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Exploring Genetic Diversity of Maize (*Zea mays* L.) Accessions in Sri Lanka by Employing SSR Markers on Photosynthetic, Canopy Architectural and Grain Yield Traits

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ABSTRACT

Purpose: *The favorable alleles identified in maize landraces are treasurable resources to enhance the genetic base of crop breeding efforts. Therefore, our pioneering research aimed to explore SSR markers related to maize canopy architectural, photosynthetic and yield traits and hence to study the genetic diversity of 19 local maize accessions compared with the high-performing maize cultivar Bhadra, as the standard variety.*

Research Method: *Genomic DNA extraction was done from young leaves of maize seedlings using standard protocols. The SSR markers employed were phi065, phi116, umc1065, umc1066, umc1222, umc1231, umc1545, bnlg155, bnlg249 and bnlg1805. Data analysis was performed using GenAlex and DARwin software.*

Findings: *Four of the used markers (umc1066, bnlg1805, phi116, and phi065) had PIC values higher than that of the overall mean (0.692), showing their potential in this genetic diversity study. These markers are mainly associated with leaf area index, plant height, leaf chlorophyll content and quantum efficiency of photosystem II. The highest genetic diversity measures were observed in SEU2, SEU17, SEU18 and SEU23, compared to Bhadra while the lowest was in SEU3. The level of inbreeding was higher in SEU8. Cluster analysis identified three major genetic groups and the landraces collected from various areas did not exhibit any geographical association.*

Research Limitations: *A large number of SSR markers are needed to explore biomass and grain yield traits.*

Originality/value: *These results will explore the potential uses of Sri Lankan maize accessions to identify the best genetic clusters of germplasm with enhanced plant canopy architecture, photosynthesis and grain yield to be utilized in maize breeding programs.*

Keywords: *Cluster analysis, Genetic diversity, Maize accessions, Maize breeding, Polymorphic Information Content, SSR markers*

INTRODUCTION

Maize (*Zea mays* L.; 2n = 2x = 20), is the most widely cultivated cereal and is regarded as a significant staple food in the world (Ranum *et al*., 2014). It is valued as an excellent staple food and feed crop and a model organism with a broad genetic diversity (Strable and Scanlon, 2009). Due to the high demand for maize caused by population growth, climatic changes, and biotic and abiotic constraints, crop breeding initiatives have become necessary to enhance the agricultural and economic qualities of maize

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plants (Xiao *et al*., 2017). Maize landraces are an invaluable source of desirable alleles that could be utilized to improve the genetic foundation of ongoing breeding programs (Lia *et al*., 2009). Farmers usually choose these genetically diverse populations based on how well they adapt to the local environment (Prasanna and Sharma, 2005). In accordance with recent data, 819 maize germplasm accessions are identified in Sri Lanka and stored in the Plant Genetic and Resource Centre, Gannoruwa, and 35 of them are landraces (Kumari *et al*., 2017). However, the genetic diversity of canopy architectural and photosynthetic traits of these landraces have been poorly understood. Narrow genetic variability in many crops especially in maize limits the production of new cultivars to achieve food security in the changing climatic conditions in the Sri Lankan context. Though, techniques such as marker-assisted breeding and mutational breeding have been employed to generate crop variability in rice and vegetable crops, in Sri Lanka, maize is still being unexplored (Parasuraman and Weerasinghe, 2021; Peiris *et al*., 2008).

Breeders can choose parental combinations to develop inbred lines with the most genetic variability by analyzing the genetic diversity of germplasm (Ertiro *et al*., 2017). Genetic diversity analysis tends to estimate the degree of genetic variability of heterotic groups when deciding core subsets chosen for particular characteristics (Semagn *et al*., 2012). Numerous techniques, including morphology, biochemistry, and molecular-based tools, can be employed to investigate the genetic diversity of various genotypes (Govindaraj *et al*., 2015). Morphological traits have traditionally been used for clone and varietal identification, though it is complex, time-consuming and ambiguous. DNA-based molecular markers have the potential to efficiently identify genetic diversity to assist with the management of plant genetic resources (Asif and Zafar, 2006). Additionally, these markers are not tissue, growth or stage-specific and are unaffected by environmental factors. Most often, markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), inter-simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), and single nucleotide polymorphism are used (SNP) techniques have been developed. Among these, SSR markers are widely adopted methods for genetic diversity studies owing to their high polymorphism, co-dominant nature, repeatability, and reliability (Wang *et al*., 2010). The widespread usage of these DNA markers in genetic research has laid the groundwork for their implementation in DNA fingerprinting (Nashath *et al*., 2023).

