




Research Article

Characterization of Sri Lankan Maize (*Zea mays* L.) Accessions Using SSR Markers Associated with Insect-resistant Traits

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ARTICLE INFO	ABSTRACT
<p>Article history Received: 24 May 2023 Accepted: 15 Jun 2023 Published: 30 Jun 2023</p> <p>Keywords Crop breeding, Genetic diversity, Insect resistance, Landraces, SSR markers</p> <p>Correspondence ANM Mubarak ✉: anmubarak@seu.ac.lk</p> <p></p>	<p>Maize genetic diversity is utmost to develop new varieties conferred with favorable agronomic traits. Landraces are priceless resources that can serve to strengthen crop breeding programs. Hence, this study aimed to characterize 19 Sri Lankan maize landraces and one elite commercial variety <i>Bhadra</i> using SSR markers linked with insect-resistant traits. Using established procedures, genomic DNA extraction was done from immature maize leaves and PCR was performed utilizing <i>bnlg1017</i>, <i>bnlg339</i>, <i>umc1021</i>, <i>umc1187</i>, <i>bnlg1346</i>, <i>bnlg1588</i>, <i>bnlg1556</i>, <i>umc1178</i>, <i>nc134</i>, <i>umc1688</i> and <i>umc1045</i> primers. GenAlex and DARwin software were used for the data analysis. According to the results, a sum of 77 alleles was amplified with the maximum number of observed (13) and effective (11.28) alleles for <i>bnlg1588</i>. The highest gene diversity and PIC values were recorded in <i>bnlg1588</i> (0.911 and 0.904 respectively), while the lowest was in <i>umc1045</i> (0.640 and 0.581 respectively). Out of the total SSR primers used, <i>bnlg1588</i>, <i>bnlg339</i>, <i>bnlg1346</i>, and <i>bnlg1017</i> linked with FAW-resistant traits were found to be more informative based on their genetic diversity parameters. The accessions <i>SEU7</i>, <i>SEU11</i>, <i>SEU16</i>, and <i>SEU20</i> showed more genetic diversity compared to <i>Bhadra</i>, whereas <i>SEU17</i> showed the lowest diversity. The dendrogram divided the 20 maize accessions into two main clusters with the mean similarity of 0.752. Similarly, the principle coordinate analysis explained 40.63 % of the genetic variation and grouped the maize accessions into two. Considering the genetic diversity parameters, the accessions <i>SEU6</i> and <i>SEU7</i> from cluster I and <i>SEU9</i>, <i>SEU11</i>, <i>SEU16</i>, and <i>SEU20</i> from cluster II could be utilized for strategic maize breeding programs in Sri Lanka.</p>
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Introduction

Maize (*Zea mays* L.) is a vital crop in the world economy and is a key source of food, animal feed, and raw materials for a range of industrial outputs (Jaiswal et al., 2019). Due to its broad genetic diversity, this crop is highly adapted to selective pressure (Costa et al., 2020). However, a number of insect pests, including fall armyworm (FAW) and pathogens, have considerable negative impact on maize productivity in Sri Lanka as well as in other maize-growing countries. The FAW outbreak, which appeared for the first time in Sri Lanka in August 2018, has already severely damaged extensive corn monocropping systems (Perera et al., 2019).

Historically, pesticide sprays are used to control insect pests in maize cultivation; however, their harsh environmental impact and evolution of resistance have hampered their efficiency (Carvalho et al., 2013). As opposed to traditional pest control approaches, the *Bt*

maize, which is a transgenic crop that expresses insecticidal proteins from *Bacillus thuringiensis* bacteria, is an alternative to farmers. Nevertheless, within three years of its commercial distribution, the majority of *Bt* crops lose their ability to effectively manage pests (Fatoretto et al., 2017). In this regard, another solution is to utilize the host plant resistance for implementing integrated pest management strategies (Dogimont et al., 2010). This requires the detailed-knowledge about the diversity of pest-resistance sources before using them in breeding research. Maize landraces are open-pollinated variants that have undergone natural and artificial selection for a long time. Farmers chose a large number of these materials based on their adaptability to environmental conditions (Cömertpay et al., 2012). Hence, these crops may contain novel beneficial genes for various agronomic properties (Fonseka et al., 2020).

