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Genetic diversity assessment and relationship among tropical-yellow endosperm maize inbred lines using SSR markers

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Abstract

Maize is the most important food crop in sub-Saharan Africa. Tropical-adapted yellow maize inbred lines can be used as source of provision of pro-vitamin A, needed by millions of African children that suffer from vitamin A deficiency. A substantial variation of pro-vitamin A content is known to exist among available inbred lines. The objective of this study was to assess the diversity and relationship among 38 tropical-adapted maize inbred lines. The lines were investigated using 87 SSR markers. Seventy-five (75) SSR markers were polymorphic across the 38 inbred lines and generated a total of 297 alleles. The polymorphic information content (PIC) values obtained for the polymorphic SSR markers varied from 0.17 to 0.84 with an average of 0.56 .The number of polymorphic alleles per locus ranged from 2 to 11 with a mean of 3.96. Genetic distance (GD) values among all pairs of inbred lines varied from 0.007 to 0.59 with an average of 0.45. Cluster and principal coordinate analyses based on genetic distance from SSR data showed two well defined main groups of the 38 inbred lines, consistent with their pedigrees. The study has revealed a considerable level of genetic diversity among the inbred lines. The genetic potential that exists will facilitate the selection of parents with diverse alleles for development of new maize genotypes with higher pro-vitamin A level.

Keywords: cluster analysis; Principal Coordinate analysis; genetic distance; SSRs; tropical-adapted yellow maize inbred lines

Introduction

Vitamin A deficiency (VAD) affects children under age five in sub-Saharan Africa (WHO, 1995). This condition has contributed to growth retardation, depressed immune response, disturbed cellular differentiation and progressive blindness which could result in total blindness in children (Sommer et al, 1980; Aguayo et al, 2004; Sommer, 2008). About 28-35% of children in these regions are vitamin A deficient (WHO, 2002). Lack of sufficient vitamin A in many staples consumed by the Africans might have contributed to VAD.

In sub-Saharan Africa, maize is widely grown for consumption and also for generating income for peasant farmers. Traditionally, it has been part of the people's diet, 85% of the maize grown is used directly as human food and average maize consumption in Africa is 106.2 g/person/day (WHO, 2003). Biofortification of staple crops such as maize with high pro-vitamin A carotenoids through conventional breeding will lead to the development of new pro-vitamin A enriched maize varieties that could be used to reduce VAD (Kurilich and Juvik, 1999; Nestel et al, 2006). Yellow maize endosperm contains varying amounts of pro-vitamin A carotenoids which include α-carotene, β-carotene, β-cryptoxanthin, but the concentrations are very low (Kurilich and Juvik, 1999). Since the tropical adapted yellow maize varieties grown in Africa contain small amounts of pro-vitamin A carotenoids, there is a need for breeding maize for high pro-vitamin A to meet the nutritional requirements (Brunson and Quackenbush, 1962; Hauge and Trost, 1928). Some variation for specific carotenoid content has been reported in maize inbred lines adapted to the tropics (Maziya-Dixon et al, 2000; Menkir et al, 2008; Harjes et al, 2008).

Recently, maize inbred lines from diverse tropical adapted crosses and backcrosses have been developed and the diversity in concentration of carotenoids has also been measured (Menkir et al, 2008). These lines possess genes for adaptation and superior agronomic performance and thus represent a good genetic base to breed tropical maize for high levels of pro-vitamin A. Among the yellow endosperm maize inbred lines, 38 lines were chosen with varying levels of carotenoids to study the extent and patterns of genetic diversity present among them. Genetic diversity assessment studies will thus be useful to identify parents for making crosses and establishment of heterotic groups to develop yellow endosperm maize hybrids having high pro-vitamin A carotenoids (Russell et al, 1997). DNA markers can be used for assessing the extent of genetic diversity present in breeding materials (Livini et al, 1992; Menkir et al, 2004, 2005; Senior et al, 1998)

Among the PCR-based markers that are available, simple sequence repeat (SSR) has been extensively used for genetic diversity assessment in maize (Ad-

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etimirin et al, 2008; Smith et al, 1997; Heckenberger et al, 2002; Reif et al, 2003; Pinto et al, 2003; Warburton et al, 2002). SSR motifs are 2, 3, or 4 nucleotides that are found in abundance in the genomes of eukaryotic plant species and these units are tandemly repeated many times in the DNA sequence (Hamada et al, 1982; Morgante and Olivieri, 1993). The SSR markers will allow detection of polymorphisms at the DNA level which will facilitate the separation of inbred lines into well defined groups based on genetic distance estimates (Enoki et al, 2002; Menkir et al, 2004).

