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Research Article

Assessment of genetic diversity in Myanmar maize inbred lines using SSR markers

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Abstract

A total of 120 maize inbred lines were subjected to genetic diversity analysis by 40 SSR markers. The study revealed total alleles of 146 across 40 simple sequence repeat (SSR) markers, ranged from 2 to 8 with the average of 4 per locus. Gene diversity was observed with the mean value of 0.55, range from 0.11 to 0.71 among maize genotypes. Polymorphism Information Content (PIC) value ranged from 0.10 to 0.76 with the mean value of 0.48. Umc1525 was the best marker in this study for identification of genotypes as revealed by its PIC values of 0.76. A wide range of genetic variability and PIC value were observed among maize genotypes designating that tested genotypes are invaluable genetic materials for the breeding program. In UPGMA classification by molecular markers, the genotypes were identified three distinct clusters. The log-likelihood exposed by the population structure with the optimum K value of 4, pointed out that genotypes could be divided into four sub-populations. Genotypes involved in sub-population IV analyzed by population structure, which was one group in cluster IIIB constructed by UPGMA, indicating that genotypes in sub-population IV were unique and diverse from other maize genotypes. Principal coordinate analysis (PCoA) was performed to identify maize inbred lines into genetically diverged and similar genotypes. Based on this finding, genotypes viz. TK.19.17, TK.19.20, TK.19.21, TK.19.27, TK.19.28, TK19.31, TK.19.33, YZSI.20.006, YZSI.20.15, YZSI.20.16, YZSI.20.17, YZSI.20.26, YZSI.20.054, YZSI.20.036 and YZSI.20.027 were genetically diverged and useful in maize breeding program for the exploitation of heterosis.

1. Introduction

In Myanmar, maize is the second most important cereal crop after rice, and its demand has steadily increased since 2009. The maize growing area has expanded and hybrid maize varieties have been widely cultivated [1]. The yields and quality of maize in most maize producing countries have been greatly improved by conventional and hybrid breeding programs [2]. In maize breeding programs, the genetically diverse elite inbred lines can play a significant role to develop hybrids with maximum expression of heterosis without making all possible crosses among them [3]. The potential hybrids can be obtained in crosses of parents from a diverse origin than hybrids formed from lines with same genetic background [4]. Lack of genetic diversity of breeding resources may lead to limitation in breeding progress and genetic advance from selection [5, 6]. To overcome this limitation, the assessment of genetic diversity and population structure is consistently carried out by using different marker techniques such

as morphological, biochemical and molecular markers [7, 8]. Consequently, accurate characterization of inbred lines using molecular technique is important for effective breeding [9]. Therefore, genetic diversity study of breeding resources by using molecular technique is essential for the development of new maize inbred lines, identification of heterotic grouping and selection of potential parents in maize breeding [10].

Molecular markers are indispensable to assess genetic resources for breeders for the collection of genetic variability and the detection of genetic differences [11]. The assessment of genetic diversity by molecular marker has a great potential to identify genetically diverged inbred lines [4, 12, 13]. In the genetic diversity study of crops like maize, wheat, rice, and barley, SSR markers have been widely used [14, 15]. In the assessment of genetic diversity, genetic structure, evolutionary origin, population structure, genome wide association mapping, fingerprinting, and plant breeding programs, application of SSR markers are also essential [3, 16-20]. SSR markers have the capacity of high variability from co-dominant and multi-allelic polymorphisms, and precise and fast detection [21]. Therefore, the classification of inbred lines by SSR markers is invaluable to select potential parents for the breeders to exploit maximum heterosis. The present study aimed to assess genetic diversity and population structure of Myanmar maize inbred lines using Simple Sequence Repeat (SSR) markers.

2. Materials and methods

2.1 Plant materials

The 120 maize inbred lines were used for genetic diversity analysis. The inbred lines were developed from introduced hybrids (Supplementary Table S1) at the Other Cereal Crops Research Section, Department of Agricultural Research (DAR), Yezin, Nay Pyi Taw and Tatkone Research Farm, DAR, Myanmar. B73 (CIMMYT) and C7 (used as common tester in Myanmar hybrid maize breeding program) were also involved in tested genotypes to observe their genetic relationship. The experiment was conducted at Biotechnology Research Section, DAR.