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The genetic diversity of crop germplasm has been evaluated using a variety of techniques, such as morphological, biochemical, and molecular techniques (Govindaraj et al., 2015). Morphological and biochemical analysis, however, do not consistently depict the true genetic relationship among the genotypes due to their low abundance, environmental impact, and inter-genic interactions, and insufficient genome sampling (Wang et al., 2020). Since the 1980s, a variety of crop species, including maize, have been effectively characterized using DNA-based molecular markers (Rakshit et al., 2011). SSR (Simple Sequence Repeat) or microsatellites are PCR-based, co-dominant, robust, and trustworthy, with good reproducibility and discriminatory capacity and broadly used in almost all crop species for large-scale DNA fingerprinting, diversification study, gene mapping, and QTL analysis (Silva et al., 2012). Moreover, a number of recent studies proved evidence that the SSR markers have been utilized by researchers all over the world to characterize maize landraces (Guevarra et al., 2022; Yousuf et al., 2021; Zulkadir and Idikut, 2021; Joshi et al., 2020; Oppong et al., 2014; Aci et al., 2013).

In accordance with recent data, 819 maize accessions are stored in Plant Genetic and Resource Centre, Gannoruwa in Sri Lanka, and 35 of them are landraces (Kumari et al., 2017). Characterization of selected local maize germplasms in Sri Lanka for their morphological and physiological traits and their relationship with the photosynthesis and biomass production are reported by Mufeeth et al. (2020). The reported that several landraces naturally possessed improved morphological traits such as plant height, leaf length, width and

arrangements, and leaf area. Further, they physiologically exhibited improved leaf development rates, photosynthesis, transpiration and quantum yield efficiencies along with larger variations in yield traits (Mubarak et al., 2023).

However, until recent times, the Sri Lankan maize accessions have not been studied based on microsatellite markers on the fall armyworm gene resistivity. Therefore, exploring the diversity of maize accessions is utmost for the sustainable food and feed production. With this in mind, the pioneering studies were carried out with the aims of 1) determining genetic variation among Sri Lankan maize accessions using SSR DNA markers related to insect-resistant traits, 2) assessing the polymorphism and applicability of the chosen SSR marker sets for molecular analysis and 3) to measure the genetic diversity parameters of the analyzed maize accessions.

Materials and Methods

Planting materials

Twenty maize accessions were selected for the genetic diversity analysis, as shown in Table 1. These accessions included 19 Sri Lankan maize accessions which were originally collected from the traditional farmers of Badulla, Ampara and Trincomalee districts (Mubarak et al., 2023). The commercial elite cultivar *Bhadra* was used as a control variety. Maize seeds of each accession were grown inside a plant net house facilities in the Department of Biosystems Technology, Faculty of Technology, South Eastern University of Sri Lanka.

Table 1. Collection site and district information of planting materials used in this study

S/N	Accession	Collection site	District	Latitude	Longitude
1	SEU 1	Ridimaliyadda	Badulla	7° 14' N	81° 6' E
2	SEU 2	Ridimaliyadda	Badulla	7° 14' N	81° 6' E
3	SEU 3	Kirawana, Padiyathalawa	Ampara	7° 4' N	81° 22' E
4	SEU 4	Udakumbure Gedara, Kandaketiya	Badulla	7° 21' N	81° 01' E
5	SEU 5	Dehigama, Kandaketiya	Badulla	7° 29' N	80° 56' E
6	SEU 6	Padiyathalawa	Ampara	7° 36' N	81° 20' E
7	SEU 7	Padiyathalawa	Ampara	7° 36' N	81° 20' E
8	SEU 8	Padiyathalawa	Ampara	7° 36' N	81° 20' E
9	SEU 9	Kandepoththawa, Baduluoya, Kandaketiya	Badulla	7° 12' N	80° 59' E
10	SEU 10	Kirawana, Padiyathalawa	Ampara	7° 4' N	81° 22' E
11	SEU 11	Kirawana, Padiyathalawa	Ampara	7° 4' N	81° 22' E
12	SEU 14	Udakumbure Gedara, Kandaketiya	Badulla	7° 21' N	81° 01' E
13	SEU 15	Kandepoththawa, Baduluoya, Kandaketiya	Badulla	7° 12' N	80° 59' E
14	SEU 16	Kandepoththawa, Baduluoya, Kandaketiya	Badulla	7° 12' N	80° 59' E
15	SEU 17	Udakumbure Gedara, Kandaketiya	Badulla	7° 21' N	81° 01' E
16	SEU 18	Baduluoya	Badulla	7° 12' N	80° 59' E
17	SEU 20	Nagadeepaya	Badulla	7° 26' N	81° 12' E
18	SEU 21	Kinniya	Trincomalee	8° 5' N	81° 18' E
19	SEU 23	Dehigama, Kandaketiya	Badulla	7° 29' N	80° 56' E
20	<i>Bhadra</i>	Department of Agriculture	-	-	-