Moreover, several studies have reported the successful utilization of SSR markers to identify critical agronomic characteristics in various crops, including rice (Khan *et al*., 2022), soybean (Li *et al.,* 2010), wheat (Haque *et al*., 2021) and maize (Belalia *et al*., 2019). Similarly, SSR markers have been used in genetic diversity studies of maize under drought conditions (Emam *et al*., 2023), identification of QTLs in oil palms (Bhagya *et al*., 2020), assessing the genetic purity of rice (Kumar *et al*., 2021) and evaluating the salt tolerance of wheat (EI-Hendawy *et al*., 2019). Further, photosynthetic traits in a population of rice deletion mutants (Mubarak *et al*., 2013), physiological and genetic evaluation in a wheat-doubled haploid population (Mubarak *et al*., 2008) and the presence of drought tolerance genes in maize (Sedhom *et al*., 2021). Therefore, the goals of the current study were to characterize 20 maize accessions, including 19 Sri Lankan maize landraces and one high-performing maize cultivar *Bhadra* using 10 SSR markers and to (i) determine the polymorphism of genes pertaining to canopy architecture, photosynthetic and yield traits and (ii) to identify promising lines for maize breeding program.

MATERIALS AND METHODS

Plant Materials

A total of 20 maize accessions, including 19 Sri Lankan maize landraces and one high-performing

maize commercial elite cultivar *Bhadra* (control variety) were utilized for this study (Table 01). These landraces were initially collected from the villages of Ampara, Badulla and Trincomalee districts of Sri Lanka. Maize seedlings were grown in plastic pots supplemented with sterilized sand, coir dust and compost (1:1:1 ratios) in the plant net house facilities 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), located in Ampara District which belongs to the low country dry zone (DL_{2b}) agro-climatic region.

DNA Extraction and Quantification

Genomic DNA extraction was done from young and damage-free leaves of three-week-old maize seedlings (Adu *et al*., 2019) using the DNeasy plant Mini Kit (Qiagen, Milano, Italy), according to manufacturer protocol. The quantity and purity of the extracted DNA samples were estimated using a UV spectrophotometer (Genesys 10S UV-Vis, Thermo Scientific Inc.). Normalization of DNA samples was done to equalize the concentrations (50 ng/µL) of all the DNA samples to be run in PCR.

SSR Markers and PCR Amplification

A total of 10 pairs of SSR markers related to maize canopy traits (Leaf area index (LAI), plant height and number of leaves) photosynthetic (Quantum efficiency of photosystem II and leaf chlorophyll content) and yield (Ear length and 100-grain weight) traits were selected based on previously published research works (Leipner *et al*., 2008; Trachsel *et al*., 2010; Wang *et al*., 2011; Li *et al*., 2017; Vathana *et al*., 2019). Marker characteristics were obtained from the maizegdb website (http://www.maizegdb.org.), primer sequences were sent and primers were imported from Integrated DNA Technologies, USA (Table 02). PCR was performed in a total of 15 µL per reaction having 2 µL of template DNA, 7.5 µL of 2X FastGene® Taq ReadyMix (1 U Taq DNA polymerase, 1X Taq buffer, 0.2mM of each dNTP, 1.5 mM $MgCl_2$ and stabilizers) (Nippon Genetics Europe GmbH), 1 µL of each forward and reverse primers and 3.5 µL of distilled water. The PCR mix was labeled, vortexed well, and loaded in a 96-well thermal cycler (Prima-96, HiMedia, LA949, China). Then DNA amplification was programmed as one cycle of initial denaturation at 94 °C for 5 minutes, followed by 35 cycles each of denaturation at 94 °C for 30 seconds, primer annealing at 52–56 °C for 30 seconds and extension at 72 °C for 30 seconds. The final extension was carried out at 72 °C for 10 minutes and maintained at 4 °C.

The amplified products were separated on 3 % Agarose gel (FastGene®, Nippon Genetics Europe) using 1X TAE buffer solution. The first well of the gel was loaded with 2 µL of 100 bp DNA ladder (Himedia) and the rest of the wells were loaded with amplified PCR products (5 µL). Electrophoresis was performed using a horizontal gel electrophoresis apparatus (Enduro, Labnet, USA) at 90 volts for 1 h and 30 min. Then the gels were stained in ethidium bromide (1µg/mL) for 15 minutes and de-stained in distilled water for 10 minutes. The gels were observed under ultraviolet light using a gel documentation system (Axygen, GD1000, USA) and photographed for further analysis.

Genotype Score and Data Analysis

Alleles (each band) of each SSR marker were scored according to a 100 bp DNA ladder. The allelic data were converted to binary data with the presence of band as 1 and absence as 0 in an Excel sheet to calculate the genetic diversity parameters and for cluster analysis. The genetic diversity parameters include the number of observed alleles (N_a), number of effective alleles (N_e), expected (H_e) and observed (H_o) heterozygosity, Shannon diversity index (I) and Fixation index (F) among populations, and SSR loci were calculated using GenAlex software

version 6.51b2 (Smouse and Peakall, 2012). The Polymorphic Information Content (PIC) was calculated using the following equation 01 (Sharma *et al*., 2009);

Table 01: Description of 20 Sri Lankan maize accessions used in this study.