2.2 SSR markers analysis

2.2.1 DNA extraction

Young fresh leaves of 3–5 plants were selected to extract DNA using the cetyl trimethyl ammonium bromide (CTAB) method. DNA concentration was measured by using Nanodrop ND-2000 Spectrophotometer. Then, DNA mother liquor was diluted to 50 ng/ µl. By preliminary screening, 40 polymorphic SSR markers covering the whole maize genome were used in this study. The primer sequence information was available from the Maize Genetics and Genomics Database (www.maizegdb.org).

2.2.2 Polymerase chain reaction (PCR) and electrophoresis

Every 10 µl of PCR reaction system contained 1.3 µl 10xBuffer, 1.0 µl (2.5 mM) dNTP, 0.3 µl (25 µM) each forward and reverse primer, 0.5 µl (5 U/µl) Taq polymerase, 1 µl (50 ng/µl) DNA, and 5.6 µl ddH_2O. PCR reaction procedures included pre-denaturation at 95 ℃ for 5min, denaturation at 95 ℃ for 30s, annealing (different marker sites) at 55-62 ℃ for 30s, extending at 72 ℃ for 30s, repeating 35 times, extending at 72 ℃ for 5 min, stop the reaction at 10 ℃, storage at 4 ℃. PCR products were detected using red safe gel electrophoresis. The PCR products along with 100bp DNA ladder as molecular marker was resolved in 3% agarose gel. Then, the allele of 40 SSR loci were scored according to the molecular weight of each amplified DNA with the 100bp DNA ladder.

2.3 Statistical analysis

Power Marker Version 3.25 Software was used to detect allele number, gene diversity and polymorphic information content (PIC). The construction of Phylogenetic tree analysis was assembled based on the Unweighted Pair-Group Method with Arithmetic Means (UPGMA) [22] dendrogram by using molecular evolutionary genetics analysis (MEGA V6) software [23]. The genetic distance of maize genotypes was assessed according to Nei coefficient [24].

The population structure was performed using the Bayesian Markov Chain Monte Carlo model (MCMC) implemented in STRUCTURE v 2.3.1 software [25]. The number of potential genetic clusters (K values) was set from 1 to 10; with 3 independent runs for each K. Analysis of Molecular variance (AMOVA) was used to observe genetic variation among individual genotypes within the same populations and also among different populations. Principal coordinate analysis (PCoA) was completed by GenAIEx V 6.5 software [26].

3. Results and discussion

3.1 Genetic diversity of maize genotypes

In this study, all the SSR markers created divergent reproducible amplification which was applied for the genetic diversity examination of maize genotypes (Table 1). The study revealed a total of 146 alleles across 40 marker loci suggesting that a high polymorphism can be foreseeable in the tested maize inbred lines. The allele number ranged from 2 to 8 with the average of 4 per locus. Nikhou et al., [27] observed that the alleles number ranges from 2 to 6 using 10 SSR markers. Synrem et al., [28] reported that the allele number ranges from 1 to 5 with mean of 3.5. Nikolić et al., [29] found that the allele number ranges from 1 to 6 with mean of 5.95. The difference in allele number observed among genotypes may be due to population size under study, the methodologies used for the discovery of polymorphic markers which affect allelic dissimilarities or equality based on pedigrees [30]. The values of genetic diversity were observed in the range of 0.11 (umc2061) to 0.79 (umc1525) with the average of 0.55. In this study, average gene diversity value (0.37) is higher than other finding [30], with the range of 0.22 to 0.47. A high range of gene diversity among genotypes may be due to diverse sources involved in their development, making them more heterogeneous. In this study, SSR markers showed high allelic variants as well as gene diversity values.