DNA extraction, SSR markers, and PCR amplification

The DNA was isolated from the leaves of 21 days maize seedlings using the DNeasy plant Mini Kit (Qiagen, Milano, Italy) in accordance with the manufacturer's instructions. A total of 11 sets of SSR markers linked with insect-resistant characteristics in maize plants, such as FAW resistance and glossy leaf surface, were selected from published research articles (Jessup et al., 2011; Brooks et al., 2005; Brooks et al., 2007; Womack et al., 2018; Womack et al., 2020). The chosen markers belonged to 3 different series; *bnlg*, *umc* and *nc* (Table 2). PCR was assembled using standard procedure. The reaction mixture (15 µl) comprised 2 µl of template DNA, 7.5 µl of 2X FastGene® Taq ReadyMix (Nippon Genetics Europe GmbH), 1 µl (10 µM) of each forward and reverse primers (Integrated DNA Technologies, USA) and 3.5 µl of distilled water. The PCR reaction was carried out in a 96-well thermal cycler (Prima-96,

HiMedia, LA949, China) under following conditions: one cycle of initial denaturation at 94 °C for 5 minutes, followed by 35 cycles each of denaturation at 94 °C for 30 s, primer annealing at 50–59 °C for 30 s, extension at 72 °C for 30 s and the final extension at 72 °C for 10 minutes and maintained at 4 °C. Using a 1X TAE buffer solution, the amplified products were separated on a 3% Agarose gel (FastGene®, Nippon Genetics Europe). The first well of the gel had 2 µl of a 100 bp DNA ladder (Himedia) placed onto it, while the remaining wells had 5 µl of amplified PCR products. Using a horizontal gel electrophoresis device (Enduro, Labnet, USA) at 90 volts for one hour and thirty minutes, electrophoresis was carried out. The gels were next stained for 15 minutes with ethidium bromide (1 g/ml), and then de-stained for 10 minutes with distilled water. The gels were photo-documented using a gel documentation system (Axygen, GD1000, USA), under ultraviolet light.

Table 2. Traits, sequences, repeat motifs and chromosome bins of selected SSR markers (source: <http://www.maizegdb.org>.)

