ISSN 1479-2621

Plant Genetic Resources: Characterization and Utilization (2020) **18**(6); 454–461
doi:10.1017/S1479262120000489

Microsatellite marker-based genetic diversity of tropical-adapted *shrunken-2* maize inbred lines and its relationship with normal endosperm inbred lines of known heterotic classification

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Received 13 July 2020; Accepted 24 November 2020 – First published online 7 January 2021

Abstract

Knowledge of the genetic diversity and relationships among maize inbred lines can facilitate germplasm management and plant breeding programmes. The study investigated the level of genetic diversity among S_6 lines developed from a tropical-adapted *shrunken-2* (*sh-2*) maize population and their relationship with normal endosperm tropical inbred lines of known heterotic groups. Ninety-one *sh-2* maize inbred lines (UI1-UI91) developed in the University of Ibadan super-sweet Maize Breeding Programme were genotyped at 30 simple sequence repeat (SSR) loci, alongside five normal endosperm maize inbred lines viz. TZi3, TZi4, TZi10, TZi12 and TZi15, four of which belong to two heterotic groups. Twenty-three SSR markers were polymorphic and detected a total of 61 alleles, with a range of 2–7 and an average of 2.65 alleles per locus. The polymorphic information content ranged from 0.12 in bnlg1937 to 0.77 in phi126, with an average of 0.36. The gene diversity (H_e) averaged 0.43. Cluster analysis resulted in five groups consisting of 16, 36, 17, 23 and 3 inbred lines, with one *sh-2* line ungrouped. TZi 12 and TZi 15, both of which are of the same heterotic group, clustered with TZi 3 of another heterotic group. Considerable genetic diversity exists among the 96 inbred lines. Only two of the five normal endosperm lines shared clusters with the *sh-2* lines. The clustering of the normal endosperm inbred lines is not related to their established heterotic patterns. Inbred lines in two clusters offer the possibility of guiding the exploitation of heterosis among the *sh-2* lines.

Keywords: genetic diversity, *shrunken-2* maize inbred lines, simple sequence repeats (SSR)

Introduction

Crop introduction has played an important role in the agriculture of different regions of the world. Its success is demonstrated by the wide distribution and high productivity of major crops in areas far from their centres of origin. Following its introduction to West Africa (WA) about 500 years ago (Adetimirin, 2008), maize (*Zea mays* L.) has become one of the major crops in the sub-region, even in drier ecological zones with short season where it has displaced some traditional cereal crops.

Maize cultivar development in WA has focussed almost exclusively on flint and dent types for diverse uses. Sweet maize, the types consumed fresh in Europe and America, are cultivated on a very limited scale using imported seeds. The WA region has one of the lowest nutrition rankings and highest population growth rates among all sub-regions in the world (Rosenbloom *et al.*, 2008; UNDESA, 2020). In recognition of the important role sweet corns could play in the nutrition of people of WA and economy

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of the sub-region, the Department of Agronomy, University of Ibadan, Nigeria introduced a broad-base *shrunken-2* (*sh-2*) maize population from South Korea to Nigeria in 2000. Before the introduction, there was no record of an active sweet maize cultivar development programme in WA. Among sweet maize types, *sh-2* maize has the highest level of sweetness and retains it for a longer period after harvest than other sweet corn mutants (Wilson *et al.*, 1993; Yousef and Juvik, 2002). These attributes make *sh-2* the ideal maize type for developing countries of WA, where refrigerated haulage infrastructure for preservation of eating quality of the produce during shipment to distant markets is rarely available or expensive.

The introduced *sh*-2 maize population was improved for adaptability to tropical environmental conditions by four cycles of mass selection (Adetimirin, 2008). Plants of the tropical-adapted population have undergone six generations of selfing to produce S_6 lines aimed at the production of hybrids. The deployment of DNA-based markers improves the efficiency of hybrid development programmes by reducing the number of lines to be tested in hybrid combinations for the exploitation of heterosis and the development of stable hybrids. Among these marker types, simple sequence repeat (SSR) markers, also known as microsatellite markers, have been used extensively for genetic analysis and diversity assessment in maize and other crops (Weber, 1990; Senior *et al.*, 1996; Yuan *et al.*, 2000; Oppong *et al.*, 2014; Nyaligwa *et al.*, 2015).