The Polymorphic Information Content (PIC) value is a significant parameter which administers the effectiveness of a marker in estimating genetic diversity of genotypes. PIC value was observed in this study with the average of 0.48 ranging from 0.10 (umc2061) to 0.76 (umc1525) (Table 1). The average PIC value is lower to compare with the finding [31] with the average of 0.55, range from 0.054 to 0.82. Nevertheless, it was higher than other research finding [30], with the average of 0.30, range of 0.20 to 0.37. Among the 40 SSR markers, umc1525, bnlg381, umc2011, phi299852, umc1754 and bnlg2248 were observed to be the most powerful markers for genetic diversity assessment among genotypes based on their highest PIC value of 0.76, 0.75, 0.74, 0.73, 0.72 and 0.71, respectively. The powerful molecular markers could be effectively used to identify genotypes into alternative heterotic groups [10]. In plant breeding programs, study on genetic variation and PIC values are enormously supportive for information on the level of polymorphisms among genotypes [17]. The present investigation provides valuable information on the genetic constitution of 120 maize genotypes studied by using 40 SSR markers.

Out of the total markers, 10 % showed low polymorphism (PIC = 0.10 to 0.21), 42.5% was with average polymorphism (PIC = 0.28 to 0.48) and 47.5% was high polymorphism (0.51 to 0.76). A PIC value of less than 0.25 directs low polymorphism; a value between 0.25 and 0.5 shows average polymorphism and a value higher than 0.5 specifies a highly polymorphic locus [32]. Several factors can influence the PIC value including population size, diversity, method of genotyping and location of primer on a chromosome, etc.

3.2 UPGMA cluster analysis based on genetic distance

One of the effective methods for plant breeders to observe the genetic variation among the genotypes is UPGMA clustering method. In this method, the 120 genotypes were firstly divided into three main clusters, cluster I included 39 genotypes, cluster II included 28 genotypes and cluster III included 53 genotypes (Fig. 1). The cluster I could be further subdivided into clusters IA with 11 genotypes and IB with 28 genotypes. The cluster II could be further subdivided into IIA with 6 genotypes and IIB with 22 genotypes.

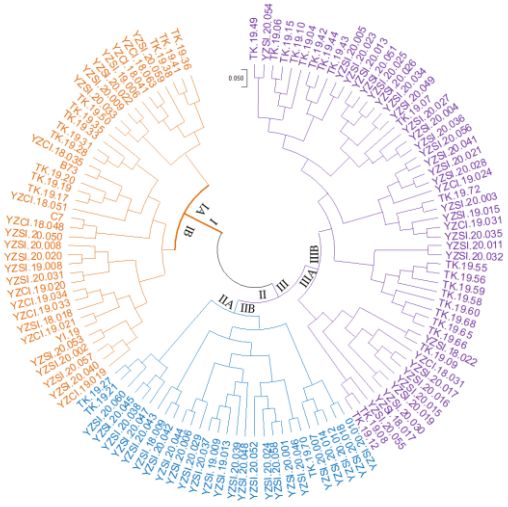

Figure 1. UPGMA clustering of maize inbred lines using Nei's genetic similarity coefficients

Table 1. Information of 40 SSRs: bins, motif, allele frequency, allele number, gene diversity and PIC value across 120 maize inbred lines