S/N	Locus	Trait	Primer sequences	Motif	Bin No*
1	<i>umc1688/glossy15</i>	Glossy leaf surface expressed after 3 rd leaf	F - AGCAGTAGCCGCAAGCAGAG R - ATCTGGAGCTGCGTGCTGTC	(GGA) ₄	9.03
2	<i>nc134</i>	Glossy leaf surface expressed after 3 rd leaf	F - CTCAGTTCTTTTCGATGGACG R - AGTCGCTGCAGCTAGCTAG	N/A	9.03
3	<i>umc1178/mir2</i>	maize insect resistance2	F - CTGTGTAAGAGCGCCAACAG R - GTCTGAACGATGAACAGTACACGC	(GGC) ₆	6.02
4	<i>umc1021</i>	FAW resistant	F - AGCCTCCTGAGACCTCTCGATT R - ACTTCGCCACCTTACATTCTTGA	(GT) ₁₄	1.03
5	<i>bnlg1017</i>	FAW resistant	F - ATTGGAAGGATCTGCGTGAC R - CAGCTGGTGGACTGCATCTA	(AG) ₁₈	2.20
6	<i>bnlg1346</i>	FAW resistant	F - CATCATGAAGCAATGAAGCC R - CCGCGCCATTATCTAGTTGT	(AG) ₂₄	5.07
7	<i>umc1187</i>	FAW resistant	F - AGAGCTACCACCCTCTTATCCC R - ATGTGCATGCTCTTGTTCCTATCA	(CCT) ₄	6.05
8	<i>bnlg339</i>	FAW resistant	F - CCAACCGTATCAGCATCAGC R - GCAGAGCTCTCATCGTCTTCTT	N/A	7.03
9	<i>bnlg1588</i>	FAW resistant	F - TAACTTGTTGTGCAGAGAGAGAG R - CCCAGAAACATCGCCAATG	(AG) ₂₁	9.07
10	<i>bnlg1556</i>	Keratin-associated protein	F - ACCGACCTAAGCTATGGGCT R - CCGGTTATAAACACAGCCGT	(AG) ₁₈	1.07
11	<i>umc1045</i>	Probable serine/threonine-protein kinase	F - GCTCGTCCATGAGCAGCATC R - AAGCTGAAGATGCGGAGGTTG	(AG) ₈	7.04

*Position in the chromosome: N/A – Not available, FAW- Fall armyworm,

Genotype Score and Data Analysis

Each band produced by the primers was considered an allele and they were scored accordingly. Genotype data analysis including 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. The Polymorphic Information Content (PIC) was calculated as previously described by Sharma et al. (2009). Additionally, using DARwin software version 6, a dissimilarity matrix based on the Jaccard coefficient was

calculated for the binary data matrix. The matrix was then used to build a UPGMA-based dendrogram and a principal coordinate analysis (PCoA).

Results and Discussion

The genetic diversity of crops is a crucial component of the agricultural system. Crop plants display a huge number of variations, making it challenging to distinguish between them based solely on morphological characteristics because these characteristics are unstable and dependent on environmental and climatic factors (Asif and Zafar,

2006). In order to identify plant genotypes, including varieties and cultivars, molecular biology techniques, particularly molecular markers, have prospective uses (Shinwari et al., 2018). Microsatellites have considerable promise for understanding the mechanisms underlying genetic variation patterns in plant populations. Hence, in this study, the genetic diversity of Sri Lankan maize accessions was evaluated using SSRs related to insect-resistant traits.

Characterization of SSR markers based on Sri Lankan maize accessions

All the SSRs were polymorphic in the present investigation and a total of 77 alleles were detected among 20 maize accessions. The number of observed alleles (N_a) per locus varied from 4 (*umc1187*, *nc134* and *umc1045*) to 13 (*bnlg1588*) averaging 7 (Table 3). This is comparable with the findings of Mathiang et al. (2022) who obtained a mean value of 7.4 alleles per SSR, ranging from 3 to 13 in 37 South Sudan maize

landraces using 27 SSRs. However, it is lower as compared with Adeyemo and Omidiji (2019), who recorded 9.15 alleles per locus using 20 SSRs among 19 Nigerian local maize varieties. The Allele of a particular locus that is present in a maximum number of studied accessions is considered the major allele (Jaiswal et al., 2019). According to our results, both *nc134* and *umc1045* markers corresponding to glossy leaf surface expressed after 3rd leaf and probable serine/threonine-protein kinase genes respectively represented the highest and considered as major allele with the frequency of 0.500, while *bnlg1588* had the lowest (0.132). The primer *bnlg1588* showed the highest effective number of alleles (N_e) (11.281) while the lowest was shown by *umc1045* (2.778) (Table 3). In this study, the average effective number of alleles was 5.295 and which is higher than the 4.8 recorded by Saavedra et al. (2013) in characterizing 28 Brazilian popcorn populations using 11 SSR loci.

