

## SSR-MARKER BASED GENETIC DIVERSITY IN SRI LANKAN TRADITIONAL MAIZE (*ZEA MAYS* L.) ACCESSIONS

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### ABSTRACT

The beneficial alleles possessed by maize landraces are valuable resources for improving novel climate resilient maize varieties *via* crop breeding initiatives. Therefore, this study investigated the genetic diversity of 20 traditional maize accessions of Sri Lanka using five SSR markers (*umc1545*, *bnlg1627*, *umc1342*, *bnlg105* and *bnlg1564*). A total of 35 alleles were identified by all the SSR markers, with observed allele numbers ranging from 4 (*umc1545*) to 10 (*bnlg1627*). The higher values of effective number of alleles, observed heterozygosity, and expected heterozygosity for markers *bnlg1627* and *bnlg1564* suggest their potential as key contributors in identifying the genetic variability within the maize accessions. The Polymorphism Information Content (PIC) values ranged from 0.670 to 0.827 with a mean value of 0.735, further highlighting the informativeness of the markers. Considering the genetic diversity among the accessions, *SEU6*, *SEU20*, and *SEU23* consistently displayed higher levels in their genetic diversity values while the genetic dissimilarity values ranged from 0.1 to 1.0 with the mean value of 0.81. The accessions *SEU3* and *SEU4* were closely linked among the pairwise combinations. Further, the cluster analysis identified five clusters of Sri Lankan maize accessions. Based on the results of this study, *SEU6* and *SEU20* can be the potential candidates for conservation and beneficial for the adaptability of maize to changing environments. These results can pave the way for targeted maize breeding strategies and highlight the need for continued efforts in improving food, feed and forage industries, with implications for enhancing crop resilience and sustainable agricultural development.

**Keywords:** Crop breeding, Genetic diversity, SSR markers, Traditional maize accessions

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## INTRODUCTION

Maize (*Zea mays* L.), a member of the Poaceae family, is a widely cultivated grass of substantial agricultural importance (Yadesa, 2021). It is a crop with a long history dating back thousands of years. Maize has risen to global prominence, becoming one of the most extensively produced crops across continents. It is grown from North and South America through Africa, Europe, and Asia, adapting to different climates and soils (Cairns et al., 2012). In many nations, maize is a staple crop, supplying necessary nutrients through its grains, which can be consumed fresh, dried, or processed into a variety of items (Ranum et al., 2014). Maize has various purposes beyond food production, such as animal feed in the form of grains and forage, an ingredient in biofuels and other industrial products (Tanumihardjo et al., 2020). Because of its adaptability and versatility, maize is an essential part of the world's agricultural landscape.

Maize farming confronts a number of significant concerns around the world (Shahzad et al., 2021). Changing weather patterns, rising temperatures, more frequent extreme weather events, and insect and disease attacks have all caused immense problems on maize productivity (Tripathi et al., 2016). Addressing these concerns requires an integrated strategy that utilizes the exploration of genetic diversity and identify the candidate genotypes with greater potentials for improved photosynthetic, biomass with rapid forage and grain yield production. In this light, maize landraces are regarded as the living heritage of agricultural diversity, embodying ancestral wisdom and local adaptation of maize farming. These traditional maize varieties, shaped by generations of farmers, have developed to survive in specific conditions, displaying incredible resistance and unique features (Nashath et al., 2023). Various landraces have had a significant impact on the world's maize genetic resources. Preserving these maize landraces by investigating their genetic diversity holds potential for improving agricultural sustainability (Mufeeth et al., 2023).

The molecular-based characterization is a revolutionary method of studying the genetic diversity of plant populations' intricate complexity (Wambugu and Henry, 2022). This accurate and effective technology enables the identification and analysis of specific genetic markers linked with desirable features, allowing for the selective breeding of plants with superior characteristics (Barcaccia, 2010). Traditional observational approaches are limited by molecular techniques, which provide a full perspective of genetic diversity at the molecular level (Wang et al., 2020). When comparing with other markers including AFLPs and RAPDs, Simple Sequence Repeat (SSR) markers, commonly known as microsatellites, offer several distinct advantages in plant genetic studies. Some of the important advantages include their high degree of polymorphism, co-dominant nature, and relative abundance throughout plant genomes, making them widely relevant and transferrable between species (Silva et al., 2012; Wang et al., 2010). Furthermore, several studies have demonstrated the utilization of SSR markers by researchers all around the world to