In this study, SSR markers were used to assess the extent of genetic diversity and relationship among selected yellow endosperm maize inbred lines. The study will be useful for the development of new maize lines in the current effort to increase pro-vitamin A content.

Materials and Methods

Plant material

In this study, we included 38 yellow endosperm maize inbred lines developed at the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria from different sources and contain varying levels of pro-vitamin A (Menkir et al, 2008). The list of the inbred lines and their genetic background are shown in Table1. Seeds were obtained from IITA maize breeding program.

DNA extraction

Seedlings were grown in the green house for 3 weeks after which fresh leaf tissue of 5-6 seedlings of each inbred line was harvested and stored in eppendorf tubes at -80°C for total genomic DNA extraction. The modified Dellaporta et al (1983) method was used with some modifications to extract DNA. The quality and quantity of DNA was determined by NanoDrop spectrophotometer machine (ND-100 Technologies, Wilmington, Delaware, USA).

PCR conditions and electrophoresis for SSR analysis

A total of 87 SSR maize primers were used for PCR amplification of the 38 inbred lines, which includes 35 SSR chosen from the MaizeGDB database (http://nucleus.agron.missouri.edu/cgi-bin/ssr_bin.pl) and the 52 core SSR primers described by Warburton et al (2002). They were all selected based on the bin locations, which provides a uniform coverage of all the ten chromosomes in the maize genome. Primer names and chromosome loci (Bin number) of the SSR loci evaluated are included in Table 2. Oligonucleotide primers were synthesized at nano-mole concentration by Integrated DNA Technologies Leuven, Belgium (IDT) Primer Company.

All oligonucleotide primers were diluted to a working concentration of 5 µM with sterile water and stored at -20°C. PCR conditions and gel visualisation were performed as described by Senior et al (1998). Variable "touch down" PCR profiles, annealing temperatures (65-55°C, 70°C-63°C and 60°C-50°C) were used for different SSR primers. The SSR loci amplified were separated on 2% (w/v) superfine agarose gels (Amresco). The gel was stained with ethidium bromide solution and photographed under UV light attached to a gel documentation system (Bio-Rad, Hercules, CA). Allele sizes of amplified fragments were scored on the basis of size in comparison with DNA molecular weight markers. Clear polymorphic SSR fragments (bands) were scored in a binary form of 1 or 0 for presence or absence of the band, respectively.

Genetic diversity evaluation

To determine the genetic variability among the 38 inbred lines, the following indicators were considered for the polymorphic SSR bi-allelic loci; allelic richness, total number of alleles in all the SSR loci and the mean number of alleles. Polymorphic information

Table 1 - Pedigree for the 38 yellow endoperm tropical-adapted maize inbred lines.