Table 3. Observed properties of SSR loci used in this study

SSR loci	Major allele frequency	Number of observed alleles (N_a)	Number of expected alleles (N_e)	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)	Shannon's Information Index (I)	Polymorphic Information Content (PIC)	Fixation index (F)
<i>bnlg1017</i>	0.225	8	6.015	0.700	0.834	1.886	0.812	0.160
<i>bnlg339</i>	0.200	11	8.511	0.800	0.883	2.249	0.871	0.093
<i>umc1021</i>	0.350	7	4.848	0.200	0.794	1.740	0.768	0.748
<i>umc1187</i>	0.425	4	2.909	0.250	0.656	1.145	0.587	0.619
<i>bnlg1346</i>	0.294	8	5.453	0.412	0.817	1.845	0.793	0.496
<i>bnlg1588</i>	0.132	13	11.281	0.632	0.911	2.481	0.904	0.307
<i>bnlg1556</i>	0.350	6	4.103	0.600	0.756	1.518	0.717	0.207
<i>umc1178</i>	0.325	5	3.810	0.950	0.738	1.414	0.691	-0.288
<i>nc134</i>	0.500	4	2.941	0.000	0.660	1.221	0.610	1.000
<i>umc1688</i>	0.250	7	5.594	0.550	0.821	1.803	0.797	0.330
<i>umc1045</i>	0.500	4	2.778	0.100	0.640	1.168	0.581	0.844
Total		77						
Maximum	0.500	13	11.281	0.950	0.911	2.481	0.904	1.000
Minimum	0.132	4	2.778	0.000	0.640	1.145	0.581	-0.288
Mean	0.323	7	5.295	0.472	0.774	1.679	0.739	0.411
SE	0.036	0.884	0.787	0.092	0.028	0.132	0.033	0.113

Maximum observed heterozygosity (H_o) was recorded by *umc1178* (0.950) and *bnlg339* (0.8) while the lowest was detected by *nc134* (0.000). The highest observed heterozygosity for *umc1178* and *bnlg339* primers, which are connected to the maize insect resistance and FAW resistant genes, respectively indicates higher heterozygous nature of Sri Lankan maize accessions for these traits. Expected heterozygosity (H_e) which is an indication of gene diversity, varied from 0.640 (*umc1045*) to 0.911 (*bnlg1588*) with an average of 0.774. The mean value obtained for H_e (0.774) is consistent with previous reports by Qi-Lun et al. (2008) for maize landraces collected from Wuling Mountain, China (0.70). Shannon's Information Index (I) ranged from 1.145 (*umc1187*) to 2.481 (*bnlg1588*) and the Fixation index (F) varied from -0.288 (*umc1178*) to 1.000 (*nc134*). All of the loci's Shannon information

index values were more than 1, indicating the markers' substantial genetic diversity. The PIC values show how useful these the markers are at detecting genetic relationships between varieties (Kim et al., 2021). The PIC values of markers ranged from 0.581 (*umc1045*) to 0.904 (*bnlg1588*) averaging 0.739. This value is close to the results of Adeyemo and Omidiji, (2019) (PIC = 0.75) among Nigerian maize landraces and higher than that of Oppong et al. (2014) for Ghanaian maize landraces (PIC = 0.51). All the markers had PIC values higher than 0.5 showing their ability to discriminate the given set of maize accessions (Varshney et al., 2007). Most of the diversity measures were higher in *bnlg1588*, *bnlg339*, *bnlg1346* and *bnlg1017* and these markers were related to FAW-resistant traits indicating the higher gene diversity for these alleles in the tested maize accessions (Table 3). Among the 11 SSRs used in this study, 6 had a

di-repeat motif, and 3 had a tri-repeat motif. Both di and tri-nucleotide repeats detected similar alleles per locus (6.667 and 7.000 respectively). Conversely, the average PIC value of tri-repeat-based SSR loci (0.766) was higher compared to di-repeat SSR loci (0.712). This is in agreement with the earlier reports (Jaiswal et al., 2019).

