Research article

DNA Fingerprinting of Selected Maize (*Zea mays* L.) Genotypes using SSR Markers

SAN KYI*
Yezin Agricultural University, Nay Pyi Taw, Myanmar
Email: sankyi747@gmail.com

KYAW KYAW WIN
Pro-Rector's Office of Administration, Yezin Agricultural University, Nay Pyi Taw, Myanmar

HLA THAN
Pro-Rector's Office of Academic, Yezin Agricultural University, Nay Pyi Taw, Myanmar

SOE WIN
Department of Plant Breeding, Physiology and Ecology, Yezin Agricultural University, Nay Pyi Taw, Myanmar

NYO MAR HTWE
Advanced Center for Agricultural Research and Education, Yezin Agricultural University, Nay Pyi Taw, Myanmar

AYE LAE LAE HLAING
Plant Molecular Biology Laboratory, Biotechnology Research Section, Department of Agricultural Research, Nay Pyi Taw, Myanmar

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Abstract Molecular marker has been used for variety identification, genetic diversity of genotypes and additional using intellectual property protection in DUS testing. DNA fingerprinting of fourteen maize genotypes had been studied at Plant Biotechnology Laboratory, DAR, Myanmar during 2020-2021. Nine hybrids and five inbreds were analyzed using 24 polymorphic SSR markers, resulted a total number of 101 alleles with a range from 2 to 9 alleles per locus. Polymorphism has been sufficiently detected with the average of 0.66 per SSR locus. Cluster analysis separated all the maize genotypes as five major groups and indicated the existence of genetic variation among the observed inbreds and hybrids. Six SSR primer pairs (dupssr12, bnlg1940, umc1248, umc1586, bnlg1518 and bnlg1028) were selected as final marker set for variety identification with the selection criteria such as detection rate of the SSR fragment, the presence of rare allele, PIC value, and reproducibility and PCR band pattern of SSR fragments. In this study, all tested genotypes have been fingerprinted with unique profile identity (ID) to support the DNA fingerprint catalogues of Myanmar Maize Molecular DUS test guidelines.

Keywords DNA fingerprinting, maize SSR markers, varietal identification

INTRODUCTION

Maize (*Zea mays* L.) is the most diverse crop species characterized at both morphological and molecular levels (Anderson and Cutler, 1942; Buckler et al., 2006; Zheng et al., 2008). Genetic identification of maize is essential in the process of crop improvement, crop quality estimation and the finding of parent components and their crosses.

SSR markers were able to discriminate maize genotypes depending upon their hybridization level and their genetic relationships among the different inbred lines (Kamal et al., 2010). It is the most powerful biotechnological tool, suitable for detecting of genetic purity status of maize hybrids.

SSR also used in DNA fingerprinting analysis for identification of individuals, populations, inbred lines, and hybrids, making the description of the species at the molecular level, to protect the breeders' Rights and its protection.

Plant varietal protection and patents are the most important end products of plant breeding institutes to get back their research investment by mean of intellectual property protection. DNA fingerprinting technology is useful for preventing counterfeit and fake varieties in the market and this fingerprinting information is additional information for DUS characterization (Wang et al., 2013). UPOV (2010) recommends SSRs for current construction of DNA fingerprint databases that have been well-defined and tested. In the present study, the SSR primers set were used to differentiate hybrids and inbreds, and to create the DNA fingerprint catalogues for supporting of Myanmar Maize DUS test guidelines for identifying maize genotypes in infringement case, and for utilizing as a source of parental line for future breeding programs.

METHODOLOGY

Plant Materials and Genomic DNA Extraction

Fourteen maize genotypes (Table 1) were used to study molecular characterization at Plant Biotechnology Laboratory, Department of Agricultural Research (DAR), Myanmar during 2020-2021. Nine hybrids (imported by private companies) and five inbred lines (developed by DAR) were used. Genomic DNA extraction, DNA quantification and qualification were carried out according to the procedure of Hlaing et al. (2017).

