convergent breeding using Simple Sequence Repeat (SSR) markers. Convergent breeding combines desirable traits from diverse genetic sources into a single genotype, enhancing maize productivity, adaptability, and resilience. The experiment was conducted at the BSIP experimental field in Maros, South Sulawesi, and involved 30 inbred lines of maize. DNA isolation was performed using the CTAB buffer-modified extraction method, and 50 SSR markers were used to analyze genetic diversity. The results revealed significant genetic variation among the maize inbred lines. The study identified 421 alleles, with an average of 8.42 alleles per marker, indicating robust polymorphism levels. The phylogenetic analysis grouped the inbred lines into five clusters, with similarity coefficients ranging from 0.57 to 0.85, reflecting varying degrees of genetic diversity. The findings underscore the importance of using SSR markers to detect genetic diversity in maize populations, particularly those derived from convergent breeding, to enhance breeding programs and develop superior maize varieties. These findings provide valuable insights into breeding programs aiming to improve agronomic traits and to develop exceptional and adaptive maize varieties from convergent-derived inbred lines.

Keywords: Maize, Inbred lines, Convergent breeding, SSR markers.

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INTRODUCTION

Maize (*Zea mays* L.) is a staple of global importance crop, serving as a vital source of food, feed, energy and industry in Indonesia (Magfiroh et al., 2018; Syahruddin et al., 2020; Wicaksana et al., 2022). Over decades, plant breeders have employed various techniques to improve maize productivity, adaptability and nutritional quality, especially in the tropics (Paterniani, 1990; Reeves & Cassaday, 2002; Lee & Tracy, 2009; Hallauer, 2011; Turner-Hissong et al., 2020). Among these techniques, convergent breeding has emerged as a promising strategy (Kist et al., 2010; Chen et al., 2022; Widiayani et al., 2025).

Convergent breeding is an approach that combines desirable traits from diverse genetic sources into a single, optimized genotype (Nur et al., 2017). It leverages the genetic diversity in various maize populations, landraces, and wild relatives to create hybrids or inbred lines with superior performance. The method often involves recurrent selection, marker-assisted selection (MAS) and advanced genomic tools to ensure the efficient combination of traits (Andorf et al., 2019). Convergent breeding has significantly increased maize yield by integrating high-yielding traits from diverse germplasm (Nur et al., 2020). By combining genetic resources from temperate and tropical maize, breeders have developed hybrids capable of thriving in various environments (Makmur et al., 2024).

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Climate change has amplified the need for crops that can withstand drought, heat and nutrient-deficient soils (Challinor et al., 2009; Hannah et al., 2020; Palmgren & Shabala, 2024). Producing inbred lines from convergent crosses will be the right solution to increase the genetic diversity of inbred lines to face climate change challenges. Convergent breeding allows for incorporating traits conferring resilience to such stresses, making maize cultivation sustainable even in marginal areas. Resistance to pests and diseases such as maize lethal necrosis (MLN), fall armyworm, and stem borers is critical for productivity. Convergent breeding strategies have facilitated the introgression of resistance genes from wild relatives and exotic germplasm and represent a powerful strategy to meet the multifaceted challenges facing maize cultivation, which requires the integration of traits from diverse genetic backgrounds (Takuno et al., 2015). By combining genetic resources, technological innovations, and global collaboration, this approach holds the potential to secure maize productivity and resilience for future generations (Zhang et al., 2023). Ensuring compatibility and stable trait inheritance while preventing linkage drag (where unwanted traits are inherited alongside desirable ones) adds significant complexity (Wang et al., 2021).

Inbred lines from convergent breeding involve crossing genetically diverse populations over several generations to combine desirable traits into a unified line (Rockman & Kruglyak, 2008; Welsh & Mcmillan, 2012). Convergent breeding has excellent potential to produce superior inbred lines, but the process also presents several challenges that plant breeders need to anticipate. The challenge of producing inbred lines from convergent breeding crosses is convergent breeding combine's diverse germplasm, leading to high genetic variability in the early generations. This can cause undesirable traits to segregate alongside desirable ones. It becomes challenging to break these associations. Achieving homozygosity through repeated selfing and selection requires significant time and resources (Technow et al., 2021). Overcoming the genetic complexity of the convergent genetic material can be helped by analyzing the genetic diversity of the crossed population to obtain information on the diversity of the population (Desai et al., 2013; Abhari et al., 2024).

Analysis of the genetic diversity of maize inbred lines resulting from convergent crosses is an essential step in maize breeding programs. High genetic diversity in maize inbred line populations is critical for improving yield potential, disease resistance and adaptation to diverse environmental conditions (Liu et al., 2003; Hassan et al., 2018). One effective method to analyze genetic diversity is using molecular markers, such as SSR (Chakravarthi & Naravaneni, 2006; Wang et al., 2011; Kumar & Singh, 2012; Wangari, 2013).

SSR markers are short DNA segments consisting of repetitive nucleotide sequences. This marker is highly polymorphic, meaning it has high variation among individuals in the population, making it very useful for identifying genetic differences. SSR has several advantages, including high polymorphism, even distribution, consistency and reproducibility. SSR markers can provide valuable information about genetic structure by obtaining necessary information about genetic variation in the population and supporting breeding efforts to produce superior and adaptive maize varieties (Kantartzi, 2013).