Genetic diversity parameters of the analyzed maize accessions

The number of observed alleles (N_a) among Sri Lankan maize accessions ranged from 4 (*SEU17*) to 9 (*SEU20*), with a mean value of 7.25. The landraces *SEU1* and *SEU2* had the highest major allele frequency (0.455). Conversely, *SEU21* had the lowest (0.227). Meanwhile, the effective number of alleles (N_e) ranged from 3.333 (*SEU17*) to 5.902 (*SEU11*). The observed heterozygosity (H_o), with a mean value of 0.461, ranged from 0.000 (*SEU17*) to 0.636 (*SEU2*, *SEU14*, *SEU16*, and *SEU20*).

Within the group of accessions, the average genetic diversity (H_e) and PIC were 0.785 and 0.756, respectively, with *SEU11* having the highest values ($H_e = 0.831$, PIC = 0.810) and *SEU17* having the lowest mean values ($H_e = 0.700$, PIC = 0.645). The average genetic diversity is similar to Swiss maize accessions (0.78) (Eschholz et al., 2010). The Shannon index, with a mean of 1.730, ranged from 0.180 (*SEU17*) to 1.909 (*SEU11*). The accessions *SEU7*, *SEU11*, *SEU16*, and *SEU20* showed more genetic diversity compared to *Bhadra*, whereas *SEU17* showed the lowest diversity based on the genetic diversity indices (H_o , H_e , and PIC). The fixation index, according to Getachew et al. (2020), assesses the degree of inbreeding between and within inbred lines. It varied from 0.115 (*SEU2*) to 1.000 (*SEU17*) with an average of 0.417. Our data showed that *SEU17* exhibits the highest level of inbreeding, as evidenced by the lowest H_o and the highest F values (Table 4).

Table 4. Genetic diversity parameters of selected Sri Lankan maize accessions

Maize accessions	Major allele frequency	Number of observed alleles (N_a)	Number of expected alleles (N_e)	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)	Shannon's Information Index (I)	Polymorphic Information Content (PIC)	Fixation index (F)
<i>SEU1</i>	0.455	7	3.559	0.182	0.719	1.553	0.685	0.747
<i>SEU2</i>	0.455	7	3.559	0.636	0.719	1.540	0.684	0.115
<i>SEU3</i>	0.364	7	4.745	0.455	0.789	1.744	0.764	0.424
<i>SEU4</i>	0.409	7	4.172	0.455	0.760	1.664	0.732	0.402
<i>SEU5</i>	0.273	7	5.149	0.455	0.806	1.775	0.779	0.436
<i>SEU6</i>	0.273	8	5.628	0.545	0.822	1.874	0.799	0.337
<i>SEU7</i>	0.273	8	5.628	0.455	0.822	1.874	0.800	0.447
<i>SEU8</i>	0.389	6	3.522	0.444	0.716	1.459	0.671	0.379
<i>SEU9</i>	0.273	8	5.628	0.455	0.822	1.874	0.799	0.447
<i>SEU10</i>	0.318	6	4.939	0.364	0.798	1.692	0.769	0.544
<i>SEU11</i>	0.273	8	5.902	0.545	0.831	1.909	0.810	0.343
<i>SEU14</i>	0.364	8	5.042	0.636	0.802	1.846	0.781	0.206
<i>SEU15</i>	0.318	8	4.745	0.364	0.789	1.770	0.761	0.539
<i>SEU16</i>	0.318	8	5.628	0.636	0.822	1.896	0.802	0.226
<i>SEU17</i>	0.400	4	3.333	0.000	0.700	1.280	0.645	1.000
<i>SEU18</i>	0.364	8	4.654	0.545	0.785	1.780	0.759	0.305
<i>SEU20</i>	0.273	9	5.261	0.636	0.810	1.871	0.785	0.214
<i>SEU21</i>	0.227	7	5.628	0.545	0.822	1.808	0.798	0.337
<i>SEU23</i>	0.273	7	4.840	0.364	0.793	1.712	0.763	0.542
<i>Bhadra</i>	0.35	7	4.444	0.500	0.775	1.678	0.744	0.355
Maximum	0.455	9	5.902	0.636	0.831	1.909	0.810	1.000
Minimum	0.227	4	3.333	0.000	0.700	1.280	0.645	0.206
Mean	0.332	7.250	4.800	0.461	0.785	1.730	0.756	0.417
SE	0.014	0.239	0.181	0.035	0.009	0.037	0.011	0.044