Inbred	Pedigree
PVL01	9450xKI 21-7-3-1-1-1-B-B-B
PVL02	9450xKI 21-7-3-1-1-3-B-B-B
PVL03	9450xKI 21-7-3-1-2-4-B-B-B
PVL04	9450xKI 21-7-3-1-2-5-B-B-B
PVL05	(9450xCM 116x9450)-5-2-2-2-B-B-B
PVL06	(9450 x KI 28)-1-2-1-2-B-B-B
PVL07	9450xKI 21-7-2-1-1-B-B-B
PVL08	9450xKI 21-7-2-1-2-B-B-B
PVL09	4001 x B73LPA x 4001-33-2-1-B-B-B
PVL10	1368 x GT-MAS-Gk-10-3-1-2-B-B-B
PVL11	(9450xCM 116x9450)-3-3-1-2-1-B-B-B-B
PVL12	9450xKI 21-3-2-2-1-3-B-B-B-B
PVL13	9450xKI 21-1-5-3-2-2-B-B-B-B
PVL14	9450xKI 21-1-5-3-2-1-B-B-B-B
PVL15	SYN-Y-STR-34-1-1-1-1-2-1-B-B-B-B-B-B-B-B
PVL16	9450xKI 21-1-4-1-1-2-B-B-B-B
PVL17	9450xKI 21-5-2-3-1-B-B-B
PVL18	ACR97TZL-CCOMP1-Y-S3-12-2-B-B-B-B-B
PVL19	ACR97TZL-CCOMP1-Y-S3-33-6-B-B-B-B-B
PVL20	ACR97TZL-CCOMP1-Y-S3-40-3-B-B-B-B-B
PVL21	KU1414-SR/NC350-4-1-B-B-B
PVL22	KU1414-SR/NC350-1-1-B-B-B
PVL23	(9450 x KI 28)-1-2-1-1-B-B-B-B
PVL24	KU1414-SR/KVI43-6-4-B-B-B
PVL25	KU1414-SR/KVI43-6-1-B-B-B
PVL26	KU1414-SR/KVI11-7-2-B-B-B
PVL27	KU1414-SR/KVI11-7-1-B-B-B
PVL28	(9450xCM 116x9450)-5-1-3-3-1-B-B-B-B
PVL29	9450xKI 21-4-2-3-1-1-B-B-B-B
PVL30	Taraba-14-2-2-4-2-B-B-B-B-B
PVL31	Z.Diplo.BC4-467-4-1-2-1-1-B-1-B-B-B-B-B-B-B
PVL32	TZE-COMP5-Y-C7-S3-61-B-B-B-B-B-B-B
PVL33	(9450x KI 21)-8-2-1-1-B-B-B
PVL34	(9450 x KI 28)-5-1-2-1-1-B-B-B
PVL35	9450xKI 21-7-2-2-1-1-B-B-B
PVL36	9450xKI 21-7-2-4-2-1-B-B-B
PVL37	9450
PVL38	KU1414-SR

Table 2 - Bin numbers, allele numbers and PIC values for SSR loci used to genotype 38 yellow endosperm maize inbred lines.

SSR locus	Bin no.	No. of alleles	PIC value	SSR locus	Bin no.	No. of alleles	PIC value
bnlg118	5.07	5	0.79	phi127	2.08	3	0.31
bnlg1014	1.01	4	0.70	phi96100	2.00	5	0.54
bnlg1721	2.08	10	0.82	phi109188	5.03	3	0.56
bnlg2162	4.08	11	0.84	phi109275	1.03	3	0.23
nc004	4.03	4	0.52	phi109642	2.04	3	0.59
nc133	2.05	3	0.42	phi227562	1.11	5	0.71
phi001	1.03	7	0.59	phi233376	8.09	4	0.68
phi002	1.07	5	0.79	phi328175	7.04	3	0.44
phi011	1.09	4	0.73	phi374118	3.02	3	0.64
phi014	8.05	4	0.56	phi420701	8.00	3	0.39
phi015	8.08	5	0.71	phi423796	6.01	2	0.23
phi021	4.03	3	0.34	Phi448880	9.05	2	0.18
phi025	6.05	5	0.75	phi453121	3.01	4	0.51
phi029	3.04	2	0.43	phi452693	6.04	2	0.31
phi032	9.04	2	0.17	umc1008	4.01	6	0.68
phi033	9.01	3	0.49	umc1026	2.04	2	0.24
phi034	7.02	4	0.71	umc1061	10.06	4	0.51
phi041	10.00	3	0.47	umc1109	4.10	4	0.53
phi042	9.04	3	0.52	umc1136	3.09	3	0.66
phi046	3.08	3	0.61	umc1143	6.00	3	0.59
phi047	3.09	5	0.69	umc1152	10.01	7	0.82
phi053	3.05	3	0.48	umc1153	5.09	4	0.69
phi056	1.01	4	0.32	umc1154	7.05	4	0.63
phi059	10.02	3	0.53	umc1161	8.06	3	0.38
phi064	1.11	6	0.75	umc1196	10.07	4	0.74
phi065	9.03	5	0.76	umc1231	9.05	5	0.66
phi072	4.01	4	0.74	umc1277	9.08	3	0.56
phi070	6.07	3	0.43	umc1279	9.00	4	0.56
phi075	6.00	5	0.67	umc1304	8.02	2	0.48
phi076	4.11	4	0.70	umc1312	10.03	3	0.36
phi077	6.01	6	0.77	umc1399	3.07	4	0.65
phi082	7.05	3	0.63	umc1403	1.03	3	0.60
phi084	10.04	3	0.46	umc1426	7.00	7	0.77
phi087	5.06	4	0.60	umc1530	8.03	2	0.39
phi089	6.08	3	0.51	umc1592	8.01	4	0.71
phi093	4.08	2	0.21	umc1593	7.03	5	0.63
phi123	6.07	3	0.47	umc1792	5.08	4	0.69
phi126	6.00	6	0.77				

content (PIC) values were calculated at each locus using the formula (Nei, 1973):

$$\mathrm{PIC} = 1 - \sum P_{ij}^2$$

Where Pij is the allele frequency of the jth allele for the ith marker summed over numbers of alleles. PIC values give an estimate of the discriminatory power of a marker by taking into account not only the number of alleles at the locus but also the relative frequencies of these alleles. The genetic diversity between pairs of inbred lines i and j was computed based on Modified Roger's Distance (MRD, Rogers, 1972).