The genetic complexity of the corn population resulting from convergent crossing necessitates molecular analysis methods to estimate the level of gene diversity unaffected by environmental factors. SSR markers are highly sensitive, reproducible, and consistently detect genetic diversity in complex populations. The combination of these two methods has never been attempted before. Therefore, we conducted this study to determine the level of gene diversity in the corn population from convergent crossbreeding using SSR markers.

MATERIALS & METHODS

Experimental Design

The experiment was conducted at Badan Standardisasi dan Instrumen Pertanian (BSIP) experimental field, DR. Ratulangi Street No. 274, Maros, South Sulawesi (5.0323° S 119.6685° E) from September to October 2024 and at the molecular biology laboratory from October to November 2024. The experiment used 45 inbred lines of maize derived from convergent crossing. The inbred seed was planted in the field and after 14 days, the young leaves were taken and put in paper bags, then labeled with the field code. Only 30 out of 45 inbred lines were selected based on the uniformity of population growth and phenotypes per inbred line and sent to a molecular biology laboratory for genetic tests. Diversity analysis used SSR markers spread across 10 maize chromosomes. The types of SSR markers used include bnlg, umc, phi, nc, and dupssr, each with different design methods and genomic target focuses (Sharopova et al., 2002). These primers are frequently utilized in genetic mapping research, genetic diversity analysis and genomic studies, particularly in maize.

Experimental Procedure

DNA Isolation

DNA isolation was performed using the CTAB buffer-modified extraction method (Ramlah et al., 2018). Young leaf samples of the F2 generation were collected approximately 14 days after planting and stored at 4ºC. Each leaf sample, weighing 0.4 g per genotype, was placed into a mortar and ground to a fine consistency using a pestle with the addition of CTAB buffer. The homogenized samples were then divided equally into two microtubes. To each microtube, 10µL of ß-mercaptoethanol was added. The microtubes were incubated in a water bath at 60ºC for 60 minutes, with the tubes being inverted every 15 minutes. After incubation, the tubes were removed from the water bath, cooled, and chloroform isoamyl alcohol (CIAA) was added. The mixture was homogenized using a vortex mixer for 10 minutes. Finally, the microtubes were centrifuged at 11,600 rpm for 10 minutes.

The result of centrifugation was the formation of three layers: supernatant, pellet and chisam. The supernatant (clear liquid at the top) was carefully

transferred to a 1.5mL microtube and cold isopropanol was added. The tube was swirled until fine strands of DNA formed. The microtube was then centrifuged for 10 minutes to settle the DNA at the bottom of the tube. The supernatant was discarded, leaving only the DNA pellet. The DNA pellet was washed by adding cold 70% ethanol and allowed to stand for 10 minutes. The ethanol was then carefully discarded to avoid losing the DNA pellet. This washing step with cold 70% ethanol was repeated, allowing it to stand for another 10 minutes before discarding the ethanol again. The DNA pellets were then dried by inverting the tube on a tray lined with paper towels. Once dried, Tris-EDTA buffer was added to the DNA pellet tube, and the mixture was incubated in a water bath for 60 minutes. After the DNA dissolved in the Tris-EDTA buffer, it was homogenized and centrifuged.

DNA Quantity Test using Spectrophotometer

The DNA quantity test was conducted by pipetting 0.7-4μL of corn DNA solution and measuring it with a spectrophotometer at 260 nm and 280 nm wavelengths. The spectrophotometer displayed the results on the monitor screen, showing the concentration values and DNA purity for each genotype measured.

DNA Amplification using PCR (Polymerase Chain Reaction)

The DNA solution measured by spectrophotometer was diluted (equivalent to 10 ng/μl) as much as 1μL was put into a microplate. Reagent solution (PCR-mix) was added consisting of nuclease-free water 2.25μL per reaction, @Primer Mix (F and R) 5 uM 0.5μL per reaction, and KAPA2G Fast HotStart ReadyMix 2x enzyme 6.25μL per reaction. PCR-mix as much as 9μL was put into a DNA microplate, one drop of mineral oil was added and the microplate was closed. This step was carried out using a total of 50 SSR primers.

The PCR program used is predenaturation at 95ºC for 5min, denaturation at 95ºC for 30s, primer attachment temperature (annealing) adjusted for each primer for 30 seconds, and extension at 72ºC for 30s, denaturation extension cycle repeated 35 times, final elongation at 72ºC for 7min and cooling at 25ºC for 4min.

After the PCR process, electrophoresis was performed on an 8% polyacrylamide gel. For electrophoresis in 2 pages with two plates, a solution of acrylamide 8% was used as much as 100μL, TEMED 100μL, and ammonium persulfate (APS) 1,000μL. The solution mixture was put into a glass plate, and a comb mold was installed between the two plates until the gel solidified (polymerized). After that, the comb molds from both plates were removed, and the plate was inserted into a series of vertical electrophoresis devices containing 1x TE solution. DNA samples that have been PCR pipetted as much as 4μL are inserted into each gel well, and 2μL marker as a marker in the first and last gel wells. The electrophoresis process was carried out at an electric voltage of 100 V for 1 hour until the first color reached the bottom of the gel. Polyacrylamide gel electrophoresis results were washed with distilled water, immersed in silver nitrate solution for 5-7min and then rinsed in distilled water ± 2 seconds. Subsequently, it was immersed in NaOH solution mixed with formaldehyde 3,000μL / l while shaking gently until DNA bands appeared. Visualization of DNA bands was done using a camera on a gel doc.