Information on the genetic diversity of lines developed from the *sh-2* maize population adapted to tropical environments will guide the choice of parents in the development of *sh-2* hybrids for the WA sub-region and reduce dependence on importation of seeds. In addition, the *sh-2* lines may benefit from the heterotic groups already established for normal endosperm maize lines in the sub-region. Therefore, the objectives of this study were to assess the extent of genetic diversity among WA's first generation of tropical-adapted *sh-2* lines and their genetic relationship with normal endosperm inbred lines of known heterotic classification based on microsatellite markers.

Materials and methods

Genetic materials used for the study

The study was conducted in the Biotechnology Laboratory of the Department of Agronomy, University of Ibadan, Ibadan, Nigeria. A total of 96 maize inbred lines were used in the study; of these, 91 (UI 1 to UI 91) were S_6 *sh-2* lines, selected from a panel of 166 *sh-2* lines from the University of Ibadan sweet corn breeding programme based on superior plant and ear aspect scores. The remaining five were normal endosperm maize inbred lines with known heterotic groupings, developed at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria viz. TZi 3, TZi 4, TZi 10, TZi 12 and TZi 15 (Table 1).

Table 1. Parentage and heterotic groups of normal endosperm inbred lines used in the study

Inbred line	Parentage	Heterotic group
TZi 3	Across 7721 × TZSR	2
TZi 4	Guana Caste 7729 × TZSR	–
TZi 10	Tlalt 7844 × TZSR	2
TZi 12	N28 × TZSR	1
TZi 15	N28 × TZSR	1

DNA sample collection

Seedlings were raised in pots in the greenhouse (Lat. 7°27′ 05.6″N; Long. 3°53′47.1″E; 208 m above sea level) for 3 weeks. Genomic DNA was extracted from leaf tissues of the 3-week-old maize plants using Whatman Flinders Technology Associates (FTA) plant cards by direct leaf press method (Whatman FTA protocol BD05, 2002). Small sections of the second or third leaf were cut from healthy plants with sterilized scissors and placed on labelled FTA plant card, with the underside of the leaf facing down. Leaf samples were overlaid with parafilm, and a pestle was applied to each sample area with moderate force until the sap was visible on the reverse side of the FTA card. Pieces of leaves stuck to the FTA cards were carefully removed. The FTA cards were dried for a minimum of 1 h at room temperature and thereafter placed in a clip top bag and stored in a desiccator containing active silica gel.

Purification of DNA samples for polymerase chain reaction (PCR)

A 2 mm disc was removed from the stained region of the dried FTA card using a clean Harris 2 mm micro-punch, and placed directly in a labelled PCR tube containing 150 µl of FTA purification reagent. The PCR tube was then incubated at room temperature for 15 min with moderate shaking, and thereafter the reagent was removed with a pipette. Polyphenols, polysaccharides, and other secondary metabolites or contaminants (alkaloids, flavonoids, quinines and terpenes) were washed off the FTA cards in two rounds of washes with FTA purification reagent. Thereafter, 200 µl of TE buffer (10 mM Tris HCl, 0.1 mM EDTA, pH 8.0) was added to the PCR tube, and incubated at room temperature for 10 min, following which the TE buffer was removed with a pipette. After two rounds of washing with TE buffer, the disc was allowed to dry for 3 h at room temperature.

https://doi.org/10.1017/S1479262120000489 Published online by Cambridge University Press

Table 2. SSR markers used for genetic diversity analysis of 91 *shrunken-2* maize inbred lines and five normal endosperm inbred lines

S/N	SSR marker	Bin	Chr. Arm	Repeat type	S/N	SSR marker	Bin	Chr. Arm	Repeat type
1	umc1568	1.02	1a	TAG(4)	16	phi126	6.00	6a	AG
2	umc1147	1.07	1b	CA(7)	17	umc1656	6.02-6.03	6a	CGGT(7)
3	phi064	1.11	1b	ATCC	18	umc2165	6.07	6b	TTC(12)
4	bnlg1297	2.02	2a	AG(32)	19	umc2177	7.00	7a	GTC(4)
5	mmc0401	2.05	2b	GGA(2)AG(27)	20	phi034	7.02	7a	CCT
6	phi127	2.08	2b	AGAC	21	phi051	7.05	7b	AGG
7	umc2369	3.02-3.03	3a	GCAC(4)	22	umc1483	8.01	8a	ACG(4)
8	phi053	3.05	3b	ATAC	23	umc1728	8.05-8.06	8b	GAC(5)
9	umc2050	3.07	3b	CGC(4)	24	umc1663	8.08-8.09	8b	ATG(8)
10	phi072	4.01	4a	AAAC	25	phi022	9.03	9a	GTGC
11	bnlg1937	4.05-4.06	4b	AG(21)	26	umc1357	9.05	9b	CTG(8)
12	phi006	4.11	4b	CCT	27	bnlg1506	9.07-9.08	9b	AG(19)
13	phi096	5.03	5a	AGGTG	28	umc1152	10.01	10a	ATAG(6)
14	phi087	5.06	5b	ACC	29	phi050	10.03	10a	AAGC
15	umc1153	5.09	5b	TCA(4)	30	umc1477	10.05-10.06	10b	GCC(4)