describe the genetic makeup of maize landraces (Mathiang et al., 2022; Vathana et al., 2019; Adeyemo and Omidiji, 2019; Belalia et al., 2019). The genetic diversity of Sri Lankan maize landraces has been explored using SSR markers related to photosynthetic, canopy architectural and grain yield traits (Nashath et al., 2024) and insect resistant traits (Nashath et al., 2023) in recent studies. The study's objectives were to determine genetic variation among 20 Sri Lankan maize accessions using 5 SSR markers, to examine the polymorphism of the chosen SSR marker set, and lastly to estimate the genetic diversity characteristics of the investigated maize accessions.

## MATERIALS AND METHODS

### Plant material

Twenty maize accessions (Nineteen Sri Lankan traditional maize landraces and one high-performing commercial cultivar *Bhadra*) were used for the study, among which 12 were collected from Badulla, six were from Ampara and one was from Trincomalee, major maize growing districts in Sri Lanka (Table 1) (Mubarak et al., 2023). The collected seeds were multiplied and stored in airtight containers at 1 °C for future usages. These collections were grown inside a net house of the Department of Biosystems Technology, Faculty of Technology, South Eastern University of Sri Lanka (7° 18'00" N and 81° 51' 41" E, 16 m above sea level) for DNA extraction.

Table 1. List of maize accessions and their collection site.

S.N	Accessions	Collected area	District	Latitude	Longitude	Name of the farmer	Quantity collected
1	SEU1	Ridimaliyadda	Badulla	7° 14' N	81° 6' E	AI Praneeth	200 g
2	SEU2	Ridimaliyadda	Badulla	7° 14' N	81° 6' E	AI Praneeth	250 g
3	SEU3	Kirawana, Padiyathalawa	Ampara	7° 4' N	81° 22' E	YM Shayama	1 kg
4	SEU4	Udakumbure Gedara, Kandaketiya	Badulla	7° 21' N	81° 01' E	AN Dharmawardana	200 g
S.N	Accessions	Collected area	District	Latitude	Longitude	Name of the farmer	Quantity collected
5	SEU5	Dehigama, Kandakatiya	Badulla	7° 29' N	80° 56' E	S Bandara	250 g
6	SEU6	Padiyathalawa	Ampara	7° 36' N	81° 20' E	WM Karunapala	2 kg
7	SEU7	Padiyathalawa	Ampara	7° 36' N	81° 20' E	WM Karunapala	2 kg
8	SEU8	Padiyathalawa	Ampara	7° 36' N	81° 20' E	I.M. Darmawardena	1 kg
9	SEU9	Kandepoththawa, Baduluoya, Kandakatiya	Badulla	7° 12' N	80° 59' E	YM Yasapala	250 g

S.N	Accessions	Collected area	District	Latitude	Longitude	Name of the farmer	Quantity collected
10	SEU10	Kirawana, Padiyathalawa	Ampara	7° 4' N	81° 22' E	YM Shayama	3kg
11	SEU11	Kirawana, Padiyathalawa	Ampara	7° 4' N	81° 22' E	YM Shayama	1kg
12	SEU14	Udakumbure Gedara, Kandaketiya	Badulla	7° 21' N	81° 01' E	AN Dharmawardana	200 g
13	SEU15	Kandepoththawa, Baduluoya, Kandaketiya	Badulla	7° 12' N	80° 59' E	AN Dharmawardana	500 g
14	SEU16	Kandepoththawa, Baduluoya, Kandaketiya	Badulla	7° 12' N	80° 59' E	AN Dharmawardana	250 g
15	SEU17	Udakumbure Gedara, Kandaketiya	Badulla	7° 21' N	81° 01' E	AN Dharmawardana	200 g
16	SEU18	Baduluoya	Badulla	7° 12' N	80° 59' E	NK	1kg
17	SEU20	Nagadeepaya	Badulla	7° 26' N	81° 12' E	NK	1kg
18	SEU21	Kinniya	Trincomalee	8° 5' N	81° 18' E	NK	1kg
19	SEU23	Dehigama, Kandaketiya	Badulla	7° 29' N	80° 56' E	S Bandara	500 g
20	Bhadra	DoA	-	-	-	-	-