S/N	Accession	Collection site	District	Kernel color
1	*SEU 1*	Ridimaliyadda	Badulla	Orange
2	*SEU 2*	Ridimaliyadda	Badulla	Orange and yellow
3	*SEU 3*	Kirawana, Padiyathalawa	Ampara	Orange and Yellow
4	*SEU 4*	Udakumbure Gedara, Kandaketiya	Badulla	Purple
5	*SEU 5*	Dehigama, Kandakatiya	Badulla	Red
6	*SEU 6*	Padiyathalawa	Ampara	Red
7	*SEU 7*	Padiyathalawa	Ampara	Yellow
8	*SEU 8*	Padiyathalawa	Ampara	Pearl white
9	*SEU 9*	Kandepoththawa,Baduluoya, Kandekatiya	Badulla	Pearl white
10	*SEU 10*	Kirawana, Padiyathalawa	Ampara	Red
11	*SEU 11*	Kirawana, Padiyathalawa	Ampara	Pearl white
12	*SEU 14*	Udakumbure Gedara, Kandaketiya	Badulla	Pearl white
13	*SEU 15*	Kandepoththawa,Baduluoya, Kandekatiya	Badulla	Yellow
14	*SEU 16*	Kandepoththawa,Baduluoya, Kandekatiya	Badulla	Light yellow
15	*SEU 17*	Udakumbure Gedara, Kandaketiya	Badulla	Purple and orange
16	*SEU 18*	Baduluoya	Badulla	Red
17	*SEU 20*	Naagadeepaya	Badulla	Pearl white
18	*SEU 21*	Kinniya	Trincomalee	Yellow-orange
19	*SEU 23*	Dehigama, Kandakatiya	Badulla	Red orange
20	*Bhadra*	Department of Agriculture	--	Yellow

Table 02: Details of SSR markers used in this study.

S/N	Locus	Trait	Primer sequences	Motif	Bin No*
1	*phi116*	Leaf area index, plant height, ear length and 100-grain weight	F - GCATACGGCCATGGATGGGA R - TCCCTGCCGGGACTCCTG	(ACTG)	7.06
2	*phi065*	Leaf area index and plant height	F - AGGGACAAATACGTGGAGACACAG R - CGATCTGCACAAAGTGGAGTAGTC	(CACTT)	9.03
3	*umc1065*	Leaf area index, plant height and number of plant leaves,	F – ACAAGGCCATCATGAAGAGCAGTA R - CACGGTCTGGCACACTAACCTTAT	$(ACA)_{17}$	2.05
4	*umc1231*	Leaf area index	F – TAGACATGTTGAAACCAGGACCG R - ACGACGTCAACAACAGCATGA	$(CTG)_8$	9.05
5	*umc1545*	Leaf area index	F – GAAAACTGCATCAACAACAAGCTG R - ATTGGTTGGTTCTTGCTTCCATTA	$(AAGA)_4$	7.00
6	*bnlg249*	Plant height	F – CCGGTCGCAGTTAGTAGATGAT R - TCGGCGTTGATTTCGTCAGTA	(AG)	6.01
7	*umc1066*	Leaf chlorophyll content	F – ATGGAGCACGTCATCTCAATGG R - AGCAGCAGCAACGTCTATGACACT	$(GCCAGA)_5$	7.01
8	*bnlg155*	Leaf chlorophyll content	F – ACCGAGTAGCCGAGACACG R - AGAGTCCTGGAGCCACATGAG	(CT)	7.03
9	*umc1222*	Quantum efficiency of photo-system II and leaf chlorophyll content	F – CTCAGAACAGAAGCCATCAAAAGC R - CGTCTTCGTGAGAGACATCCTGT	$(AG)_{20}$	1.01
10	*bnlg1805*	Quantum efficiency of photo-system II	F – GCCCGTTTGCTAAGAGAATG R - TGTTCGAGCATTTGCTCTTG	$(AG)_{29}$	7.03

**Position in the chromosome*

$PIC= 1- \Sigma xi^2$ Equation 01

Where *x*i is the relative frequency of the ith allele of the SSR loci.

Analysis of molecular variance (AMOVA) was also performed to obtain genetic variation among and within individuals using GenAlex software version 6.51b2 as previously described by Yousuf *et al*. (2021). Furthermore, the binary data matrix was subjected to calculate dissimilarity matrix based on the Jaccard coefficient using DARwin software version 6 (Khan *et al*., 2022). The resultant distance matrix was then employed to construct a UPGMA (unweighted pair group method of arithmetic clustering) based on the dendrogram and principal coordinate analysis (PCoA) to supplement the grouping patterns disclosed by the dendrogram.