Genetic distances were computed using Winboot software. Genetic distance matrices generated from the SSR data sets were then subjected to cluster analysis using a sequential agglomerative hierarchical nested clustering method (SAHN), based on the unweighted pair-group method with arithmetic averages (UPGMA) as suggested by Sneath and Sokal (1973). In addition, the cophenetic correlation coefficient was calculated to test correspondence between original distances and the distances defined in the dendrogram using the MXCOMP procedure of NTSYS-PC 2.01 (Rohlf, 1997). Further, assessment of the relationships among maize inbred lines was determined using principal coordinate analysis (Gower, 1966). The UPGMA and PCoA analyses were per-

formed with version 2.01 of the NTSYS-PC package (Rohlf, 1997; Exeter Software, Setauket, USA).

Results and Discussion

The SSR markers were used to reveal the genetic diversity among the 38 yellow endosperm tropical maize inbred lines. Among the 87 SSR loci used for genotyping, 75 were polymorphic across the 38 inbred lines and produced a total of 297 alleles. The number of polymorphic alleles per locus varied from 2 to 11 with a mean of 3.96. The PIC values obtained for the polymorphic SSR markers varied from 0.17 (phi032) to 0.84 (bnlg2162) with an average of 0.56. Nearly 70% of these markers had more than 0.5 PIC value. The number of alleles detected among the 38 inbred lines using SSR markers and PIC values for polymorphic loci are presented in Table 2.

The SSR markers generated a low level of polymorphism in spite of using a core set of highly informative SSR markers. The level of allelic richness obtained among the 38 maize inbreds was moderate, possibly due to high level of relatedness among most of the lines. The mean allele per SSR marker in this study was lower than those detected by previous SSR studies. Warburton et al (2002) and Lu and Bernardo (2001) reported an average of 4.9 allele for 57 CML lines and 40 U.S maize inbreds respectively, while Senior et al (1998) recorded an average of 5.0 alleles for 94 elite U.S. maize inbreds. In another study, Xia et al (2004), found an average of 7.4 alleles per SSR marker for 155 tropical lowland adapted maize inbreds. The mean value from our study, however, was higher than the 3.85 average alleles per locus per SSR locus reported by Legesse et al (2007),