NK – Not Known

### Maize DNA extraction

Using the DNeasy plant Mini Kit (Qiagen, Milan, Italy), genomic DNA was extracted from young, three-weeks-old maize seedlings (three seedlings per accession) in accordance with the manufacturer's instructions. A UV spectrophotometer (Genesys 10S UV-Vis, Thermo Scientific Inc.) was used to verify the DNA's purity and calculate its concentration. Purified water was used to dilute the DNA samples to 50 ng/l, and they were then kept in the freezer at -20 °C for subsequent usage.

### SSR analysis

In this study, five SSR primer pairs from Singode and Prasanna, (2010), Vathana et al. (2019) and Chen et al. (2008) were chosen based on their high performance in maize genetic diversity studies (Table 2). A total of 15 µl of materials were used for the PCR, including 2 µl of DNA template, 7.5 µl of Taq ReadyMix (2X FastGene®), 1 µl of forward and reverse primers, and 3.5 µl of distilled water. PCR reaction was performed in a PCR machine (Prima-96, HiMedia, LA949, China) and the DNA amplifications were carried out in 35 cycles of 30 seconds at 94 °C, 30 seconds at 52 - 55 °C, 30 seconds at 72 °C, and a 10 minute final extension period at 72 °C. The

PCR products were dissolved on a 3% Agarose gel (FastGene®, Nippon Genetics Europe) and separated using a horizontal gel electrophoresis apparatus (Enduro, Labnet, USA) at 90 volts. Then staining was done for 15 minutes with ethidium bromide (1 g/ml), and then de-staining for 10 minutes using distilled water. A gel documentation system (Axygen, GD1000, USA) was used to examine the gels under ultraviolet light and photographs were taken for later examination (Nashath et al., 2023).

Table 2. SSR primer details used in this study

Locus	Primer sequences	Motif	T <sub>A</sub> (°C)
<i>umc1545</i>	F – GAAAACACTGCATCAACAACAAGCTG R - ATTGGTTGGTTCCTTGCTTCCATTA	(AAGA) <sub>4</sub>	54
<i>umc1342</i>	F – TCTAATCCAATCGACATCGACAGA R - TCGCCCTCTTTTCTTTTCTTTTCT	(AG) <sub>8</sub>	52
<i>bnlg105</i>	F – GACCGCCCGGGACTGTAAGT R - AGGAAAGAAGGTGACGCGCTTTTC	N/A	55
<i>bnlg1627</i>	F – CGGACGGGGGTATTAAAAT R - TGTGTTTCGAGAATCTCTCG	(AG) <sub>19</sub>	54
<i>bnlg1564</i>	F – ACGGGAGAACAAAAGGAAGG R - CTCTCCCTCACATCCGCC	(AG) <sub>24</sub>	54

### Data analysis

The bands that the primers produced were graded individually based on 100 bp DNA ladder and the data were scored as binary data (0 for absence and 1 for presence) in an Excel sheet separately for SSR markers and maize landraces for data analysis. The observable number alleles ( $N_a$ ), effective number of alleles ( $N_e$ ), expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosity, Shannon diversity index ( $I$ ), and Fixation index ( $F$ ) among maize accessions and SSR markers were all determined by GenAlex (6.51b2) software (Smouse and Peakall, 2012). The Polymorphic Information Content (PIC) values were calculated according to Sharma et al. (Sharma et al., 2009). The genetic distances were estimated for the 20 maize germplasm sets using DARwin 6 software. The binary data matrix was converted into a dissimilarity matrix based on the Jaccard coefficient. A UPGMA-based dendrogram and a principal coordinate analysis (PCoA) were then constructed using the matrix (Khan et al., 2022).