RESULTS AND DISCUSSION

Polymorphism of SSR Markers

Table 03 provides the genetic characteristics of the analyzed markers. With a mean value of 5.1 alleles per locus, 51 total alleles were generated from all the markers. These values are lower compared to previous studies carried out with Algerian maize landraces that had 87 alleles with a mean of 5.8 per locus by employing 18 SSR markers (Aci *et al*., 2013) and European maize accessions that had 61 total alleles with an average of 6.5 alleles per locus by using 10 SSR markers (Vivodík *et al*., 2017). Conversely, the mean number of alleles in this study is higher compared to Joshi *et al*. (2020) who characterized 23 Nepalese maize landraces using 5 SSRs and obtained an average of 2 alleles per locus, and Wietholter *et al*. (2008) who reported 2.7 alleles per locus for 37 Brazilian maize accessions with 21 SSRs. The particular allele of a locus that appears most frequently in the population is described as the "major allele" (Singode and Prasanna, 2010). According to our results, *umc1065* corresponding to LAI, several leaves and plant height in maize crops represented the highest and was considered as major allele (frequency = 0.500), while *phi065* had the lowest (0.250). In a previous study, *umc1065* was reported as the major allele (frequency of 0.456) among Chinese maize inbred lines (Vathana *et al*., 2019) while, *phi065* had 0.48 among Kashmir maize landraces (Yousuf *et al*., 2021). This seems major allele frequencies subjected to alteration due to the breeding pattern of maize accessions. The number of observed alleles (N_a) in this study ranged from 4 (*umc1222, umc1231* and *umc1545*) (Figure 01) to 6 (*umc1066, umc1065, bnlg1805* and *bnlg155*) (Figure 02). The term "number of effective alleles" refers to the number of equally frequent alleles necessary to attain the same expected heterozygosity as in the population under study (Hauser *et al*., 2011). With an average of 3.918 alleles per locus, the number of effective alleles (N_e) found varied from 2.880 (*umc1222*) to 5.023 (*bnlg1805*). The average effective number of alleles obtained here resembles the Philippine traditional maize population (3.781) (Guevarra *et al*., 2022) and is comparatively higher than reported by Belalia *et al*. (2019) among African Sahar maize populations (2.315) and temperate maize landraces (2.60) in Kashmir region (Yousuf *et al*., 2021). The proportion of heterozygous individuals at a locus in the population is referred to as the "observed heterozygosity" (Vigouroux *et al*., 2002). In our study, lower values of overall observed heterozygosity (H_o) (0.38) were obtained compared to expected heterozygosity (H_o) (0.735) in all the accessions and it indicates the homozygote nature of Sri Lankan maize accessions. However, the greatest observed heterozygosity for *phi065* and *umc1066* (0.750) related to leaf chlorophyll content, LAI and plant height traits shows increased heterozygous nature for these traits. Moreover, the chance that two randomly selected alleles in a sample are distinct from one another is known as "genetic diversity" (Navascués and Emerson, 2005). The examined genetic diversity is similar to the expected heterozygosity values for diploid maize (Adu *et al*., 2019) and the mean value obtained (0.735) is consistent with earlier reports by Beyene *et al*. (2006) for Ethiopian maize accessions and Yao *et al*. (2008) for Chinese maize landraces. The *bnlg1805* ((H_e = 0.801) locus linked to the

quantum efficiency of photosystem II trait and *phi065* (H_e = 0.796) linked to LAI and plant height, had an increased expected heterozygosity, indicating that the higher gene diversity of these alleles in the tested maize accessions.

Table 03: Genetic parameters of the SSR markers used in this study.

SSR marker	Major allele frequency	N_a	N_e	H_o	H_e	*I*	PIC	F
umc1066	0.275	6	4.678	0.750	0.786	1.628	0.753	0.046
umc1222	0.444	4	2.880	0.333	0.653	1.179	0.588	0.489
umc1065	0.500	6	3.100	0.389	0.677	1.392	0.639	0.426
bnlg1805	0.278	6	5.023	0.611	0.801	1.696	0.772	0.237
bnlg155	0.400	6	3.493	0.050	0.714	1.425	0.666	0.930
umc1231	0.375	4	3.347	0.400	0.701	1.270	0.644	0.430
phi116	0.300	5	4.545	0.400	0.780	1.557	0.745	0.487
umc1545	0.350	4	3.636	0.100	0.725	1.335	0.675	0.862
phi065	0.250	5	4.908	0.750	0.796	1.600	0.764	0.058
bnlg249	0.350	5	3.571	0.000	0.720	1.399	0.672	1.000
Total		51						
Maximum	0.500	4	5.023	0.750	0.801	1.696	0.772	1.000
Minimum	0.250	6	2.880	0.000	0.653	1.179	0.588	0.046
Overall Mean	0.080	5.1	3.918	0.378	0.735	1.448	0.692	0.497
SE	0.025	0.277	0.250	0.086	0.017	0.053	0.021	0.108

N_a - No. of observed alleles, N_e - No. of effective alleles, H_o - Observed Heterozygosity, H_e - Expected Heterozygosity/Genetic diversity, I - Shannon's Information Index, PIC - Polymorphic information Content, F = Fixation Index.