Marker	Bins	Motif	Allele Frequency	Allele Number	Gene Diversity	PIC
bnlg1014	1.01	AG(14)	0.9	2	0.24	0.21
bnlg1614	1.02	AG(15)	0.4	4	0.65	0.58
bnlg1083	1.02	AG(29)	0.5	3	0.64	0.57
umc1754	1.06	(CGAT)5	0.3	6	0.76	0.72
dupssr12	1.08	(AC)15	0.5	4	0.62	0.54
bnlg1297	2.02	AG(32)	0.3	3	0.67	0.59
bnlg2248	2.03	AG(30)	0.3	6	0.75	0.71
bnlg381	2.04	—	0.3	6	0.78	0.75
bnlg2328	2.05	AG(33)	0.6	2	0.47	0.36
umc2372	2.06	(TC)6	0.6	2	0.48	0.37
mmc0271	2.07	(GA)39	0.9	2	0.18	0.16
umc1525	2.09	(CGA)4	0.3	8	0.79	0.76
umc2369	3.03	(GCAC)4	0.4	5	0.67	0.60
umc1501	3.05	(AAG)5	0.8	2	0.34	0.28
bnlg1035	3.05	AG(13)	0.4	6	0.67	0.60
bnlg1160	3.06	AG(13)	0.6	3	0.58	0.51
umc1140	3.08	(AAGAA)4	0.5	3	0.57	0.48
umc2061	4.05	(CTG)8	0.9	2	0.11	0.10
umc1847	4.07	(CGC)6	0.5	3	0.56	0.46
umc1101	4.09	(CT)6	0.5	4	0.64	0.58
umc1940	4.09	—	0.6	3	0.51	0.41
umc2011	4.10	—	0.3	8	0.77	0.74
umc2291	5.00	(CCT)5	0.4	4	0.68	0.61
umc1056	5.03	(AGCA)4	0.3	7	0.72	0.67
phi331888	5.04	AAG	0.7	2	0.39	0.31
umc1591	5.04	CA	0.5	2	0.50	0.37
umc1153	5.09	(TCA)4	0.6	2	0.49	0.37
Umc1350	6.07	(GCT)5	0.9	3	0.16	0.15
phi299852	6.09	AGC	0.3	8	0.76	0.73
umc1671	7.05	(AGC)7	0.6	2	0.49	0.37
umc1904	8.03	(TAAGC)5	0.7	3	0.49	0.44
bnlg1812	8.05	AG(22)	0.5	3	0.63	0.55
bnlg1065	8.07	AG(21)	0.6	2	0.48	0.36
umc1586	9.03	(ATC)5	0.5	2	0.50	0.37
umc2359	9.07	(AAAAG)4	0.7	2	0.42	0.33
umc1137	9.08	(CT)15	0.4	6	0.68	0.62
umc1432	10.02	(AG)6	0.4	3	0.66	0.59
umc1166	10.02	(CT)10	0.5	3	0.52	0.41
bnlg1028	10.06	AG(12)	0.5	3	0.51	0.40
bnlg2190	10.06	AG(31)	0.7	2	0.44	0.34
Total				**146**		
Mean			**0.50**	**4**	**0.55**	**0.48**

PIC = polymorphism information content

The clusters III could be further subdivided into IIIA with 12 genotypes and IIIB with 41 genotypes to evaluate genetic diversity and relationship among tested genotypes. B73 and C7 were involved together in cluster I, and thus breeder could assume that genotypes in cluster I were great genetic different from another cluster II and III. The genotypes involved together in one cluster, we can assume that they are genetically similar while genotypes included in different clusters, we can assume that they are genetically different. In this study, the breeders can develop hybrids by crossing the diverged genotypes from different clusters.

3.3 Genetic relationships and population structure analysis

The log-likelihood values exposed by the population structure, the optimum Delta K for 120 genotypes were observed at K=4 (Fig. 2a). This result pointed out that the genetic structure of 120 genotypes had the

most probable number of populations at K = 4 (four distinct subpopulations). Based on it, the genotypes could be divided into four groups such as sub-population I, II, III and IV (Fig. 2b). The genotypes in each group were determined by their inferred genome fraction value >70% (Supplementary Table S2). In sub-population I included 30 genotypes (25.00%), sub-population II included 18 genotypes (15.00%), sub-population III included 14 genotypes (11.67%) and sub-population IV included 20 genotypes (16.67%), respectively. According to inferred genome fraction value <70%, 38 genotypes (31.67%) were found as admixture individuals. It may be due to some genetic materials were developed from wider genetic sources. Admixture may be advantageous by foremost to heterosis and lifting inbreeding depression [33]. Based on this finding, 82 out of 120 genotypes from four discrete groups could be effectively utilized as genetic resources for heterosis breeding to develop hybrids with breeder's desired traits.