SSR primer selection

Thirty SSR markers, three from each linkage group, and with at least one marker per chromosome arm, were selected. Twenty-five of these markers are SSRs with longer repeat motifs (tri-, tetra- and penta-nucleotides), while five are di-nucleotide SSRs. All the selected markers have reported polymorphic information content (PIC) values of a minimum of 0.75 (Senior *et al.*, 1998; Vigouroux *et al.*, 2005; Krishna *et al.*, 2012). The 30 SSR primers were diluted to a stock concentration of 100 µM with molecular biology grade water and stored at −21°C. Details about map positions, chromosomal location and repeat type for the SSRs are given in Table 2. The primer sequences were obtained from the Maize Genetics and Genomics database (www.maizegdb.org) and synthesized at Inqaba Biotech, South Africa.

PCR amplification and electrophoresis

PCR was carried out in a thermal cycler (Applied Bio-systems 2720) in a 25 µl volume reaction mixture containing washed 2 mm FTA disc (assuming disc punch + DNA constitute zero added volume), 0.4 µM each of forward and reverse primer for each SSR locus, 1.0 U of heat-stable *Taq* DNA polymerase, 0.6 mM of deoxyribonucleoside triphosphate mix, 3.5 µl of ×1 PCR buffer (10 mM Tris HCl, 20 mM $MgCl_2$, 30 mM KCl, pH 9.0) and 17.8 µl of molecular biology grade water. Amplifications were carried out under conditions of an initial denaturing step at 94°C for 2 min, a touch down programme of nine cycles (93°C for 15 s, 65°C for 20 s with a −1°C increment per cycle and 72°C for 1 min) followed by 30 cycles at 93°C for 15 s, 55°C for 20 s and extension at 72°C for 1 min. In the last cycle, there was a final extension step at 72°C for 10 min to ensure completion of the final amplification products, followed by termination of the cycle at 4°C. After PCR, 10 µl of the amplification products from each genotype-marker combination was loaded into the wells of two 1.5 mm wide 50-well comb per gel, spaced 15 cm apart, and were separated by electrophoresis in a horizontal gel system (Bio-Rad, Model 96), using 1.5% super-fine agarose gel in ×1 TBE buffer stained with 0.15 µg/ml ethidium bromide solution at a constant power of 80 V for 4 h. All 96 lines, amplified by a single SSR primer pair, were run on the same gel divided evenly among the two combs. For easy viewing under the trans-illuminator, the gels were divided into two sections, each section containing the samples loaded into one each of the two combs, after which the image was visualized under a high-performance bench-top ultraviolet trans-illuminator and captured with a SONY digital camera (Model No. DSC-H7). The size of the amplified fragment (in base pairs) was determined based on their position relative to a standard 100 bp molecular DNA ladder.

Gel scoring

Allele scoring was carried out manually in terms of the position of the bands relative to the DNA ladder and sequentially from the smallest to the largest sized bands; for a given primer pair, the products of different sizes were considered different alleles. Only clear and unambiguous polymorphic

https://doi.org/10.1017/S1479262120000489 Published online by Cambridge University Press

bands amplified by the SSR markers were qualitatively scored for their presence (1) or absence (0) among the maize lines. Diffused bands or bands that were ambiguous for scoring were considered as missing data and designated 9. In cases where a line showed two allelic bands of varying intensities, the most intense band was scored as 1. Genotypes showing two allelic bands with equal intensity were considered heterozygous for that locus. Also, null allele, scored as 9, was assigned to a genotype for a microsatellite locus whenever an amplification product was not detected for a particular genotype-marker combination. The data were entered into an Excel spreadsheet which was used to generate a binary data matrix that was subjected to statistical analysis.