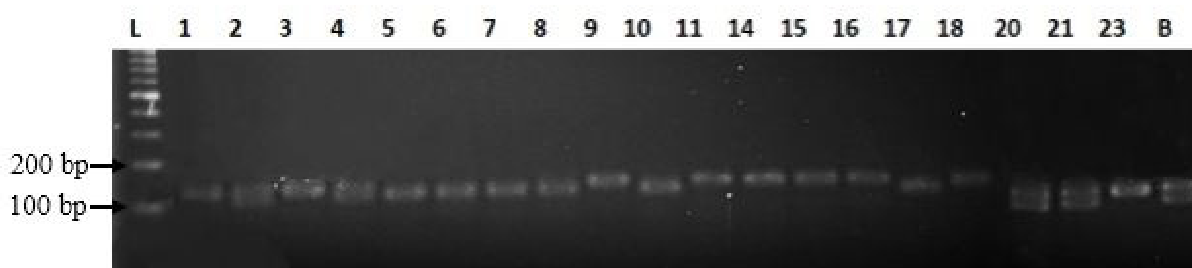

Figure 01: PCR amplification profile of 20 Sri Lankan maize accessions with the SSR marker *umc1222*. L: 100 bp ladder, the numbers in the gel correspond to the identification of maize accessions as indicated in Table 01.

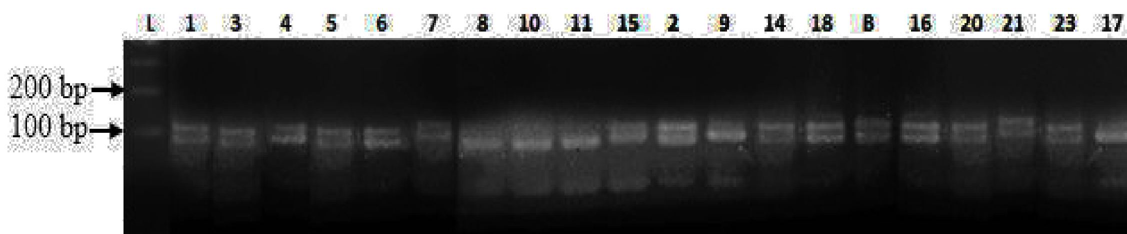

Figure 02: PCR amplification profile of 20 Sri Lankan maize accessions with the SSR marker *umc1066*. L: 100 bp ladder, the numbers in the gel correspond to the identification of maize accessions as indicated in Table 01.

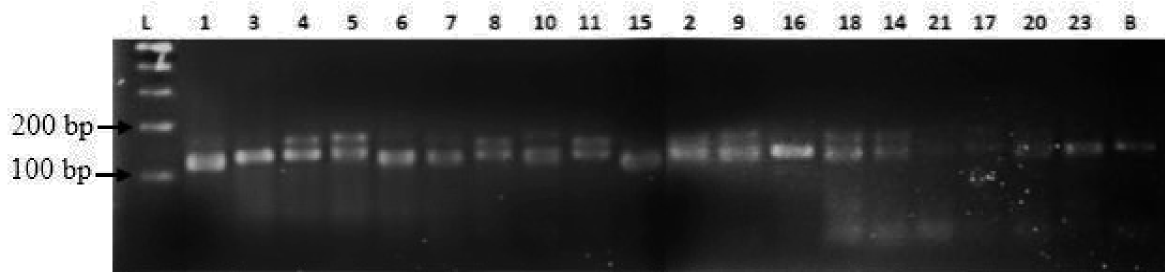

Figure 03: PCR amplification profile of 20 Sri Lankan maize accessions with the SSR marker *phi116*. L: 100 bp ladder, the numbers in the gel correspond to the identification of maize accessions as indicated in Table 01.

In diversity analysis, the Shannon index (*I*) examines the abundance and evenness of the species under study (Wang *et al*., 2018). The values ranged from 1.179 (*umc1222*) to 1.696 (*bnlg1805*), with a mean value of 1.448. *umc1066, bnlg1805, phi116* (Figure 03), and *phi065* markers all displayed greater than the lowest typical *I* value of 1.5. Each SSR locus's discriminatory potential is measured by its "polymorphism information content", which also measures the allelic diversity within a locus (George *et al*., 2004). It evaluates an SSR marker's capacity to generate polymorphism data from a set of alleles (Perseguini *et al*., 2012). When a computation results in a value of >0.50, PIC values are deemed highly polymorphic; moderately polymorphic (0.25 to 0.49); and poorly polymorphic values are 0.25. (Botstein *et al*., 1980). The PIC values varied from 0.588 (*umc1222*) to 0.772 (*bnlg1805*), with 0.692 as the mean. Similar to *me*, four of the loci (*umc1066, bnlg1805, phi116*, and *phi065*) used in this study showed a PIC value above the average. All the SSR markers used in this research produced PIC values higher than 0.5, indicating their wide polymorphism range and exhibiting the SSR markers' reliability to assess the genetic diversity of Sri Lankan maize landraces. The study's average PIC value (0.692) is similar to those determined by Adu *et al*. (2019), who used 31 SSRs to assess the genetic characterization of 70 white maize populations, as well as the traditional Philippine maize (Guevarra *et al*., 2022). Our PIC value is also higher than the reported value on Turkish local popcorn populations (PIC = 0.57) (Zulkadir and Idikut, 2021) and Ghanaian maize landraces (PIC = 0.51) (Oppong *et al.*, 2014). The SSR markers selected for this study represent different chromosomes of the maize genome. Based on the PIC values, *phi116, phi065, bnlg1805* and *umc1066* markers had the highest values where three of the markers (*phi116, bnlg1805* and *umc1066*) primarily associated with leaf chlorophyll content, quantum efficiency of photosystem II, leaf area index and plant height characteristics are positioned in chromosome number 7 and *phi065* is located in chromosome number 9, respectively. The fixation index (*F*) ranged from 0.046 (*umc1066*) to 1.0 (*bnlg249*) with a mean of 0.497. Of all the diversity measures, *bnlg1805* was the highest, while *umc1222* had the lowest values (Table 03).