Table 1 List of selected maize genotypes for molecular characterization

No.	Genotypes	Type	Source	No.	Genotypes	Type	Source
V1	Asia Seed (A.55)	Hybrid	China	V8	NK-621	Hybrid	Syngenta
V2	Asia Seed (A.99)	Hybrid	China	V9	TSF-1633	Hybrid	Thailand
V3	AA-737	Hybrid	Thailand	V10	YZI-10-054	Inbred	DAR
V4	GT-722	Hybrid	Thailand	V11	YZI-10-095	Inbred	DAR
V5	NK-625	Hybrid	Syngenta	V12	PAC-999	Inbred	Thailand
V6	KMHE-3550	Hybrid	India	V13	C7	Inbred	DAR
V7	CP-111	Hybrid	Thailand	V14	YZCI-16-019	Inbred	DAR

PCR Amplification and Gel Electrophoresis

Twenty-four SSR primer pairs were used to identify the studied maize genotypes. These highly polymorphic primer sets, PCR amplification and agarose gel electrophoresis were conducted following the methods reported by previous research findings (Hlaing et al., 2017).

Statistical Analysis on Marker Data

The SSR allele segregation data were used to construct Nei distance dendrogram (Nei, 1972) using NTSYSpc 2.1 software (Rohlf 2000). Polymorphism Information Content (PIC) for each SSR was calculated by the formula $PIC=1-\Sigma X^2k/n$ developed by Ni et al. (2002) where, X^2k represents the frequency of the k^{th} allele, and n represents the number of genotypes.

Development of Core SSR Marker Set for DNA Fingerprinting (DUS testing)

Based on their specific discrimination capacity of SSR markers, the core marker set was developed with the following criteria: (1) PCR banding pattern of SSR fragments (2) the presence of rare allele (3) PIC values and (4) marker's reproducibility. Furthermore, the genetic fingerprint map of tested maize genotypes was constructed using the coding-based system with the original allele size coded by the assigned numeric values which set in two numeric codes from "01", "02", "03", etc. The transformation was generated to all cores SSR ID set based on allele size (bp) range. The final SSR fingerprinting map of maize genotypes was produced to support variety identification and DUS testing on PVP system.

RESULTS AND DISCUSSION

Amplification Profile, SSR Polymorphism and Cluster Analysis on Diversity Assessment

SSR markers have been proven to be powerful tools in the assessment of genetic variation within and among the species. A total of 24 SSR primers were used for identification of maize genotypes through evaluation of DNA fragment polymorphisms. A total number of 101 alleles were detected with a range from 2 to 9 alleles per locus. These varied number of alleles indicated that there is a high level of genetic diversity among tested genotypes. The primer pairs bnlg1028 showed the highest number of 9 alleles per locus, umc1248 showed 8 alleles in all tested genotypes and umc1586, bnlg1940 and dupssr12 showed 6 alleles in all samples (Table 2).

Table 2 Data on number of alleles, total number of alleles, and PIC value obtained among 14 maize genotypes for 24 SSR markers

No.	SSR marker	No. of alleles	Total no. of alleles	PIC value	No.	SSR marker	No. of alleles	Total no. of alleles	PIC value
1.	umc1397	4	20	0.63	13.	bnlg1617	4	14	0.72
2.	umc2234	3	20	0.62	14.	umc1520	5	22	0.71
3.	dupssr12	6	30	0.77	15.	umc1248	8	38	0.85
4.	umc1542	3	20	0.62	16.	umc1393	3	15	0.66
5.	umc2372	4	19	0.70	17.	umc1671	5	24	0.59
6.	bnlg1940	6	28	0.74	18.	umc2395	3	16	0.53
7.	bnlg1160	3	23	0.63	19.	bnlg1065	3	20	0.65
8.	umc1501	4	16	0.73	20.	umc1586	6	48	0.80
9.	umc2061	4	16	0.72	21.	umc2359	2	14	0.41
10.	umc1101	3	19	0.61	22.	umc1962	5	34	0.77
11.	umc1752	2	14	0.50	23.	bnlg1518	4	18	0.67
12.	umc1792	2	15	0.48	24.	bnlg1028	9	36	0.78

The Polymorphism Information Content (PIC) value reflects the evidence of allele diversity and allele frequency among the tested genotypes (Pervaiz et al., 2009). In present study, PIC value ranged from 0.41 to 0.85 with an average value of 0.66 (Table 2). The highest PIC value was obtained for umc1248 followed by umc1586, bnlg1028, dupssr12, umc1962, bnlg1940, respectively. The PIC values of umc1248, umc1586, bnlg1028, dupssr12, and umc1962 were higher than 0.75 that were considered as the best markers for studied genotypes.