Cluster analysis and Principle component analysis

With a range of 0.27 to 0.84, the mean pair-wise genetic dissimilarity value was 0.75, indicating the high genetic diversity among the accessions. The closest pairings were *SEU6* and *SEU7*, which had a dissimilarity value of 0.27, and *SEU5* with *SEU6* and *SEU7*, which had a dissimilarity value of 0.41. The UPGMA-based cluster analysis classifies the 20 maize accessions into two main clusters; Cluster I and II consisting of 7 (*SEU1*, *SEU2*, *SEU3*, *SEU4*, *SEU5*, *SEU6* and *SEU7*) and 13 (*SEU8*, *SEU9*, *SEU10*, *SEU11*, *SEU14*, *SEU15*, *SEU16*, *SEU17*, *SEU18*, *SEU20*, *SEU21*, *SEU23* and *Bhadra*) accessions,

respectively with the mean dissimilarity value of 0.752 (Figure 1). A factorial analysis was performed to support the clustering pattern, and the first 2 principal components explained 25.59 % and 15.03 % of variability, respectively (Figure 2). The scatter plot distinctly divided all maize accessions into 2 main groups, similar to the cluster analysis. The different landraces that were collected did not exhibit any regional relationship. Similar to this, inbred maize line classifications in a previous study were unaffected by the source of the data collection (Nyaligwa et al., 2022). Overall, considering the genetic diversity parameters

(H_o , H_e , and PIC), heterosis nature and genetic distance estimates, the accessions *SEU6* and *SEU7* from cluster I and *SEU9*, *SEU11*, *SEU16* and *SEU20* from cluster II

could be utilized for strategic maize breeding programs against fall armyworm in Sri Lanka.