## RESULTS AND DISCUSSION

### SSR Polymorphism

Table 3 provides a summary of the genetic characteristics of the SSR markers examined in this investigation. These variables offer important information about the

genetic diversity of the studied samples. There were a total of 35 alleles produced by all the SSR markers. The observed allele number ( $N_a$ ) ranged from 4 (*umc1545*) to 10 (*bnlg1627*), with an average of 7. The markers effective allele numbers ( $N_e$ ), varied from 3.505 for *umc1342* to 6.446 for *bnlg1627*, indicating variations in the allele distribution and frequencies. The observed heterozygosity ( $H_o$ ) values in this study ranged from 0.000 (*bnlg105* and *umc1342*) to 0.368 (*bnlg1627*). Moreover, the  $H_e$  values ranged from 0.715 (*umc1342*) to 0.845 (*bnlg1627*), with higher  $H_e$  values for markers *bnlg1627* and *bnlg1564*, indicating their importance in maintaining genetic diversity. The PIC values varied from 0.670 (*umc1342*) to 0.827 (*bnlg1627*). Further, the fixation index ( $F$ ) values ranged between 0.564 (*bnlg1627*) and 1.000 (*bnlg105*).

The marker *bnlg1627* showed the greatest  $N_a$  value, indicating a locus with a comparatively higher level of allelic diversity. Notably, the absence of any observable heterozygosity was completely evident for the possible genetic fixation or inbreeding within the population. Based on allele frequencies, expected heterozygosity ( $H_e$ ) depicts the genetic diversity within a population. The PIC value of a marker is a measure of how informative it is in identifying alleles and provide insights into the marker's utility for genetic studies (Kim et al., 2021). The PIC values of all the markers used in this study were higher than 0.5 expressing the potential of those in classifying the given maize accessions sets (Varshney et al., 2007). Markers with higher PIC values, such as *bnlg1627* and *bnlg1564*, offer greater discriminatory power in distinguishing alleles, making them valuable tools for genetic analyses and selection strategies. The higher values of  $N_a$ ,  $N_e$ ,  $H_o$ , and  $H_e$  for markers *bnlg1627* and *bnlg1564* suggest their potential as key contributors to maintaining genetic variability within the population. According to Guo et al. (2018), marker *bnlg1564* is linked with ear length and *bnlg1627* (Figure 1) is linked with shoot dry weight traits of maize plants. Markers *umc1342* and *bnlg105* exhibited lower levels of genetic diversity, as indicated by their absence of observed heterozygosity and lower expected heterozygosity and due to the fixation of certain alleles within these loci.

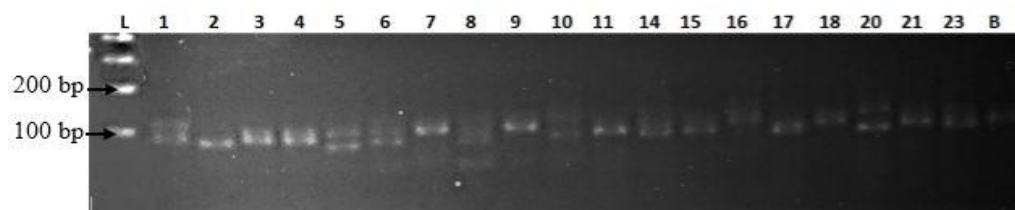


Figure 1. PCR amplification profile of 20 Sri Lankan maize accessions with the SSR marker *bnlg1627*. L: 100 bp ladder, the numbers in the gel correspond to the identification of maize accessions as indicated in table 1.

Table 3. Summary statistics of genetic parameters of the SSR markers

SSR marker	No of observed alleles ( $N_a$ )	No of effective alleles ( $N_e$ )	Observed Heterozygosity ( $H_o$ )	Expected Heterozygosity/ Genetic diversity ( $H_e$ )	Polymorphic Information Content (PIC)	Fixation Index ( $F$ )
<i>bnlg1627</i>	10	6.446	0.368	0.845	0.827	0.564
<i>bnlg1564</i>	8	4.819	0.200	0.793	0.770	0.748
<i>umc1342</i>	5	3.505	0.000	0.715	0.670	1.000
<i>bnlg105</i>	8	4.255	0.000	0.765	0.733	1.000
<i>umc1545</i>	4	3.636	0.100	0.725	0.675	0.862
Average	7	4.532	0.133	0.768	0.735	0.835
Maximum	10	6.446	0.368	0.845	0.827	1.000
Minimum	4	3.505	0.000	0.715	0.67	0.564