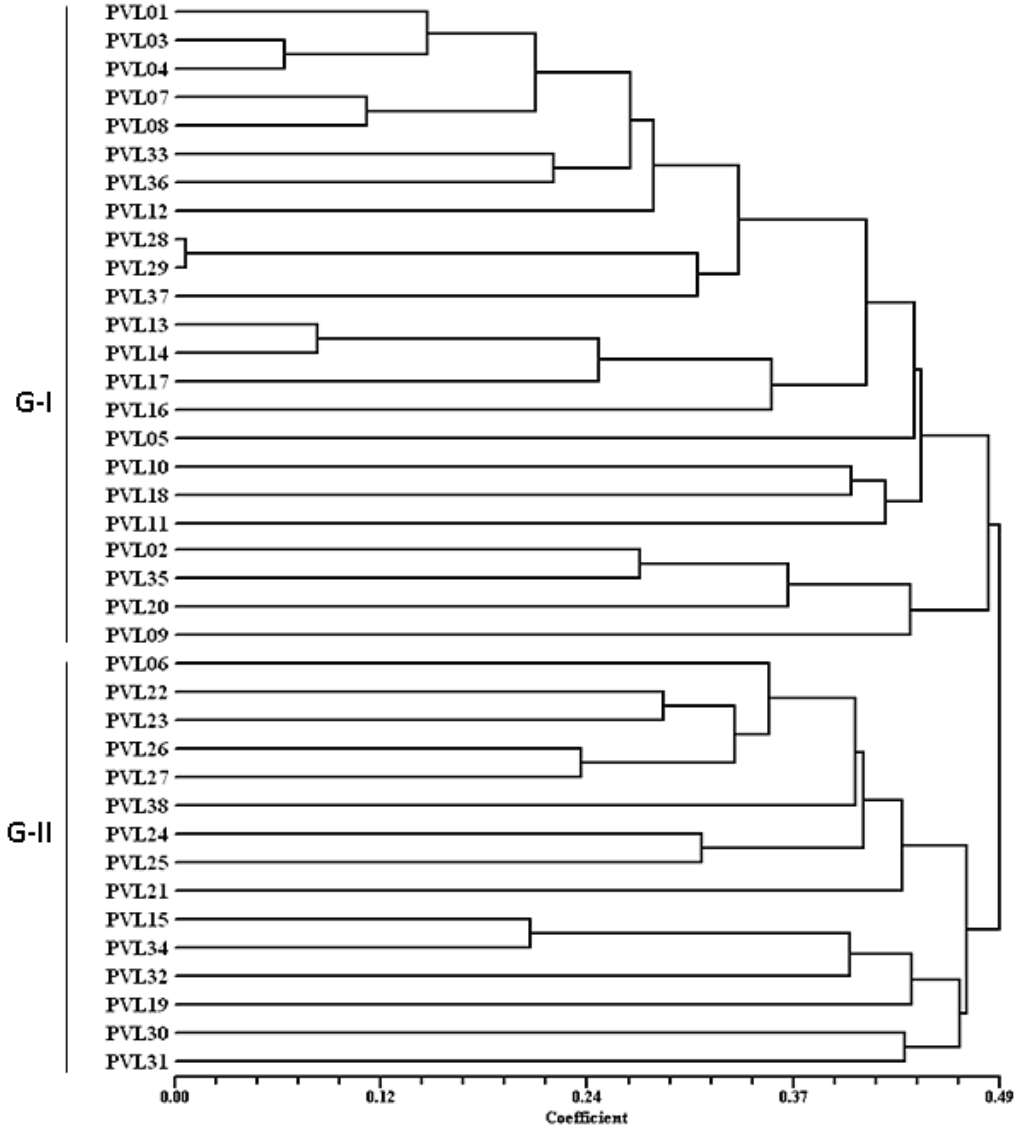

Figure 1- Dendrogram of 38 tropical-adapted yellow maize inbred lines obtained using SSR markers.