The SSR markers with PIC value of 0.5 or higher indicate that these are highly informative and extremely useful in distinguish the polymorphism rate of a marker at a specific locus (DeWoody et al., 1995). In this study, the genetic diversity of each SSR locus appeared to be associated with number of alleles detected per locus, i.e. the higher the PIC value of a locus, the higher the number of allele detected.

Moreover, the amplification profile of the 14 maize genotypes using 24 SSR markers sets showed alleles of different molecular weight, and displayed the higher polymorphism among the tested genotypes (Fig. 1). Our study highlighted the detection of genetic variability among the tested maize genotypes and also agreed the finding of polymorphic SSR markers by Liu and Muse (2005).

Cluster Analysis is a classification to determine whether the genotypes could be regarded as consisting of a number of partially dissociated groups. Genetic dissimilarities obtained from SSR marker data of 9 hybrids and 5 inbred maize genotypes were used to create a cluster diagram using Nei's genetic distance and the unweighted pair group method (UPGMA). Clustering analysis for 14 maize genotypes could be fully distinguished from one another and grouped into five major clusters (Fig. 2).

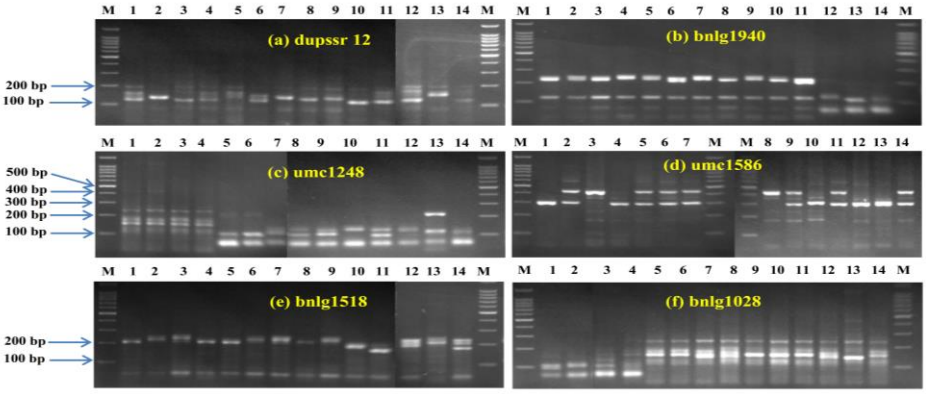

Fig. 1 Amplification of DNA profiles of the 14 maize inbreds and hybrids generated by SSR primers

(a) dupssr 12 (b) bnlg1940 (c) umc 1248 (d) umc 1586 (e) bnlg 1518 and bnlg 1028. Lane M = 100bp DNA Ladder.

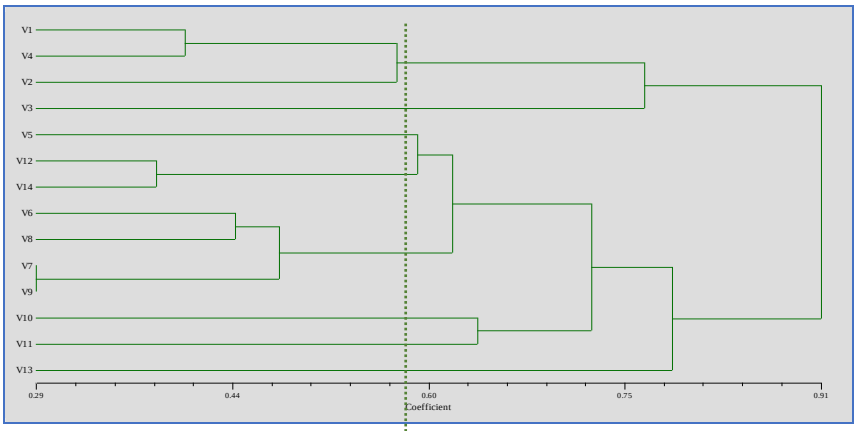

Fig. 2 UPGMA clustering tree of 14 maize genotypes based on Nei's genetic distance