Remarkably, genotypes viz. TK.19.04, TK.19.06, TK.19.07, TK.19.10, TK.19.42, TK.19.43, TK.19.44, YZSI.20.004, YZSI.20.005, YZSI.20.021, YZSI.20.026, YZSI.20.027, YZSI.20.028, YZSI.20.036, YZSI.20.041, YZSI.20.049, YZSI.20.051, YZSI.20.054 and YZSI.20.056 included together in sub-population IV in population structure approach (Supplementary Table S2). These genotypes were also involved in one group in cluster IIIB by UPGMA method (Fig. 1) indicating that these inbred lines were unique and diverse from other maize inbred lines and thus these genotypes could be useful in maize breeding program.

3.4 Analysis of molecular variance (AMOVA)

According to AMOVA, there was a highly significant genetic difference among tested maize inbred lines within the population, within individual and among population. The four populations attained from the structural analysis were consecutively exposed to AMOVA to observe the variation across and within populations. The genetic variation among individual was 78%, meanwhile 18% occurred within individuals, and 4% among populations (Table 2).

3.5 Principal coordinate analysis (PCoA)

PCoA was performed from the genetic distances of maize genotypes from the three main component factors of the eigenvalues. The percentage of variation was 5.39% (axis 1), 4.94% (axis 2) and 4.61% (axis 3),

Table 2. Analysis of molecular variance among and within populations

Source	df	SS	MS	Est. Var	Percent variation (%)	F-stati-stic
Among Pops	2	94.77	47.39	0.52	4%	0.009
Among indiv	117	2366.49	20.23	9.07	78%	0.001
Within indiv	120	251.5	2.1	2.1	18%	0.001
Total	239	2712.77		11.68	100%	

d.f = degree of freedom; SS = sum of square; MS = mean square; Est. Var = estimated variance; Pops = populations, Indiv = individuals.

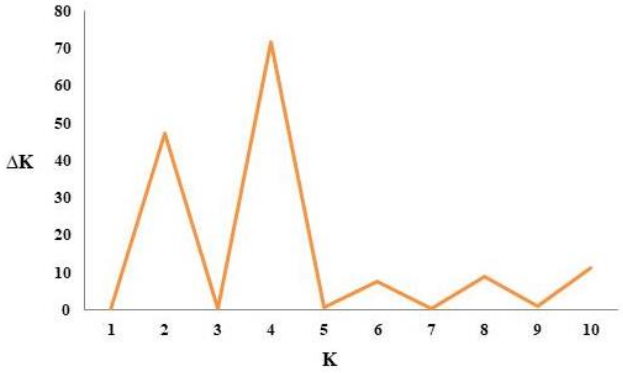

Figure 2a

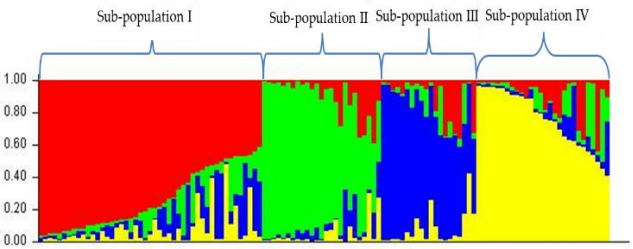

Figure 2b

Figure 2. Population structure analysis of 120 maize inbred lines; (**2a**) Values of delta K, with its modal value used to detect true K of the group (K = 4) (**2b**) sub-population sorted by kinship matrix

respectively. According to the highest percent variation, PCoA was constructed by two dimensional graphical views of axes 1 and 3 (Fig. 3). The genotypes viz. B73, TK.19.17, TK.19.20, TK.19.21, TK.19.27, TK.19.28, TK19.31, TK.19.33, YZSI.20.006, YZSI.20.15, YZSI.20.16, YZSI.20.17, YZSI.20.26, YZSI.20.054, YZSI.20.036 and YZSI.20.027 were far away from centroid and the rest of the genotypes were around the centroid.