Observation and Data Analysis

The scoring data were subsequently analyzed using the Unweighted Pair-Group Method with Arithmetic (UPGMA) - Sequential Agglomerative Hierarchical and Nested (SAHN) program on NTSYS software version 2.1 (Rohlf, 2000). Clustering was performed by selecting the clustering feature in the NTSYS ver 2.1 program to obtain a grouping dendrogram. The dendrogram thus obtained revealed the genotypes that exhibited a considerable genetic distance. Subsequently, the scoring data were subjected to analysis using Power Marker 3.25 software (Liu & Muse, 2005) to ascertain statistics such as the value of the main allele frequency, genetic diversity, heterozygosity, and PIC (Polymorphism Information Content) produced by the markers utilized to assess gene diversity (Cahyono et al., 2023). The PIC value was calculated according to the formulation by Shehata et al. (2009). PIC values range from 0 to 1, with values closer to 1 indicating greater genetic diversity.

RESULTS

The most extensive allele distribution was observed on chromosome 6, with an average of 11 alleles, while the most minor distribution was observed on chromosome 2, with 6.27 alleles (Table 1). The distribution of alleles on chromosomes indicates a situation where the amount of genetic variation (alleles) spread along a chromosome is very limited, as is the case on chromosome 2. This observation suggests that the inbred line population under study exhibits significant conservation of alleles, indicating a narrow genetic variation in the alleles on chromosome 2. Consequently, the potential for generating novel genetic combinations that would affect traits located on chromosome 2 is constrained.

The calculation of the number of alleles for each inbred line for 50 SSR primers revealed a range of 46 to 171 alleles per inbred line. The inbred line G33 exhibited the lowest number of alleles, while the inbred line G16 demonstrated the highest number (Fig. 1). An inbred line with a low number of alleles suggests that it has achieved a high degree of genetic homozygosity due to the inbreeding process (i.e., repeated self-pollination) designed to enhance homozygosity. A single allele at each locus characterizes a homozygous inbred line because both copies of the allele on homologous chromosomes are identical.

In the population of inbred lines resulting from convergent crossing, primers with high PIC values generally have numerous alleles with a comprehensive distribution of allele frequencies. Primer Phi028 has a low PIC value (0.43) due to several dominant alleles (Fig. 2). Primer nc009 exhibits a moderate PIC value (0.68) due to the presence of dominant alleles and alleles distributed in

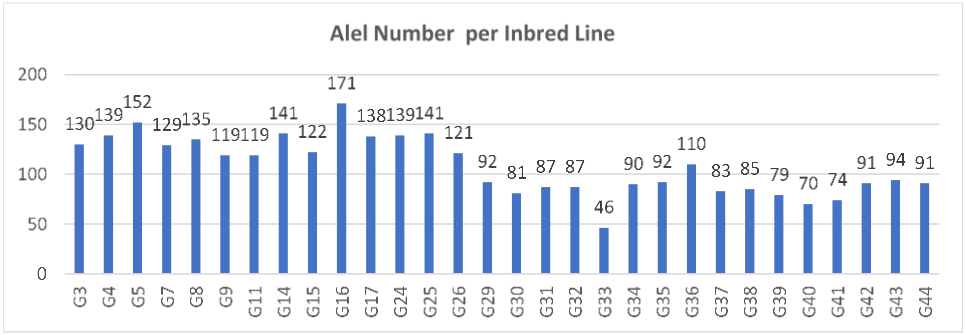

Fig. 1: Alelle number per inbred line using 50 SSR Markers.

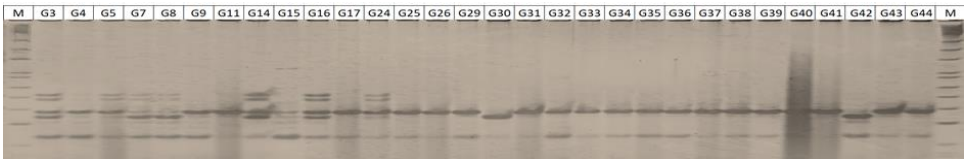

Fig. 2: Electrophoregram of DNA Amplification of Inbred lines with Phi028. M: marker G3-44: Inbred lines.

Table 1: List of SSR markers used to characterize 30 derived-convergent breeding maize inbred lines