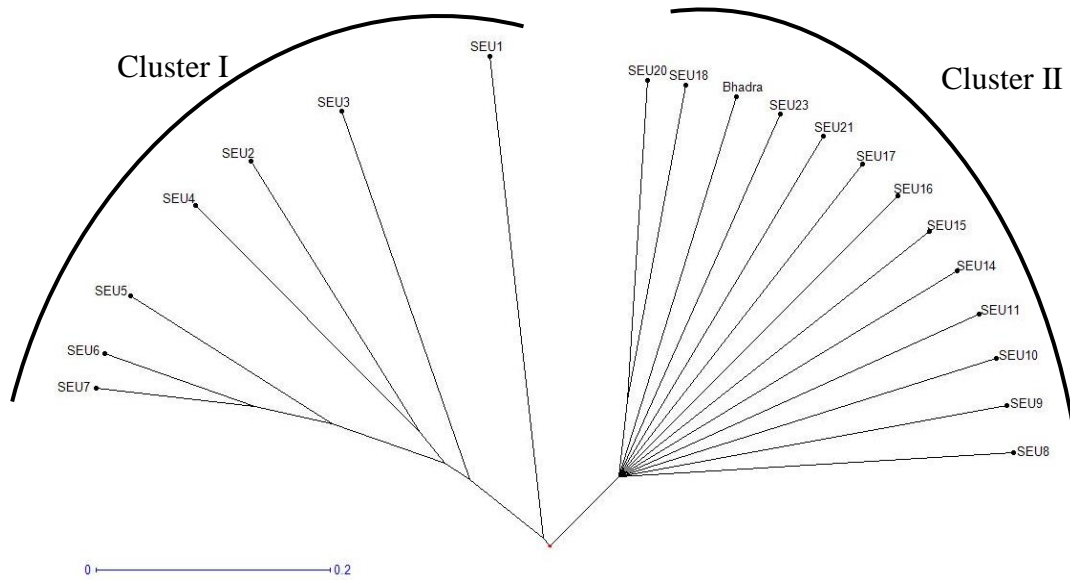


Figure 1. Dendrogram based on SSR marker data

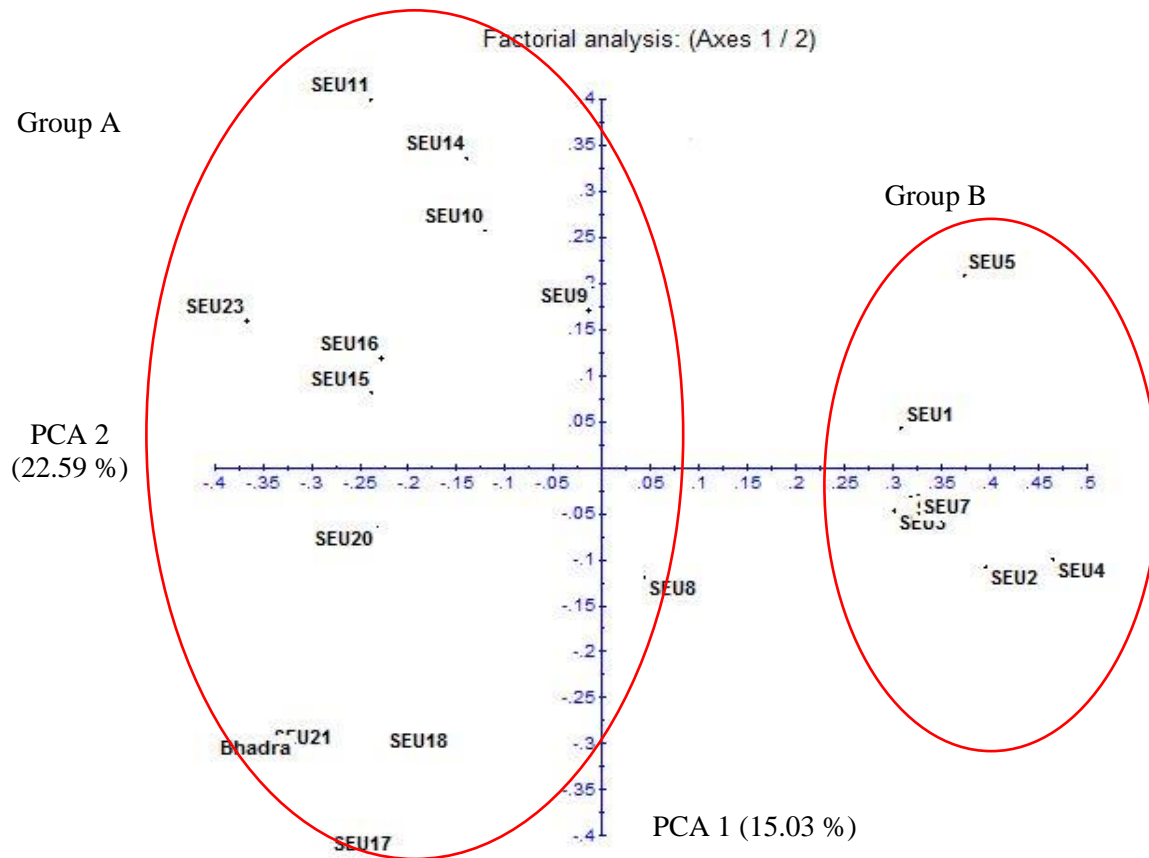


Figure 2. Principal coordinate analysis

Conclusion

Molecular analysis of Sri Lankan maize accessions using SSR markers to explore the diversity of insect resistance has revealed that the markers used in this study had a higher discrimination capacity. Among the tested microsatellite markers, *bnlg1588*, *bnlg339*, *bnlg1346* and *bnlg1017* linked with fall armyworm resistant trait had increased values for genetic diversity measures while marker *umc1045* linked with probable serine/threonine-protein gene had the lowest values. Moreover, the maize accessions *SEU7*, *SEU11*, *SEU16*, and *SEU20* displayed increased genetic diversity compared to *Bhadra*, whereas *SEU17* showed the lowest diversity. Hence, the existence of genetic diversity was seen among the Sri Lankan maize accessions and revealed for the first time through DNA marker-based genetic study. Hence, these results would be valuable to develop new maize accessions through maize breeding programs.

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Competing interests

The authors have declared that no competing interests exist

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