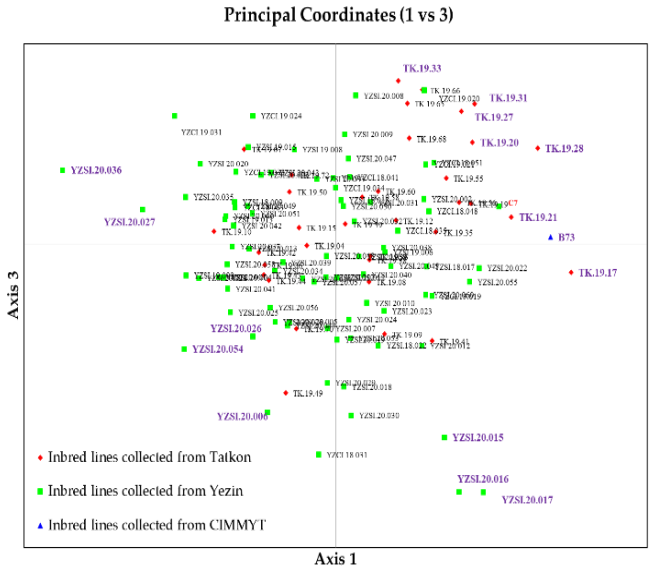

Figure 3. Principal coordinate analysis of 120 maize inbred lines illustrated based on 40 SSR markers

The genotypes were far away from the centroid; we can assume that they are genetically diverged. The genotypes were allocated near the centroid; we can assume that they are genetically more similar among tested populations [34].

Interestingly, PCoA supported the dendrogram assembled by UPGMA clustering method (Fig. 1), demonstrating that these two methods complement each other to the group classification. In UPGMA approach, TK.19.17 involved in cluster IA, TK.19.28, TK19.31, TK.19.33, TK.19.20, B73 and C7 included in cluster IB. Moreover, TK.19.21 and TK.19.27 involved in Cluster IIA, YZSI.20.006 comprised in IIB, YZSI.20.15, YZSI.20.16, YZSI.20.17 involved in cluster IIIA. The genotypes viz. YZSI.20.26, YZSI.20.054, YZSI.20.27 and YZSI.20.36 involved in cluster IIIB, respectively. These promising inbred lines could be utilized in breeding strategies for the exploitation of maximum heterosis. Genotypes from Cluster IB and Cluster IIA approached by UPGMA were also involved in one group by PCoA indicating that these inbred lines were unique and diverse from other maize inbred lines and thus these genotypes could also be useful in maize breeding program.

4 Conclusions

Among 40 SSRs, 38 markers were observed as the greatly informative markers and they adequately distinguished 120 maize genotypes for genetic diversity analysis. The genetically diverged genotypes viz. TK.19.17, TK.19.20, TK.19.21, TK.19.27, TK.19.28, TK19.31, TK.19.33, YZSI.20.006, YZSI.20.15, YZSI.20.16, YZSI.20.17, YZSI.20.26, YZSI.20.054, YZSI.20.036 and YZSI.20.027 could be effectively used as breeding materials for better estimation of heterosis and creating of new hybrids with wider adaptation to different ecosystems. Therefore, the information obtained by this research is invaluable for maize breeders with an exceptional concentration on the development of potential hybrids and generate wide variability in genetic architecture.

Supplementary Data

Supplementary Table S1

Supplementary Table S2

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Authors' contributions

This research was carried out in collaboration among all authors. Author Nay Aung performed the research, analyzed the data, interpreted the results and prepared original manuscript. Dr. Nyo Mar Htwe wrote the protocol, executed the research and improved the manuscript. Dr. Myint Aye reviewed and revised all draft of the manuscript. Dr. Kyi Moe, Dr. Thida and Dr. Soe Win reviewed and revised the final draft. All authors read and approved published version of the manuscript.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

Authors have declared that no competing interests exist.