No	Primer	Bin no.	PIC	Alleles number	Size range (bp)
1	bnlg1007	1	0.76	9	79.09-151
2	bnlg2238	1	0.82	9	142.75-427
3	bnlg1627	1	0.66	6	115.75-249
4	bnlg1884	1	0.83	6	213.36-297,22
5	bnlg1556	1	0.79	10	145.5-311
6	bnlg1025	1	0.83	12	89.2-134,5
7	umc1292	1	0.79	8	151-240.83
8	bnlg1258	2	0.67	9	132.67-191.83
9	umc1736	2	0.64	4	123.5-151
10	bnlg1225	2	0.75	7	131.75-269.67
11	phi109642	2	0.66	5	132.67-191.83
12	bnlg1035	3	0.76	5	118-369
13	bnlg1447	3	0.86	10	96.4-236.75
14	dupssr17	3	0.82	8	71.33-129
15	umc1504	3	0.79	7	142.75-200
16	umc1776	3	0.69	9	135.11-191.83
17	phi072	4	0.75	7	140-235
18	umc2039	4	0.81	9	88.55-167.33
19	umc1008	4	0.86	8	151-427
20	umc2281	4	0.61	10	145.5-300.67
21	umc1109	4	0.49	6	112-151
22	bnlg1118	5	0.77	6	200-311
23	nc013	5	0.70	9	109-200
24	phi109188	5	0.76	10	130.22-249
25	phi048	5	0.78	7	159.17-249
26	umc2373	5	0.90	12	138-244.55
27	bnlg1154	6	0.87	12	151-255.89
28	bnlg1371	6	0.85	10	82-236.75
29	nc013	6	0.65	10	104.5-200
30	bnlg1740	6	0.90	15	110-249
31	nc009	6	0.68	8	104.5-287.75
32	phi034	7	0.80	10	120.44-221.78
33	phi328175	7	0.85	9	148.25-427
34	bnlg1200	7	0.85	10	104.5-242.88
35	bnlg339	7	0.86	11	183.67-330.33
36	phi057	7	0.70	6	140-224.5
37	phi080	8	0.86	9	142.75-249
38	phi233376	8	0.62	5	140-173.62
39	bnlg1350	8	0.77	8	122.89-173.62
40	umc1161	8	0.75	7	133.4-204.9
41	umc2042	8	0.87	10	100-280
42	phi065	9	0.78	10	130.83-240.09
43	bnlg1583	9	0.80	12	140-427
44	umc2337	9	0.79	9	111.25-218.38
45	phi016	9	0.61	3	145.5-170.6
46	phi028	9	0.43	4	74-102.57
47	bnlg1028	10	0.77	8	141.1-287.75
48	bnlg1655	10	0.76	9	113.5-242.88
49	umc1061	10	0.66	7	100-151
50	phi118	10	0.77	11	91-179.58
	Total		37.83	421	74-427
	Average		0.76	8.42	

Note: bp = base pair.

only 50% of the population (Fig. 3). Primers bnlg1740 and 1371 demonstrate high PIC values, as evidenced by the substantial number of alleles and their uniformly distributed distribution within the tested population (Fig. 4 and 5). The DNA Electropherogram image reveals that the band distribution of inbred lines G3 to G26 is more varied than inbred lines G29 to G44. This variation is further substantiated by the results depicted in Fig. 1, which show that inbred lines G29 to G44 have a limited number of alleles and do not demonstrate significant variation. Our investigation revealed that inbred lines G29 to G44 have a more substantial number of self-cross generations than inbred lines G3 to G26.

As illustrated in Table 2, inbred line G11 exhibited the highest number of pairs with the lowest similarity coefficient correlations, with inbred lines G30, G32, G33, G34, G37, G40, G41, G42, G43, and G44. Inbred line G5 demonstrated the lowest similarity coefficient correlation with G32, G42, and G43. Inbred line G14 exhibited the lowest similarity coefficient correlation to G36.These pairs can be utilized to create crosses that generate extensive diversity due to their low similarity values, indicating that the alleles belonging to these pairs are significantly divergent.

As depicted in Table 2, a genetic similarity matrix is presented for various genotypes, with each value in the matrix serving to quantify the degree of genetic similarity between two genotypes. The numerical values in the matrix represent genetic similarity coefficients, with values ranging from 0.57 to 0.85. Higher values in the matrix indicate more substantial genetic similarity, while lower values indicate less similarity. Genotypes with values closer to 1 are more genetically similar. For instance, G24 and G14 (0.81) exhibit a high degree of similarity, while G33 and G32 (0.85) demonstrate a very high level of similarity. Conversely, values closer to the lower end of the scale, such as 0.57, indicate a reduced degree of genetic similarity. For example, G16 and G42 (0.58) are less similar, and G11 and G24 (0.65) also exhibit a low level of similarity compared to other pairs. The clusters of similarity for all genotypes in the table suggest the presence of clusters or groups of genotypes with high intra-group similarity (Fig. 6). G32, G33, and G31 are relatively similar, and G7, G8, and G5 also form a closely related cluster. However, the presence

Table 2: The correlation coefficient of the similarity of each inbred line to the other inbred lines