Similar to previous research, the SSR markers in this study exhibited polymorphism, generating a substantial number of alleles, showcasing the diversity within the population. Joshi et al. (2020) analyzed 23 maize landraces cultivated in Nepal with 5 SSR markers and determined the average allele number as 2 per locus,  $H_o$  as 0.687,  $H_e$  as 0.445 and the PIC value as 0.345. A sum of 65 alleles were identified in the 40 maize populations studied by Vivodik et al. (2017) using 10 SSR markers. The number of these alleles fluctuated between 4 and 8 (mean value of 6.5), while the PIC value varied from 0.734 to 0.848 (mean PIC of 0.810). In another investigation, nine SSR markers with 0.297 PIC and 0.373 gene diversity values were used to identify a total of 19 polymorphic alleles in eight maize genotypes (Kumari et al., 2018). According to PIC values (0.367), the *phi064* and *phi053* were shown to be the best markers for genotype detection. Using 12 SSR markers, a total of 40 alleles were found in 38 maize hybrids, with an average of 3.33 alleles per locus (Shiri, 2011). The 12 SSR loci in that study had a mean PIC of 0.53 and a PIC ranging from 0.23 (*Phi080*) to 0.79 (*UMC2359*).

### Maize diversity

A detailed analysis of the genetic diversity parameters found in a group of Sri Lankan traditional maize accessions is provided in Table 4. These variables offer insightful information on the genetic diversity present in the examined maize germplasm. The observed alleles ( $N_a$ ) detected ranged from 2 (*SEU4*) to 6 (*SEU6*, *SEU20*, and *SEU23*), which reflects different levels of allelic diversity. The highest  $N_a$  values were found in three accessions suggests a comparatively higher level of allele diversity within these accessions. Effective alleles ( $N_e$ ), with a mean value of 3.834, ranged from 1.515 (*SEU1*) to 5.556 (*SEU6* and *SEU20*). The observed heterozygosity ( $H_o$ ) values ranged from 0.000 to 0.400. Significantly, the accessions *SEU3*, *SEU20*, and *SEU23* showed higher observed heterozygosity values, indicating greater genetic diversity within these accessions. Expected heterozygosity ( $H_e$ ) and

PIC values were highest in *SEU1* (0.820 and 0.794, respectively), and lowest in *SEU6* and *SEU20* (0.314 and 0.340, respectively). The fixation index ( $F$ ) values which measures the inbreeding level among the population ranged from 0.259 to 1.000.

The observed variations in  $N_a$ ,  $N_e$ ,  $H_o$ ,  $H_e$ , PIC, and  $F$  provide a comprehensive view of the genetic makeup of the tested maize accessions. Accessions with higher  $N_a$  and  $N_e$  values (*SEU6* and *SEU20*) exhibit greater genetic diversity. These accessions are potential candidates for conservation and breeding due to their diverse allele repertoire. Higher observed and expected heterozygosity suggests a higher level of genetic variation within the population. This is beneficial for the adaptability and resilience of maize to changing environments. Moreover, the degree of  $H_o$  was discovered to be lower than  $H_e$ , probably due to inbreeding (Vahdati et al., 2015) since landraces are restricted to particular ecological niches with limited geographic ranges, which causes significant fixation of alleles (Yousuf et al., 2021). This is also proven by the higher  $F$  values in most of the maize landraces analyzed in this study. Additionally, the farmers grow landraces from their own preserved seed, frequently from little seed samples that could have led to random drift and thus increased homozygosity. The accessions *SEU6*, *SEU20*, and *SEU23* consistently displayed higher levels of genetic diversity, as indicated by their higher genetic diversity values. These accessions stand out as potential reservoirs of genetic diversity, making them valuable resources for future breeding programs.