References

1. Htwe K.K. Export, Market Potential, and Maize Production in Myanmar. In Conference Paper, Second Annual Agricultural Research Conference, Myanmar. 2020.
2. Trung, K.H.; Tran, Q.H.; Bui, N.H.; Tran, T.T.; Luu, K.Q.; Tran, N.T.T.; Nguyen, L.T.; Nguyen, D.T.N.; Vu, B.D.; Quan, D.T.T. A weak allele of *FASCIATED EAR 2 (FEA2)* increases maize kernel row number (KRN) and yield in elite maize hybrids. J. Agro., 2020, 10(11), 1774.
3. Adeyemo, O.; Menkir, A.; Melaku, G.; Omidiji, O. Genetic diversity assessment and relationship among tropical yellow endosperm maize inbred lines using SSR markers. Maydica, 2012, 56(1).
4. Menkir, A.; Melake-Berhan, A.; The, C.; Ingelbrecht, I.; Adepoju, A. Grouping of tropical mid-altitude maize inbred lines on the basis of yield data and molecular markers. Theor. Appl. Genet., 2004, 108, 1582-1590.
5. Cornelious, B.K.; Sneller, C.H. Yield and molecular diversity of soybean lines derived from crosses of northern and southern elite parents. Crop Sci., 2002, 42(2), 642-647.
6. Onaga, G.; Edema, R.; Asea, G. Tolerance of rice germplasm to iron toxicity stress and the relationship between tolerance, Fe2+, P and K content in the leaves and roots. Arch. Agron. Soil Sci., 2013, 59(2), 213-229.
7. Govindaraj, M.; Vetriventhan, M.; Srinivasan, M. Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. Genet. Res. Int., 2015.
8. our Badu-Apraku, B.; Fakorede, M.A.B. Advances in genetic enhancement of early and extra-early maize for sub-Saharan Africa. 2017.
9. Xu, J.I.E.; Liu, L.I.N.; Xu, Y.U.N.; Chen, C.H.U.; Rong, T.I.N.; Ali, F.A.R.; Zhou, S.H.U.; Wu, F.E.N.; Liu, Y.A.X.; Wang, J.I.N.; et al. Development and characterization of simple sequence repeat markers providing genome-wide coverage and high resolution in maize. DNA Res., 2013, 20(5), 497-509.
10. Xia, X.C.; Reif, J.C.; Hoisington, D.A.; Melchinger, A.E.; Frisch, M.; Warburton, M.L. Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers: I. Lowland tropical maize. Crop Sci., 2004, 44(6), 2230-2237.
11. Arabzai, M.G.; Gul, H. Application techniques of molecular marker and achievement of marker assisted selection (MAS) in three major crops rice, wheat and maize. Int. J. Res. Appl. Sci. Biotechnol., 2021, 8(1), 82-93.
12. Badu-Apraku, B.; Oyekunle, M.; Akinwale, R.O.; Aderounmu, M. Combining ability and genetic diversity of extra-early white maize inbreds under stress and non-stress environments. Crop Sci., 2013, 53(1), 9-26.
13. Geleta, N.; Labuschagne, M.T.; Viljoen, C.D. Genetic diversity analysis in sorghum germplasm as estimated by AFLP, SSR and morpho-agronomical markers. Biodivers. Conserv., 2006, 15(10), 3251-3265.
14. Cao, W.; Hucl, P.; Scoles, G.; Chibbar, R.N. Genetic diversity within spelta and macha wheats based on RAPD analysis. Euphytica, 1998, 104, 181-189.
15. Sun, Q.; Ni, Z.; Liu, Z.; Gao, J.; Huang, T. Genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat revealed by RAPD markers. Euphytica, 1998, 99, 205-211.
16. Aci, M.M.; Revilla Temiño, P.; Morsli, A.; Djemel, A.; Belalia, N.; Kadri, Y.; Khelifi-Saloui, M.; Ordás, L.B.; Khelifi, L. Genetic diversity in Algerian maize (*Zea mays* L.) landraces using SSR markers. 2013.
17. Eltaher, S.; Sallam, A.; Belamkar, V.; Emara, H.A.; Nower, A.A.; Salem, K.F.; Poland, J.; Baenziger, P.S. Genetic diversity and population structure of F3: 6 Nebraska winter wheat genotypes using genotyping-by-sequencing. Front. Genet., 2018, 9, 76.
18. Nyaligwa, L.; Hussein, S.; Amelework, B.; Ghebrehiwot, H. Genetic diversity analysis of elite maize inbred lines of diverse sources using SSR markers. Maydica, 2015, 60(3), 29-36.
19. Shayanowako, A.I.T.; Shimelis, H.; Laing, M.D.; Mwadzingeni, L. Genetic diversity of maize genotypes with variable resistance to Striga asiatica based on SSR markers. Cereal Res. Commun., 2018, 46, 668-678.
20. Lanes, E.C.; Viana, J.M.; Paes, G.P.; Paula, M.F.; Maia, C.; Caixeta, E.T.; Miranda, G.V. Population structure and genetic diversity of maize inbreds derived from tropical hybrids. 2014.
21. Ahmad, A.; Wang, J.D.; Pan, Y.B.; Sharif, R.; Gao, S.J. Development and use of simple sequence repeats (SSRs) markers for sugarcane breeding and genetic studies. Agronomy, 2018, 8(11), 260.
22. Sokal, R.R.A. Statistical method for evaluating systematic relationships. Univ. Kans. sci. bull., 1958, 38, 1409-1438.
23. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol., 2013, 30(12), 2725-2729.
24. Nei, M.; Tajima, F.; Tateno, Y. Accuracy of estimated phylogenetic trees from molecular data: II. Gene frequency data. J. Mol. Evol., 1983, 19, 153-170.
25. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. Genetics, 2000, 155(2), 945-959.