	G3	G4	G5	G7	G8	G9	G11	G14	G15	G16	G17	G24	G25	G26	G29	G30	G31	G32	G33	G34	G35	G36	G37	G38	G39	G40	G41	G42	G43
G4	0.72																												
G5	0.66	0.72																											
G7	0.66	0.66	0.72																										
G8	0.66	0.64	0.68	0.74																									
G9	0.63	0.68	0.67	0.69	0.70																								
G11	0.61	0.64	0.66	0.63	0.62	0.69																							
G14	0.66	0.64	0.69	0.70	0.74	0.67	0.66																						
G15	0.60	0.61	0.66	0.69	0.67	0.73	0.67	0.63																					
G16	0.65	0.67	0.71	0.67	0.67	0.63	0.60	0.72	0.63																				
G17	0.61	0.70	0.67	0.69	0.67	0.70	0.70	0.67	0.73	0.70																			
G24	0.67	0.64	0.69	0.68	0.69	0.65	0.65	0.81	0.63	0.71	0.67																		
G25	0.63	0.72	0.70	0.67	0.68	0.69	0.67	0.71	0.67	0.72	0.79	0.75																	
G26	0.64	0.67	0.64	0.66	0.65	0.62	0.66	0.63	0.66	0.66	0.69	0.65	0.70																
G29	0.66	0.69	0.66	0.66	0.64	0.72	0.71	0.62	0.67	0.64	0.68	0.64	0.69	0.72															
G30	0.64	0.62	0.66	0.68	0.68	0.67	0.67	0.67	0.67	**0.59**	0.66	0.65	0.66	0.67	0.72														
G31	0.67	0.66	0.62	0.69	0.67	0.69	0.70	0.67	0.66	0.61	0.66	0.69	0.66	0.72	0.76	0.74													
G32	0.65	0.65	**0.58**	0.64	0.62	0.67	0.67	0.65	0.68	**0.58**	0.65	0.64	0.64	0.70	0.74	0.75	0.78												
G33	0.68	0.68	0.62	0.66	0.65	0.70	0.71	0.67	0.66	**0.58**	0.64	0.66	0.66	0.72	0.80	0.78	0.84	0.85											
G34	0.64	0.64	0.62	0.65	0.60	0.68	0.68	0.63	0.65	**0.58**	0.63	0.62	0.63	0.70	0.78	0.71	0.74	0.78	0.81										
G35	0.63	0.65	0.62	0.64	0.64	0.68	0.67	0.61	0.67	0.60	0.62	0.63	0.65	0.68	0.75	0.74	0.75	0.73	0.78	0.79									
G36	0.61	0.64	0.63	0.64	0.61	0.63	0.65	**0.59**	0.67	0.61	0.63	0.60	0.67	0.67	0.72	0.67	0.68	0.68	0.72	0.74	0.72								
G37	0.66	0.63	0.63	0.64	0.63	0.66	0.68	0.62	0.66	**0.58**	0.61	0.65	0.64	0.67	0.77	0.74	0.74	0.71	0.79	0.74	0.79	0.70							
G38	0.65	0.60	0.63	0.65	0.65	0.64	0.66	0.64	0.69	0.60	0.65	0.64	0.65	0.68	0.75	0.76	0.75	0.76	0.78	0.74	0.75	0.74	0.75						
G39	0.67	0.65	0.62	0.68	0.66	0.69	0.69	0.67	0.68	0.60	0.68	0.69	0.68	0.68	0.76	0.77	0.76	0.75	0.78	0.79	0.79	0.70	0.83	0.79					
G40	0.66	0.66	0.64	0.67	0.66	0.68	0.66	0.66	0.68	**0.58**	0.63	0.65	0.69	0.71	0.77	0.77	0.77	0.72	0.81	0.74	0.79	0.71	0.82	0.77	0.82				
G41	0.64	0.65	0.62	0.64	0.63	0.68	0.66	0.62	0.69	**0.57**	0.62	0.63	0.61	0.65	0.77	0.73	0.74	0.72	0.81	0.71	0.76	0.70	0.76	0.74	0.77	0.78			
G42	0.61	0.61	**0.57**	0.61	0.61	0.67	0.63	0.62	0.65	**0.58**	0.62	0.63	0.63	0.66	0.71	0.70	0.70	0.72	0.75	0.74	0.76	0.72	0.75	0.72	0.77	0.77	0.78		
G43	0.67	0.63	**0.59**	0.64	0.64	0.68	0.66	0.64	0.65	**0.59**	0.62	0.64	0.65	0.67	0.71	0.70	0.73	0.69	0.79	0.73	0.77	0.70	0.78	0.72	0.79	0.77	0.77	0.80	
G44	0.65	0.65	0.61	0.64	0.64	0.68	0.68	0.63	0.67	**0.59**	0.65	0.65	0.68	0.70	0.76	0.71	0.74	0.72	0.76	0.78	0.76	0.72	0.81	0.75	0.80	0.79	0.72	0.76	0.80

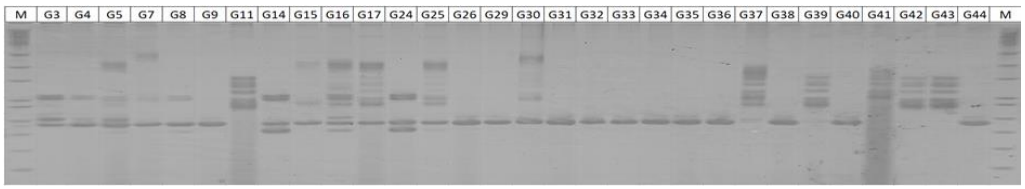

Fig. 3: Electrophoregram of DNA Amplification of Inbred lines with nc009. M: marker G3-44: Inbred lines.

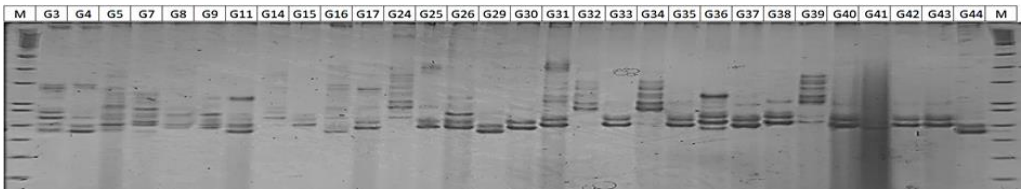

Fig. 4: Electrophoregram of DNA Amplification of Inbred lines with Bnlg1740 M: marker G3-44: Inbred lines.

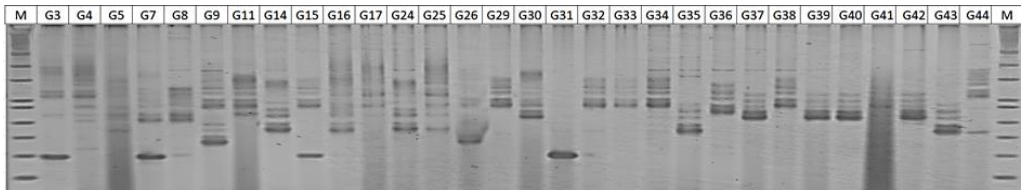

Fig. 5: Electrophoregram of DNA Amplification of Inbred lines with Bnlg1371. M: marker G3-44: Inbred lines.