Data analysis

The PIC, proportion of polymorphic loci (*P*), average number of alleles per locus, major allele frequency, allele and genotype frequencies, average observed heterozygosity (H_o) and average expected heterozygosity (H_e) were estimated following appropriate procedures in PowerMarker version 3.25 (Liu and Muse, 2005).

The genetic similarity (GS) among all possible pairs of inbred lines were estimated according to the method of Nei and Li (1979) using Numeric Taxonomy System of Statistic (NTSYS) software package version 2.1 (Rohlf, 2000). GS was estimated as:

$$GS_{ij} = 2N_{ij}/(2N_{ij} + N_i + N_j),$$

where GS_{ij}, N_{ij}, N_i and N_j is the genetic similarity between inbreds *i* and *j*, number of alleles present in both inbreds *i* and *j*, number of alleles present only in inbred *i*, and number of alleles present only in inbred *j*, respectively.

Cluster analysis was carried out based on the matrix of GS to generate a dendrogram, which is a graphical representation of the genetic relationship among the inbred lines, using Unweighted Paired Group Method with Arithmetic averages (UPGMA) clustering algorithm in the NTSYS software package version 2.1 (Rohlf, 2000). To check for the efficiency and goodness of fit of the clustering algorithm for the matrix on which it was based, the cophenetic correlation coefficient was estimated. This was done to assess the strength of the evidence for the clustering obtained, and to determine how accurately the dendrogram represent the estimates of the GS among the inbred lines.

Results

Twenty-three of the 30 SSR markers were polymorphic with few or no missing data; the remaining seven either had large proportions of missing data (umc 1147 and phi 087) or were monomorphic (phi 091, umc 1477, umc 1357, umc 1663 and umc 2177) and therefore excluded from the analyses. Missing data among the 23 SSRs ranged from 0 to 42.7% per marker with a mean of 6%. The proportion of polymorphic loci (*P*), also referred to as polymorphism rate, was 77%.

The 23 SSR markers detected a total of 61 alleles ranging from two alleles per locus to seven alleles per locus (phi126), with an average of 2.65 alleles per locus. The total gene diversity (H_e) was lowest for bnlg1937 (0.12) and highest for phi126 (0.79) with an average of 0.43. Percentage heterozygosity (H_o) was 0% in 11 of the 23 markers viz. mmc0401, phi127, phi072, phi064, phi006, phi051, umc1152, umc1153, umc1483, umc2165 and phi053. In another four loci, percentage heterozygosity was <3.2%. In general, heterozygosity was lower than 10% except for phi126 and bng1506 and averaged 6.9%. PIC was lowest in bnlg1937 with a value of 0.12 and highest in phi126 with a value of 0.77; average PIC value was 0.36. Four SSR markers (bnlg1297, mmc0401, umc2369 and phi126) had PIC values ≥0.5, an indication that they were more informative and useful in detecting differences among the inbred lines. The genetic diversity indices for each of the markers and averaged across all markers are presented in Table 3.

GS among all pairs of the 96 maize inbred lines ranged from 0.20 to 1.00, with an average of 0.40. Twenty-two pairs (0.48%) of inbred lines of a total of 4560 pairs had a GS of 1, indicating that they may be similar at the 23 loci where they were genotyped. These consisted of four pairs of *sh-2* lines (UI 14 and UI 15; UI 46 and UI 48; UI 64 and UI 65; UI 75 and UI 76) and another six sets each comprising three *sh-2* lines (UI 4, UI 5 and UI 6; UI 9, UI 10 and UI 11; UI 26, UI 27 and UI 28; UI 40, UI 41 and UI 42; UI 43, UI 44 and UI 45; UI 52, UI 53 and UI 54).

The lines were clustered into five major groups, with one white kernelled *sh-2* line forming a singleton. The *sh-2* lines were grouped into four of the five groups, with the fifth group composed exclusively of three of the five normal endosperm inbred lines. Groups I, II, III and IV included 16, 36, 15 and 23 of the *sh-2* inbred lines, respectively.