Genetic Variability among Maize Accessions

Among Sri Lankan maize accessions, the number of alleles per locus varied from 1.6 (*SEU8*) to 2 (all the accessions except *SEU7, SEU8, SEU10* and *SEU15*) with a mean value of 1.94 (Table 04). The highest major allele frequency (0.750) was found in *SEU3*, while the lowest was in *SEU23* (0.200). Meanwhile, *SEU23* had the highest effective number of alleles (N_e) (5.714) conversely, *SEU3* had the lowest (1.709). Observed heterozygosity (H_o) varied from 0.125 (*SEU8*) to 0.556 (*SEU7* and *SEU15*) with a mean value of 0.380. Shannon index (*I*) was in the range of 0.826 (*SEU3*) to 1.765 (*SEU23*), with an average value of 1.467. The mean genetic diversity (H_e) and PIC within the set of accessions were 0.733 and

0.6936 respectively, being highest for *SEU23* (H_e = 0.825, PIC = 0.8003) and lowest for *SEU3* (H_e = 0.415, PIC = 0.3894). The average genetic diversity within Sri Lankan maize accessions (H_e = 0.733) is lower than that of Swiss maize accessions (0.78) (Eschholz *et al*., 2010), though almost identical to Chinese maize (0.70) reported by Yao *et al*. (2008). However, compared to maize from other regions of the world, these values (H_e) are significantly higher, as reported by Sharma *et al*. (2009) for Himalayan accessions (0.63) and Aci *et al*. (2013) for Algerian maize (0.40). Based on the genetic diversity parameters (H_o, H_e and PIC), *SEU2, SEU17*, *SEU18* and *SEU23* exhibited higher diversity compared to *Bhadra* while the lowest was in *SEU3*. The fixation index (F), measures the inbreeding level among and within inbred lines (Getachew *et al*., 2020). The fixation index varied significantly from 0.036 (*SEU3*) to 0.816 (*SEU8*) with a mean of 0.473. The average value exceeds that of Saharan maize (F = 0.22) (Belalia *et al*., 2019) and Indian maize landrace populations (F = 0.36) (Wasala and Prasanna, 2013). According to our findings, the lowest observed heterozygosity and the highest fixation index values observed in *SEU8* indicate the maximum level of inbreeding in this accession (Table 04). AMOVA results revealed about 72 % of the overall variance within individuals, and 24 % among populations (Table 05). Belalia *et al*. (2019) and Singode and Prasanna (2010) reported similar findings where they obtained increased genetic diversity within the tested maize accessions compared to among the maize accessions.

Table 04: Genetic parameters of 20 maize accessions studied.

Accessions	Number of alleles per locus	Major allele frequency	N_e	H_o	H_e	*I*	PIC	F
SEU1	2	0.600	2.299	0.300	0.565	1.033	0.5094	0.469
SEU2	2	0.250	5.405	0.500	0.815	1.735	0.7886	0.387
SEU3	2	0.750	1.709	0.400	0.415	0.826	0.3894	0.036
SEU4	2	0.350	4.000	0.500	0.750	1.470	0.7085	0.333
SEU5	2	0.400	3.390	0.400	0.705	1.349	0.6533	0.433
SEU6	2	0.450	3.175	0.400	0.685	1.331	0.6351	0.416
SEU7	1.8	0.333	4.154	0.556	0.759	1.509	0.7207	0.268
SEU8	1.6	0.375	3.122	0.125	0.680	1.223	0.6179	0.816
SEU9	2	0.300	4.651	0.300	0.785	1.640	0.7526	0.618
SEU10	1.8	0.333	3.522	0.222	0.716	1.311	0.6627	0.690
SEU11	2	0.350	4.000	0.400	0.750	1.470	0.7085	0.467
SEU14	2	0.300	4.545	0.300	0.780	1.614	0.7457	0.615
SEU15	1.8	0.333	4.154	0.556	0.759	1.509	0.7207	0.268
SEU16	2	0.400	3.774	0.300	0.735	1.458	0.6950	0.592
SEU17	2	0.300	5.000	0.300	0.800	1.696	0.7675	0.625
SEU18	2	0.250	4.878	0.500	0.795	1.597	0.7622	0.371
SEU20	2	0.300	4.348	0.400	0.770	1.584	0.7331	0.481
SEU21	1.8	0.333	4.378	0.333	0.772	1.542	0.7359	0.568
SEU23	2	0.200	5.714	0.300	0.825	1.765	0.8003	0.636
Bhadra	2	0.300	4.878	0.500	0.795	1.683	0.7658	0.371
Maximum	2	0.750	5.714	0.556	0.825	1.765	0.8003	0.816
Minimum	1.6	0.200	1.709	0.125	0.415	0.826	0.3894	0.036
Mean	1.94	0.360	4.055	0.380	0.733	1.467	0.6936	0.473
SE	0.025	0.028	0.222	0.026	0.021	0.053	0.0221	0.040

N_e - No. of effective alleles, H_o - Observed Heterozygosity, H_e - Expected Heterozygosity/Genetic diversity, I = Shannon's Information Index, PIC - Polymorphic information Content, F - Fixation Index.

