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DNA FINGERPRINTING OF SELECTED MAIZE (*Zea mays* L.) GENOTYPES USING SSR MARKERS

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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. Nine hybrids and five inbreds were analyzed using 24 polymorphic SSR markers, resulted six SSR primer pairs 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. Cluster analysis separated all the maize genotypes as five major groups. 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.

Introduction

DNA fingerprinting technology is useful for preventing counterfeit and fake varieties in the market and this fingerprinting information is additional information for DUS characterization. UPOV 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. The specific objectives were to apply these result findings as the standard check to identify maize genotypes in infringement case, and to utilize as a source of parental line for future breeding programs.

Materials and Methods

Fourteen maize genotypes were used to study molecular characterization at Plant Biotechnology Laboratory, Department of Agricultural Research, Myanmar. The number of alleles per locus and total number of alleles were calculated by using with GenAIEx ver.6.5 (Peakall and Smouse, 2006 & 2012). Polymorphism Information Content (PIC) for each SSR was calculated by the formula $PIC = 1 - \sum X^2 k/n$. The similarity index was used for clustering the genotypes based method using NTSYSpc 2.1 software. The core SSR marker set was constructed using the coding-based system with the original allele size and transformed to two numeric codes from "01", "02", "03", etc.

Results and Discussion

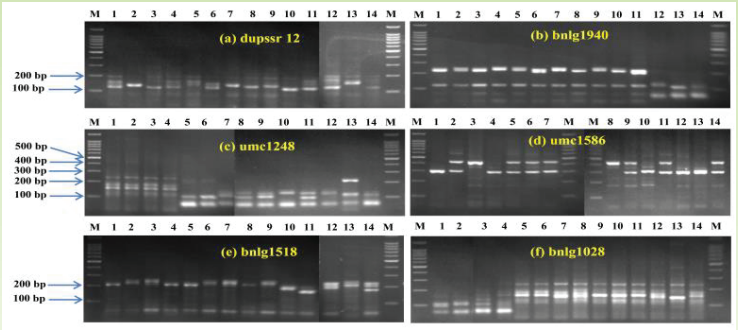

Fig. 1 Amplification of DNA profiles of the 14 maize inbreds and hybrids generated by SSR primers (a) dupssr 12 (b) bnlg 1940 (c) umc 1248 (d) umc 1586 (e) bnlg 1518 and bnlg 1028. Lane M = 100bp DNA Ladder.

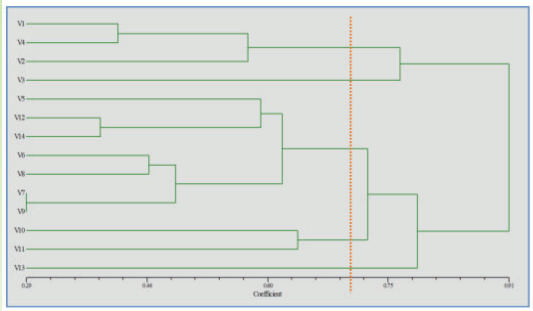

Fig. 2 UPGMA clustering tree of 14 maize genotypes based on Nei's genetic distance

Table 1. 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 2. 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

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.