26. Peakall, R.O.D.; Smouse, P.E. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes, 2006, 6(1), 288-295.
27. Nikhou, F.; Ebrahimi, A.; Shiri, M. Genetic diversity assessment among maize hybrids with using SSR markers. Tech. J. Engin. Appl. Sci., 2013, 3, 3831-3834.
28. Synrem, G.J.; Marker, S.; Ramteke, P.W.; Charan, A.A. Simple sequence repeat (SSR) markers for molecular diversity and heterozygosity analysis in maize (*Zea mays* L.) inbred lines. J. Pharmacogn. Phytochem., 2017, 6(6), 732-737.
29. Nikolić, A.; Ignjatović-Micić, D.; Kovačević, D.; Čamdžija, Z.; Filipović, M.; Mladenović Drinić, S. Genetic diversity of maize inbred lines as inferred from SSR markers. Genetika, 2015, 47(2), 489-498.
30. Kumari, A.; Sinha, S.; Rashmi, K.; Mandal, S.S.; Sahay, S. Genetic diversity analysis in maize (*Zea mays* L.) using SSR markers. J. of Pharmacogn. Phytochem., 2018, 7(1S), 1116-1120.
31. Kumar, A.; Rakshit, A.; Mangilipelli, N.K.; Varalaxmi, Y.; Vijayalakshmi, T.; Vanaja, J.M.; Yadav, S.K.; Venkateswarlu, B.; Maheswari, M. Genetic diversity of maize genotypes on the basis of morpho-physiological and simple sequence repeat (SSR) markers. Afr. J. Biotechnol., 2012, 11(99), 16468-16477.
32. Botstein, D.; White, R.L.; Skolnick, M.; Davis, R.W. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genetics, 1980, 32(3), 314.
33. Escobar, J.S.; Nicot, A.; David, P. The different sources of variation in inbreeding depression, heterosis and outbreeding depression in a metapopulation of Physa acuta. Genetics, 2008, 180(3), 1593-1608.
34. Azam, M.G.; Islam, S.; Hossain, M.G.; Rohman, M.M. Molecular assessment of maize inbred lines (*Zea mays* L.) using microsatellite markers. Bangladesh J. Agric. Res., 2018, 43(4), 533-542.