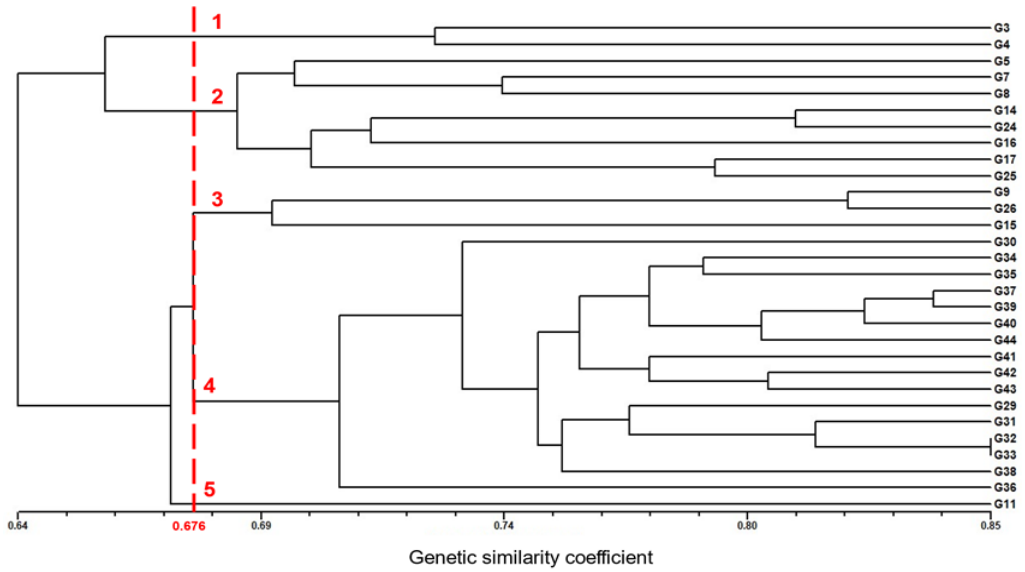

Fig. 6: Phylogenetic tree of 30 convergent-derived maize (Zea mays) inbred lines based on SSR molecular markers using NTSYS-pc software version 2.02i.

of an outlier is evident. Some genotypes, such as G11, tend to have lower values than others (e.g., G11 and G8 = 0.62, G11 and G14 = 0.66).

Phylogenetic analysis of 30 samples of maize inbred lines based on SSR molecular markers revealed a similarity coefficient level ranging from 0.57 to 0.85, with a correlation value of r = 0.88. At a similarity coefficient level 0.676, maize inbred lines were grouped into five distinct categories. Group I consists of two lines, G3 and G4, with a similarity coefficient of 0.655. Group 2 comprises eight lines, namely G5, 7, 8, 14, 24, 16, 17, and 25, with a similarity level of 0.655. Group 3 consists of three inbred lines, namely G9, 26, and 15, with a similarity coefficient of 0.676. Group 4, which contains the most inbred lines (17), is notable for its high genetic similarity (0.676). This group includes G30, 34, 35, 37, 39, 40, 44, 41, 42, 43, 29, 31, 32, 33, 38, and 36. Conversely, Group 5, which only has an inbred line (G11), exhibits a lower level of genetic similarity (0.676). Within Group 4, it is evident that there exists a pair of inbred lines, designated as G32 and G33 that exhibit a remarkably high degree of genetic similarity, with a similarity coefficient of 0.85. This level of similarity indicates that 85% of the alleles present in the two genotypes are identical at the 50 SSR markers utilized in this study.

DISCUSSION

It is hypothesized that the number of SSR primers used was inadequate for thoroughly detecting diversity in the population of convergent-derived maize inbred lines. This is due to the lack of information regarding the number of alleles and allele size obtained despite using markers with high PIC values. It is recommended that more markers be employed to detect genetic diversity in convergent breeding populations, thereby preventing errors in the selection of lines. While the extent and quality of kinship between parental plants are crucial in assessing genetic diversity, our findings underscore the pivotal role of polymorphic primers. This underscores the need for further investigation, particularly given the multifaceted nature of factors influencing cross outcomes, including genetic, environmental, and interplay. This is particularly relevant in anticipating inbreeding depression in crosses involving lines for which the genetic background is not fully understood (Suganthi et al., 2020). This inbreeding depression factor can influence the diversity of alleles and the number of alleles possessed by an individual plant in the selling process. This phenomenon is exemplified by the lines G29-G44, which exhibit a more advanced selfing generation than the lines G3-G26. The lines G29-G44 demonstrate a reduction in allele size diversity, resulting in fewer alleles than the lines G3-G26. We identified lines that can maintain allelic diversity from the two groups with different selfing generations, such as lines G16 and G36. Further investigation is necessary to determine how these lines perform in the field and their stability as potential inbred lines.

The high similarity coefficients exhibited by the convergent-derived maize inbred line populations suggest that these populations are still quite closely related genetically (Mathiang et al., 2022). Convergent crosses were performed on diverse parents, with interchangeable crosses of male and female parents. This crossing process did not result in significant diversity in most of the population. Although some lines were quite distant from the common lines, not enough lines like the G11 lines were obtained. This outcome may be attributed to the selection criteria employed during the previous population's formation, which can narrow the genetic diversity within the population and result in a high degree of similarity, as evidenced by the lines G32 and G33. These lines share traits in the field that have become selection standards, such as large cob size.