Table 05: Analysis of molecular variance based on SSR markers for maize accessions.

Source	Degree of freedom	Sum of squares	Mean sum of squares	Estimated variance	Percentage of variation	P
Among Populations	19	238.717	12.564	1.253	24%	0.001
Among Individuals	40	91.833	2.296	0.156	4%	0.006
Within Individuals	60	119.000	1.983	3.963	72%	0.001
Total	119	449.550		5.372	100%	

Cluster and Principal Component Analysis

The 20 maize accessions were resolved into three broad groups (cluster I, II and III) using the UPGMA dendrogram, which was constructed using the distance matrix based on Jaccard's coefficient. Cluster I included 9 landrace accessions (*SEU1, SEU3, SEU4, SEU5, SEU6, SEU8, SEU10, SEU11* and *SEU15*), cluster II included 5 landrace accessions *(SEU2, SEU9, SEU14, SEU16* and *SEU18*) and cluster III had 6 maize accessions (*SEU7, SEU17, SEU20, SEU21, SEU23* and Bhadra) (Figure 04). The pairwise genetic dissimilarity varied from 0.3 to 0.81. Most closely related landraces were between *SEU16* and *SEU18, SEU1* and *SEU6* followed by *SEU13* and *SEU14* while *SEU23* has the most distant relationship with the rest of the maize accessions with a dissimilarity range of 0.72 - 0.81. The landraces gathered from various areas did not show any geographic association. Similarly, in a prior study, inbred maize line categories were independent of the collection source (Nyaligwa *et al*., 2022). However, Beyene *et al*. (2006) and Aci *et al*. (2013) found a correlation between geographic areas and the genetic similarity between maize populations. The Badulla district's maize accessions were discovered to be included in all three clusters, indicating a broader genetic base. In comparison, it was found that maize landraces collected from the Ampara district had a limited genetic base, with most of them constrained into a single group (Cluster II). Cluster III was a diverse collection that included the high-performing *Bhadra* maize population in addition to the three landraces from Badulla (*SEU17, SEU20*, and *SEU23*) and one from Trincomalee district (*SEU21*). It implies that although the populations mentioned above have distinct genetic similarities, they may share a broader genetic base.

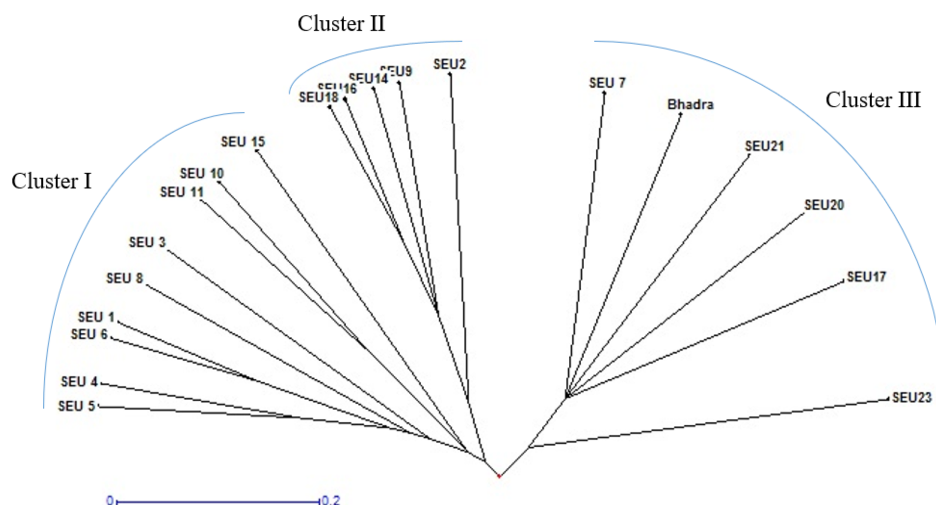

Figure 04: The UPGMA dendrogram was constructed based on Jaccard's similarity coefficient distance matrix based on SSR markers data.