Table 4. Genetic information of Sri Lankan traditional maize accessions

Accessions	Number of observed alleles ( $N_a$ )	Number of effective alleles ( $N_e$ )	Observed Heterozygosity ( $H_o$ )	Expected Heterozygosity/ Genetic diversity ( $H_e$ )	Polymorphic Information Content (PIC)	Fixation Index ( $F$ )
<i>SEU1</i>	3.000	1.515	0.200	0.340	0.314	0.412
<i>SEU2</i>	3.000	2.462	0.250	0.594	0.511	0.579
<i>SEU3</i>	3.000	2.174	0.400	0.540	0.466	0.259
<i>SEU4</i>	2.000	1.724	0.200	0.420	0.332	0.524
<i>SEU5</i>	4.000	3.333	0.200	0.700	0.645	0.714
<i>SEU6</i>	6.000	5.556	0.200	0.820	0.794	0.756
<i>SEU7</i>	5.000	5.000	0.000	0.800	0.768	1.000
<i>SEU8</i>	4.000	4.000	0.000	0.750	0.703	1.000
<i>SEU9</i>	5.000	5.000	0.000	0.800	0.768	1.000
<i>SEU10</i>	5.000	4.545	0.200	0.780	0.745	0.744
<i>SEU11</i>	4.000	3.571	0.000	0.720	0.672	1.000
<i>SEU14</i>	3.000	2.778	0.000	0.640	0.563	1.000



Accessions	Number of observed alleles ( $N_a$ )	Number of effective alleles ( $N_e$ )	Observed Heterozygosity ( $H_o$ )	Expected Heterozygosity/ Genetic diversity ( $H_e$ )	Polymorphic Information Content (PIC)	Fixation Index ( $F$ )
<i>SEU15</i>	5.000	4.545	0.200	0.780	0.745	0.744
<i>SEU16</i>	5.000	5.000	0.000	0.800	0.768	1.000
<i>SEU17</i>	4.000	3.571	0.000	0.720	0.672	1.000
<i>SEU18</i>	4.000	3.571	0.000	0.720	0.672	1.000
<i>SEU20</i>	6.000	5.556	0.400	0.820	0.794	0.512
<i>SEU21</i>	3.000	2.778	0.000	0.640	0.563	1.000
<i>SEU23</i>	6.000	5.000	0.400	0.800	0.768	0.500
<i>Bhadra</i>	5.000	5.000	0.000	0.800	0.768	1.000
Mean	4.250	3.834	0.133	0.699	0.652	0.787
Maximum	6.000	5.556	0.400	0.820	0.794	1.000
Minimum	2.000	1.515	0.000	0.340	0.314	0.259

### Cluster analysis and genetic relationship

The genetic dissimilarity values based on the SSR markers and 20 maize accessions varied from 0.1 to 1.0 with the average value of 0.81. Of the pair wise combinations, *SEU3* and *SEU4* were closely related accessions. The dendrogram discriminated and clustered the Sri Lankan maize accessions into five clusters. Cluster I comprised 3 landraces (*SEU14*, *SEU18* and *SEU21*), cluster II had 3 landraces (*SEU10*, *SEU11* and *SEU15*) and *Bhadra*, cluster III consisted of 5 landraces (*SEU5*, *SEU6*, *SEU8*, *SEU17* and *SEU20*), cluster IV and V had of 2 (*SEU9* and *SEU16*) and 6 (*SEU1*, *SEU2*, *SEU3*, *SEU4*, *SEU7* and *SEU23*) landraces respectively (Figure 2). Further, principal coordinate analysis was constructed to examine the genetic clustering using genetic distances. The clustering of maize accessions was further demonstrated by two primary coordinates. The percentage of variance for the first two principal coordinates were 23.86 % and 18.41 % (Figure 3).

The close resemblance of accessions *SEU3* and *SEU4* in the pair-wise combinations stands out, indicating possible genetic relatedness or common ancestry between these two kinds. The grouping of the maize populations into five different clusters was made easier by the dendrogram construction, exhibiting patterns of genetic relatedness and differentiation. The distinct combinations of landraces shown in Clusters I, II, III, IV, and V highlighted the variety of genetic ancestries and linkages found in the Sri Lankan maize germplasm. In a previous study, Mufeeth et al. (2020) classified 17 Sri Lankan landraces into three groups based on morphological and physiological characters. In their study, *SEU4* and *SEU7* expressed close relationship followed by *SEU8* and *SEU9*.