The results of this study have revealed several critical factors that must be considered when making crosses to obtain genetic diversity. SSR markers have proven to be a highly effective method of detecting the genetic diversity of maize populations that experience inbreeding depression due to genetic repetition originating from crossing parents whose genetic background is unknown. This can be detected by the number of allelic variations and the number of alleles possessed by each plant. Furthermore, this method can determine the optimal selfing generation to terminate self-pollination. After this determination, a trial cross should be executed to avert the erosion of diverse alleles caused by inbreeding depression resulting from excessive selfing (Ali et al., 2023).

The study utilized 50 SSR primers distributed across 10 maize chromosomes, each containing 4 to 6 primers. This distribution helps ensure that the genetic analysis covers a wide range of the maize genome. The total Polymorphic Information Content (PIC) value of 37.83, averaging 0.76 per marker, indicates a high level of genetic diversity. The high average PIC value suggests that the markers can effectively distinguish between different genetic variations in the derived-convergent maize population. Four hundred twenty-one alleles were identified, averaging 8.42 alleles per marker, which signifies robust polymorphism levels. Alleles are different forms of a gene found at the same place on a chromosome. A high number of alleles per marker indicates good polymorphism, meaning there is a lot of genetic variation in the derived-convergent maize population. This is particularly significant for breeding programs and genetic studies, as it furnishes a substantial source of genetic diversity for further investigation. The observed range of allele sizes varied from 74 to 427 base pairs, thus exhibiting a broad size distribution. This extensive range of allele sizes can be advantageous for various genetic analyses, as it offers more detailed insights into the genetic composition of the maize inbred lines under consideration. Concurrent findings from diverse research groups have reported analogous outcomes in maize, employing a range of SSR markers for PIC values (Shehata et al., 2009; Suteu et al., 2014). An average of 4.9 alleles was derived from 83 SSR loci in 40 U.S. maize inbred lines (Lu and Bernardo, 2001). Furthermore, an analysis of 85 SSR loci in 416 bands of CIMMYT maize inbred lines revealed an average of 4.9 alleles, with a range of 2 to 14 alleles per locus (Warburton et al., 2002). 260 US maize inbred lines used 94 SSR loci result an average of 21.7

alleles per locus (Liu et al., 2003). The low genetic variation on chromosome 2 in our population must be balanced by crossing with inbred lines that have a high allelic variation on chromosome 2 (Charlesworth & Willis, 2009; Kiani et al., 2015; Hassan et al., 2018; Ilyas et al., 2020).

In this study, we examined the genetic diversity of several marker genes (e.g., umc2373, bnlg1740, bnlg1154, umc2042, bnlg1200, phi328175) using the polymerase chain reaction (PCR) method. The analysis revealed that markers such as umc2373, bnlg1740, bnlg1154, umc2042, bnlg1200, and phi328175 exhibit PIC values close to 0.9, indicating their high level of genetic polymorphism. This finding underscores their significant value for genetic studies, as they can reveal more differences between individual plants, making them a valuable tool for maize genetics research (Sathua et al., 2018). Conversely, markers such as umc1109 and phi028 exhibited lower PIC values, indicating poor allele distribution within the population genome. This finding is analogous to the genetic assessment of 70 maize landraces, which also exhibited the lowest PIC value for marker Phi (Yousuf et al., 2021). Furthermore, markers exhibiting a high number of alleles, including bnlg1025, umc2373, bnlg1740, bnlg1154, phi118, bnlg1583, and bnlg339 (11-15 alleles per primer), have been shown to reflect significant genetic diversity. The SSR markers utilized in this study are highly effective for assessing genetic diversity in inbred lines of maize derived from convergent breeding, with several markers demonstrating substantial polymorphism and allele diversity (Jambrovic et al., 2018). The PIC value and the number of alleles are contingent on the extent of the genetic diversity present within the population under study. Consequently, it is imperative to select the most suitable marker to effectively capture the population's genetic diversity, particularly in cross-breeding, to develop novel hybrids in maize (Oyenike et al., 2018; Zebire, 2020). Some previous studies, such as analyzing five inbred lines, used 50 polymorphic SSR markers, including bnlg and umc markers. PIC values ranged from 0.164 to 0.672, with the bnlg marker showing higher PIC values due to its ability to detect more allelic variation (Neelothpala et al., 2022). In evaluating 24 maize genotypes using 14 SSR primers, we found that the SSR primer bnlg had the highest PIC value of 0.75, indicating its high polymorphism potential and effectiveness in detecting allelic variation. Another study reported that the average PIC value for SSR markers was 0.49, with the bnlg marker having a PIC value as high as 0.73, and the umc marker highlighting its efficiency in identifying genetic diversity (Kyi et al., 2022).