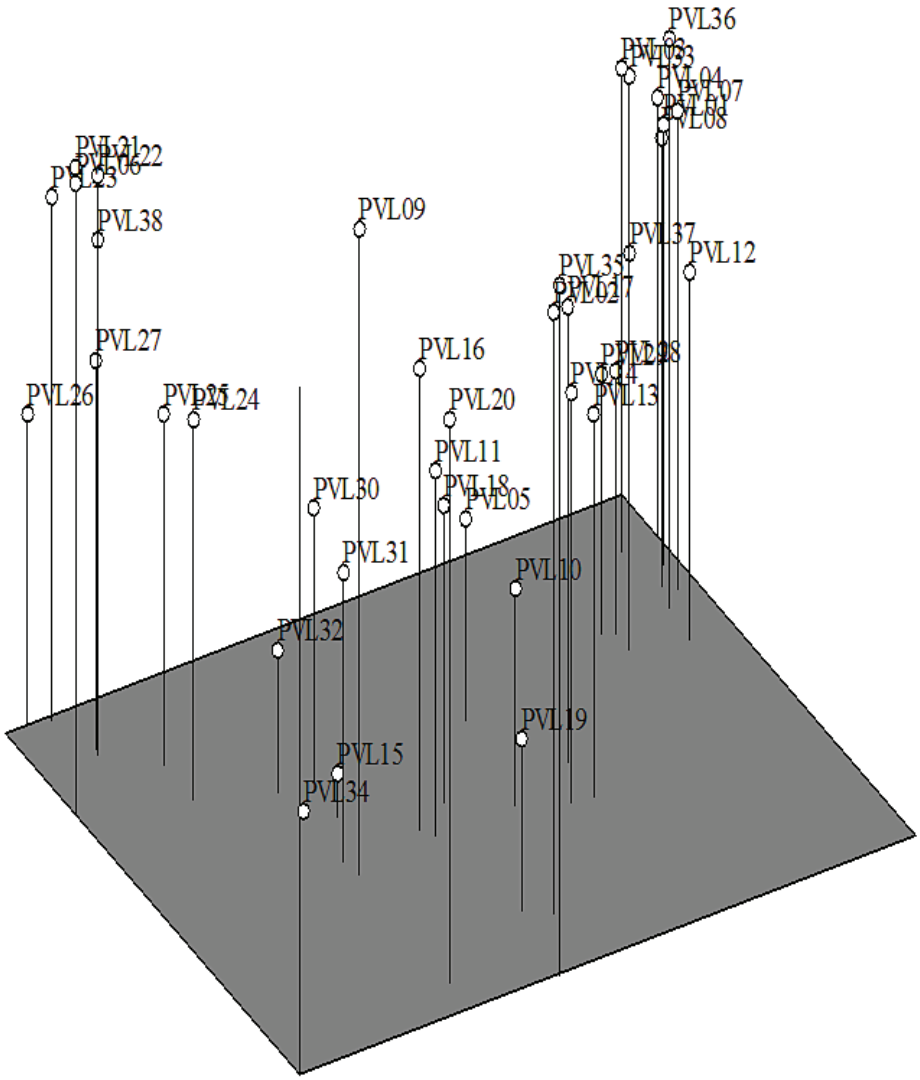

Figure 2 - 3D plot of 38 tropical-adapted yellow maize determined on the basis of Principal coordinate analysis of SSR-based estimates of Modified Roger's (1972) distance.

for 56 highland and mid-altitude adapted maize inbreds. Such considerable differences in the number of alleles detected may arise from difference in (i) the diversity of the lines used, (ii) the number of lines examined, and (iii) the genotyping method used. SSR markers exhibit relative abundance and codominant inheritance. Our results showed that the SSR markers were able to detect the extent of genetic diversity present in the 38 inbred lines. The marker detected considerable level of genetic diversity among the yellow endosperm maize inbred lines.

The genetic distance estimates of MRD between all pairs of inbred lines varied from 0.007 to 0.59, with an average of 0.45. The relationship among the 38 lines was investigated using a cluster analysis based on genetic distance values. The UPGMA cluster analysis of 38 maize inbred lines is shown in Figure 1. This analysis separated the lines into two well defined groups, consistent with their pedigrees (Table 1). Twenty-one of the 23 inbred lines found in group I were developed from bi-parental crosses and backcrosses containing temperate lines such as 9450, B73 and GT-MAS-gk (Table 1). Among the 11 inbred lines included in group II, seven were developed from crosses involving inbred lines introduced from Thailand as their parents (Figure 1, Table 1). The remaining four inbred lines found in group II were developed from diverse source germplasm. The correlation between the original genetic distance matrix and the cophenetic matrix generated from the dendrogram was 0.89, showing a moderately good fit. Principal coordinate analysis (PCoA) based on SSR genetic distance showed clearer separation of the lines into two groups consistent with cluster analysis (Figure 2).

The separation of the lines derived from different source germplasm into well defined groups suggests

that they can be used as parental lines to develop new lines having a higher level of pro-vitamin A content. The complex directional mutations that produce SSR loci and its high mutation rates (Morgante and Olivieri, 1993; Vigouroux et al, 2002) may have led to their optimal detection of genetic diversity among diverged lines. SSR markers efficiently separated the tropical-adapted yellow maize inbred lines into groups consistent with their pedigrees. Gerdes and Tracy (1994) pointed out that pedigree relationship can be used as a benchmark to test the effectiveness of markers in determining relationships among breeding lines. This observation was similar to other findings (Smith et al, 1997; Legesse et al, 2007; Reif et al, 2003), indicating that SSRs are efficient markers to classify closely related lines. PCoA based on MRD clearly showed that inbred lines were also separated into well defined groups. Thus these inbred lines can be utilized as potential parents for hybridization to develop inbred lines belonging to different heterotic groups, useful to form hybrids that optimize expression of heterosis in pro-vitamin A concentration in maize breeding programs.

In summary, SSR markers separated the yellow endosperm maize inbred lines into well defined groups. The SSR markers provided reliable information on the genetic diversity of the 38 inbred. The results from this work has aided in the identification of potential sources of genetic diversity for development of new maize genotypes with improved nutritional value. The GD estimates can be used as the basis for effective utilization of the inbred lines with diverse genetic backgrounds for high pro-vitamin A in maize breeding programs.

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