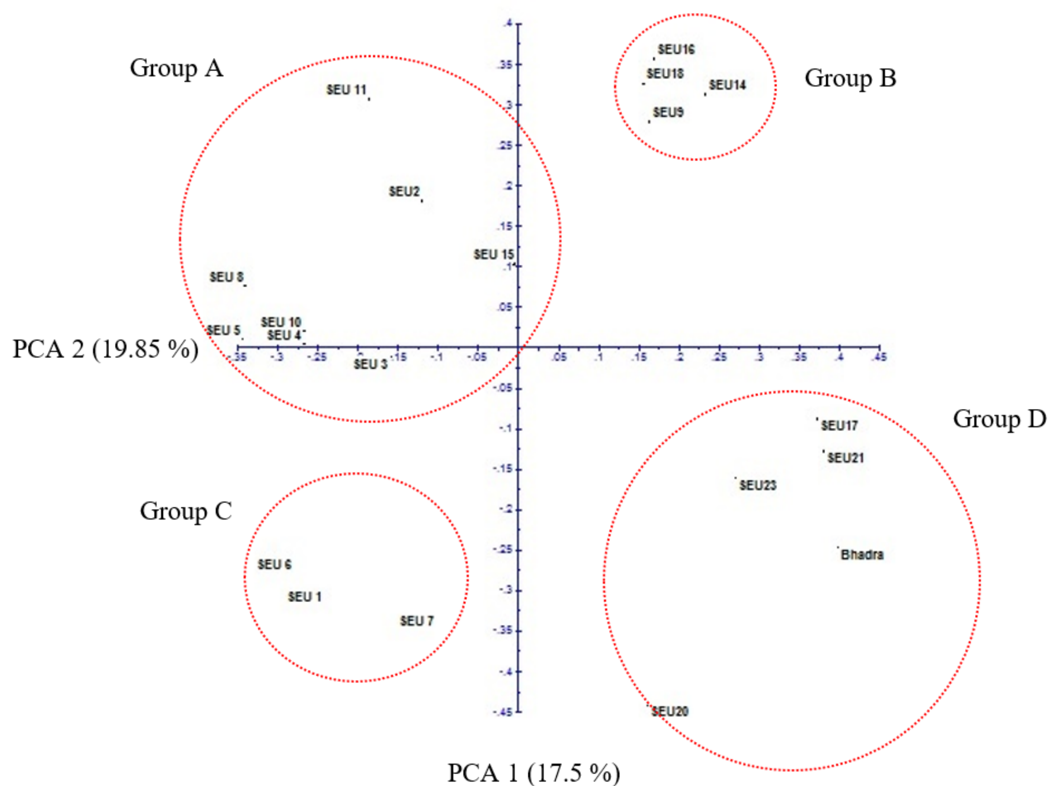

Figure 05: Principal coordinate analysis (PCoA) based on SSR data of selected 20 maize accessions.

A principal coordinate analysis was built to further investigate the genetic grouping of the 20 maize accessions using genetic distances. The first two main coordinates had marker variance percentages of 19.85 % and 17.5 %, for a total variance of 37.35 %. Moreover, a scatter plot distinctly divided all maize accessions into four main groups (Groups A, B, C, and D) based on the principal coordinate analysis (Figure 05). The predominant Group-A contained eight accessions (*SEU2, SEU3, SEU4, SEU5*, *SEU8, SEU10, SEU11* and *SEU15*), Group B comprised of four (*SEU9, SEU14, SEU16* and *SEU18*), Group C with three (*SEU1, SEU6* and*SEU7*), and Group D with five (*SEU17, SEU20, SEU21, SEU23* and *Bhadra*).

The findings of the present study represent a first step in investigating the genetic diversity of Sri Lankan maize accessions. Due to their adaptation to the local conditions in Sri Lanka, these accessions have excellent diversity and high potential for maize breeding. The selected markers for this study were able to successfully group the Sri Lankan maize accessions based on photosynthetic, canopy architectural and yield traits. Based on the genetic diversity parameters, *SEU4, SEU11* and *SEU15* (cluster I), *SEU2, SEU9* and *SEU18* (cluster II) and *SEU7, SEU17, SEU20* and *SEU23* (cluster III) could be utilized for strategic maize breeding or conservation. Hence, the results of this research can be used as a guide when selecting the parental lines for generating inbred lines and subsequently utilized in maize breeding programs. Future research must be focused on other agronomic characteristics of major diseases, pests pest-resistant traits among those examined maize accessions. Although markers related to photosynthesis and canopy architectural traits exhibited higher potential in the genetic diversity study of Sri Lankan maize accessions, yield-related markers used in this study (*phi116*) are limited and need to be increased in future studies.

CONCLUSIONS

The study successfully demonstrated the genetic

diversity among 20 Sri Lankan maize landraces selected using the molecular data from 10 SSR markers. The microprosatellites loci *umc1066, bnlg1805, phi116* and *phi065* exhibited higher PIC values indicating their efficacy and promise in genetic analysis. The high levels of variation found among the Sri Lankan maize populations could be investigated through breeding programs utilizing both traditional and novel breeding techniques. Plant breeders can use the data to help them choose parents for various breeding tasks, including hybrid breeding, creating heterotic groups, genome mapping, and identifying quantitative trait loci for critical biotic and abiotic characteristics. Using the established set of SSR markers, additional research on maize populations could be focused on biomass traits, grain partitioning and pest and disease resistance to withstand biotic and abiotic stresses in agricultural ecosystems.

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