The present study examined the performance of SSR markers concerning their design and functionality. The study focused on bnlg, a primer that has been demonstrated to exhibit a higher PIC compared to other types of SSR primers. This superior performance of bnlg is attributed to its design, which targets SSR loci with high polymorphism potential, long and varied SSR motifs, resulting in more allelic variation, even distribution of loci throughout the genome, and effective detection of many alleles with even frequencies. The analysis revealed that these factors contribute to the higher PIC value of bnlg primers, leading to more frequent production of high allelic variation. In contrast, UMC SSR primers possess high PIC values due to their efficient design, which targets polymorphism-rich SSR loci. The development of UMC primers involved a structured approach, utilizing genomic and cDNA libraries. The determination of SSR loci is achieved through the selection of sequences that are rich in polymorphism and exhibit high diversity among individuals. UMC SSRs target unique loci in the genome that vary between genotypes, thereby enhancing the ability to detect allelic variation. They prioritize short SSR motifs that mutate rapidly. The validity of UMC primers has been demonstrated in numerous genetic mapping, QTL, and genetic diversity analysis studies. These primers have been shown to detect high polymorphism across a wide range of populations and genetic conditions, making them among the most reliable SSR primers available. In a study of convergent-derived inbred lines, it was demonstrated that SSR primers BNLG and UMC were more sensitive to detecting diversity. These SSR markers have also been employed in constructing fingerprinting for various maize-inbred lines in China (Wang et al., 2011). This outcome is attributable to the inbreeding process (repeated self-pollination), which aims to enhance homozygosity. A homozygous inbred line is characterized by the presence of a single allele at each locus, as both copies of the allele on homologous chromosomes are identical. The objective of establishing inbred lines is to attain homozygosity; however, this results in diminished genetic diversity, rendering inbred lines more vulnerable to environmental stress and the accumulation of deleterious recessive traits (Caballero & García-Dorado, 2013).

Maize inbred lines with few alleles are subject to a loss of genetic flexibility, rendering them more susceptible to environmental stress and pathogens. This results in reduced agronomic potential, including yield, vigour and resistance. Furthermore, limited genetic diversity impedes breeding, as it reduces the potential for heterosis and genetic innovation. To address these limitations, it is recommended to either cross inbred lines with germplasm from diverse populations or employ genetic enrichment through modern breeding technologies for specific trait improvement (Senior et al., 1998; Zhao et al., 2024). The number of alleles can also be a descriptor of the genetic diversity present in every genotype, as well as the genetic drift of a given population (Greenbaum et al., 2014). Our findings indicate that the number of crossing elders does not serve as a determining factor in the diversity of alleles possessed by a genotype. Instead, it is the number of generations of self-crossing that has the capacity to determine the diversity of alleles in an individual. This is due to the effect of inbreeding depression, which leads to the loss of alleles from an individual plant.

The genetic similarity matrix is a tool used in hierarchical clustering to generate a dendrogram, which organizes the genotypes into clusters based on their pairwise similarity values (Levenstien et al., 2003). This matrix facilitates breeding decisions by allowing researchers to identify closely related genotypes, thereby preventing inbreeding or selecting genetically diverse

pairs for enhanced hybrid vigor (Lawson & Falush, 2012). The genetic similarity coefficient facilitates the analysis of genetic diversity and the assessment of overall diversity within a population. Higher similarity values indicate lower diversity. The coefficient can be used to validate genetic grouping hypotheses or compare results with other clustering methods (Bocianowski et al., 2024). The coefficient value can vary depending on the number of primers used in a given population (Lamboy, 1994). Conversely, G11 is classified in a distinct group, suggesting notable differences between this inbred line and the others.

Populations resulting from convergent breeding can have narrow diversity for several reasons. Strict selection in the convergent breeding process may cause only individuals with highly desirable traits to be selected for mating. This can reduce genetic variation as only a small portion of the initial population is used. Limited use of parental lines in a cross has low genetic diversity, the resulting cross will also show limited diversity. Intensive selection processes can lead to a genetic bottleneck, where only a few individuals contribute to the next population, reducing overall genetic variation (Zafar et al., 2022; Zafar et al., 2024). The goal of convergent breeding is to combine superior traits from different parental lines into one homogeneous genotype. This process can reduce genetic variation as the focus is on homogenizing certain traits. By understanding these factors, plant breeders can take steps to maintain genetic diversity, such as using more diverse parental lines or combining other breeding methods (Nur et al., 2017; Zafar et al., 2023). Using appropriate markers such as SSR can improve the accuracy in the selection of potential maize strains obtained from the inbreeding process of the convergent breeding population, as we obtained from our current research results, which, of course, still need field confirmation to support the data we obtained at this time.

Conclusion

The genetic diversity analysis of maize inbred lines derived from convergent breeding using SSR markers revealed significant genetic variation within the population. The study demonstrated that SSR markers are highly effective in detecting genetic diversity, with high sensitivity, reproducibility, and consistency. The results showed that the inbred lines exhibited varying levels of genetic similarity, with some lines displaying high genetic diversity and others showing limited variation. The findings highlight the importance of using sufficient SSR markers to assess genetic diversity and accurately avoid errors in line selection. The study also emphasized the need for further investigation into the performance and stability of inbred lines in the field. Overall, the combination of convergent breeding and SSR marker analysis provides valuable insights into the genetic diversity of maize populations, supporting the development of superior and adaptive maize varieties for sustainable agriculture Lines.

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Author's Contribution: MF, MA, RE and AN led the project's conceptualization. KS, WBS, NNA, and MFA handle data curation. KS and MFA carried out a formal analysis. Funding acquisition was the responsibility of MF and MA. RE, NNA, SBP, MM, and MFA conducted the investigation. MF, MA, RE, AN, MFA, and KS developed the methodology. MF and MA were in charge of project administration. MF, RE, SBP, and AN provided resources. KS and MFA managed the software. MF, MA, AN, and RE supervise. MFA, SBP, KS, and WBS performed validation. Visualization by MFA and KS. Writing the original draft involved MF, KS, and MFA. MA, AN, RE, WBS, NNA, and SBP did review and editing.

Data availability: All the data is available in the article.

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