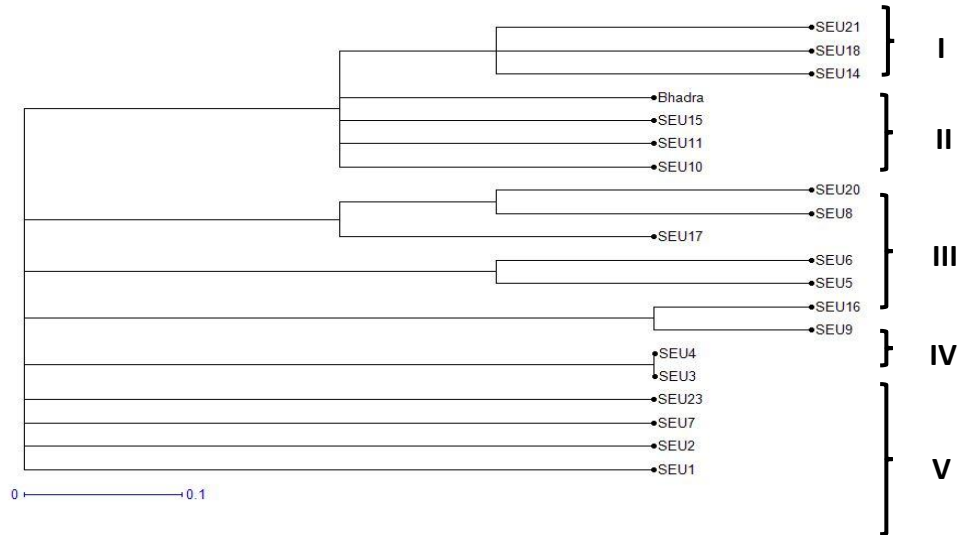


Figure 2. Distribution of Sri Lankan traditional maize accessions shown by cluster analysis

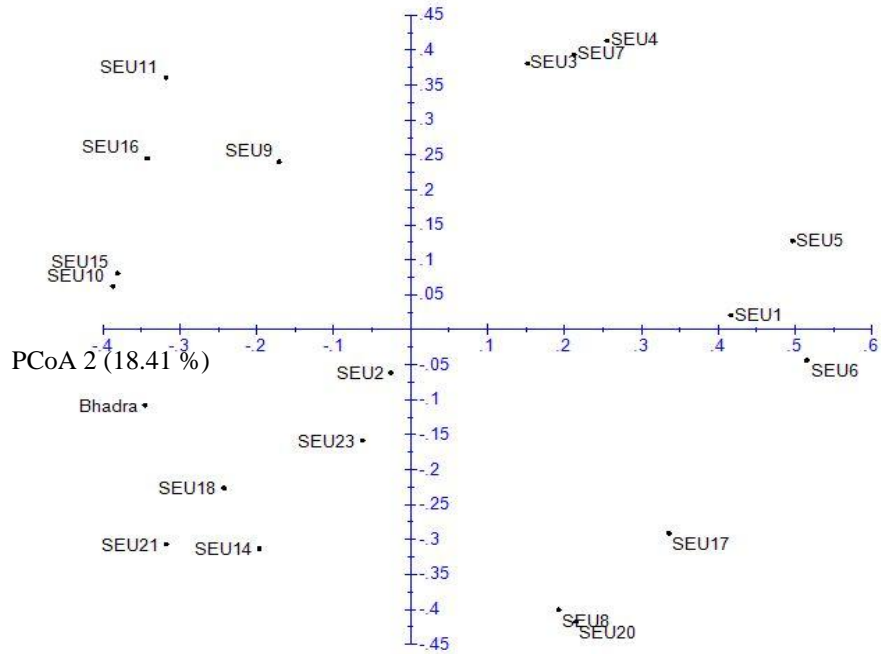


Figure 3. Principal Coordinate Analysis

## CONCLUSION

Using five SSR markers, the study sought to evaluate the genetic diversity of 20 Sri Lankan maize accessions. There were found to be 35 alleles in total, with the markers *bnlg1627* and *bnlg1564* being particularly notable for their contribution to genetic variability. The informativeness of the markers was demonstrated by their PIC scores. Notably, *SEU3* and *SEU4* were closely related, although *SEU6* and *SEU20* showed significant genetic diversity. Among the accessions, the study found five clusters. Two landraces that are considered as viable options for conservation and environment adaptation were *SEU6* and *SEU20*. These results lay the foundation for future research into conservation, breeding, and genetic improvement techniques for Sri Lankan maize accessions.

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