Development of Core SSR Marker Set for DNA Fingerprinting (DUS testing)

The goal of development of core SSR marker set for genotype identification is to maximize distinguishing ability of each marker with a minimum number of markers set in DNA fingerprinting. In this study, the core marker candidate was decided with the selection criteria such as detection rate of the SSR fragment, the presence of rare allele, PIC value, and reproducibility and PCR band pattern of SSR fragments. As a result, six SSR primer pairs were selected as a final marker set for variety identification, i.e., dupssr12, bnlg1940, umc1248, umc1586, bnlg1518 and bnlg1028 due to their characterization and discrimination capacity. These polymorphic marker

pairs revealed that there were four to nine differentiated loci among all genotypes with a fragment length of 50-57 bp to 600 bp across loci (Table 3).

Table 3 Coding system of selected six SSR marker set based on allele size (bp) range

Code	dupssr12	bnlg1940	umc1248	umc1586	bnlg1518	bnlg1028
01	110-115	60-64	50-57	166-182	162	75
02	120-128	106-118	100	237	180	100
03	135-140	121-125	112-120	282-310	190-213	110
04	150-160	212-220	125	341-358	225-238	138
05	170-175	225-234	150	400-415		162
06	197-200	237-240	175-180	600		150
07			200-210			175-187
08			230-232			200
09						246-250

Table 4 SSR fingerprinting map of 14 maize genotypes based on their allele size codes

Genotype name	dupssr12	bnlg1940	umc1248	umc1586	bnlg1518	bnlg1028
Asia Seed (A.55)	020406	0205	050608	03	03	0103
Asia Seed (A.99)	03	0205	050608	030506	04	0104
AA-737	0206	0205	050608	01020405	04	020409
GT-722	0206	0206	050608	010304	03	020609
NK-625	040506	0206	010207	0102030405	03	060709
KMHE-3550	0203	0204	010207	01030405	04	060709
CP-111	03	0205	0103	030405	04	060709
NK-621	0206	0204	010204	010405	03	060809
TSF-1633	0206	0205	010203	0102030405	04	0609
YZI-10-054	0106	0204	0104	01020304	02	0609
YZI-10-095	010304	0204	010204	030506	0102	060709
PAC-999	020506	0103	0104	01030406	0304	0609
C7	0406	0102	010307	0304	03	0509
YZCI-16-019	0206	0103	0103	01030405	020304	060709

This allele size can be assigned as a specific DNA fingerprinting band of the genotypes and can be used as the maize variety identification. The primer bnlg1028 was found the best marker for the identification of studied genotypes as revealed by higher PIC values and showed the highest polymorphism. In other study, DNA fingerprinting with SSR set of tested maize genotypes were fully distinguished from one another compared to the specific SSR markers which differentiated maize hybrids from their parental inbreds (Jhansi et al., 2015). In this study, the genetic fingerprint map of 14 maize genotypes was constructed using the coding-based method with the original allele size coded by the assigned numeric values and the specific marker's allelic fragments were transformed into combine codes as final identity of the genotype, shown in Table 3 and Table 4. By using the core marker set, the results of assigning numeric code or ID described a specific identity of the variety (genotype) that can quantitatively differentiate it from the others. In this study, 14 maize varieties had unique profile ID to support plant variety protection and breeder right, complementing tools for DUS testing purpose. It is noted that these findings not only met the requirements for the minimum number of primers but were also sufficient to fully distinguish the 14 varieties from one another.

CONCLUSION

The goal of this research is to develop DNA fingerprinting catalogue of maize genotypes (both inbreds and hybrids) using SSR polymorphic information and their genetic variance supporting Myanmar maize DUS guidelines. In this study, the six pairs SSR sets generate the genetic fingerprint map (coding system) of typical maize genotypes which can be used in DUS testing for identification of hybrids and inbreds at any stage of crop growing cycle. Furthermore, molecular based SSR testing provide a higher degree of detection efficiency in DUS testing than traditional morphological based DUS method with regard to the verification of new varieties or genotypes.

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