The dendrogram from the cluster analysis showing relationships among the 96 inbred lines based on GS values is shown in Fig. 1. The clustering of the *sh-2* lines was largely on the basis of their pedigree; many S_6 lines derived from the same S_4 plant clustered together. However, few lines derived from similar S_4 plants were placed in different groups. UI 33 and UI 32, which were derived from the same S_4 plant, clustered into Groups II and III, respectively. Six S_6 lines derived from the same S_4 plant were distributed into Group I (four lines – UI 46, UI 47, UI 48 and UI 49), Group III (one line – UI 73) and Group IV (one line – UI 8). Similarly, three lines derived from the same S_4 plant were clustered into Group I (UI 1) and Group IV (UI 2

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Table 3. Genetic diversity indices of the polymorphic SSR markers used in the study

SSR marker	No. of alleles	Gene diversity	Heterozygosity (%)	PIC
umc1568	2	0.42	3.13	0.33
phi064	2	0.46	–	0.35
bnlg1297	3	0.61	5.21	0.53
mmc0401	4	0.59	–	0.50
phi127	2	0.41	–	0.32
umc2369	3	0.61	8.33	0.54
phi053	2	0.49	0.00	0.37
umc2050	3	0.25	2.53	0.23
phi072	2	0.50	–	0.37
bnlg1937	3	0.12	5.75	0.12
phi006	2	0.29	–	0.25
umc1153	2	0.40	–	0.32
phi126	7	0.79	13.68	0.77
umc1656	3	0.54	9.38	0.43
umc2165	2	0.50	–	0.37
phi034	3	0.41	7.37	0.35
phi051	2	0.49	–	0.37
umc1483	2	0.30	–	0.26
umc1728	2	0.09	7.37	0.09
phi022	3	0.32	1.09	0.28
bnlg1506	2	0.50	92.70	0.37
umc1152	3	0.52	–	0.44
phi050	2	0.40	2.08	0.32
Average	**2.65**	**0.43**	**6.90**	**0.36**

PIC, polymorphic information content.

and UI 3). There were lines in each cluster that were not directly related by ancestry; the 16 S_6 lines in Group I were derived from seven S_4 plants.

Relationships between known heterotic groups for the five normal endosperm lines (TZi 3, TZi 4, TZi 10, TZi 12 and TZi 15) and groups formed based on the SSR data were different. The lines TZi 3 and TZi 15 belong to different heterotic groups identified for the WCA sub-region, and are parents of Oba Super-1, a high yielding commercial white hybrid maize variety in Nigeria. The clustering together of these lines in Group V, and the clustering of TZi 3 and TZi 10, two lines belonging to the same heterotic group identified for the WCA sub-region, in separate groups, clearly indicates that the groupings resulting from the SSR markers were not on the basis of heterotic effects. Lines TZi 3, TZi 12 and TZi 15 were clustered into Group 5 while TZi 4 and TZi 10 clustered into Group III.

The cophenetic correlation coefficient value (r) was 0.66, an indication that the cluster analysis showed a moderately good fit to the original GS matrix.

Discussion

An insight into crop genetic diversity is critical for the planning and successful execution of a plant breeding programme. Information on molecular diversity complements morphological and agronomic traits considerably in improving breeding efficiency and genetic gain. The *sh-2* inbred lines genotyped at the 23 SSR marker loci in this study are the first generation of super-sweet maize inbred lines developed in the WA sub-region. In the future, hybrids with heterosis, developed from the *sh-2* inbred lines, will provide seed companies that produce and market maize seeds a unique opportunity to diversify the maize value chain, bringing with it the potential for increased profitability and sustainability.

The five SSR markers that were monomorphic in the present study were polymorphic in previous diversity studies among American normal endosperm maize germplasm (Senior *et al.*, 1998; Vigouroux *et al.*, 2005). Although sweet corns have a narrow genetic base (Tracy, 1997; Tracy, 2001), selection for adaptability to the tropical environment and superior agronomic characteristics in the parent population and during the inbreeding process could have contributed to the monomorphism at these loci, especially if the SSR loci are linked to regions of the chromosome under selection.

The average number of alleles obtained in the present study (2.65) was comparable to the 2.70 reported by Wietholter *et al.* (2008) using 21 SSR markers and 3.25 obtained by Bantte and Prasanna (2003) based on 36 SSR markers. It was, however, considerably lower than the five alleles per locus reported for 94 elite US maize inbreds detected with 70 SSR markers (Senior *et al.*, 1998); 4.90 alleles per locus obtained for 50 CML lines genotyped at 85 SSR loci (Warburton *et al.*, 2002); 7.36 alleles per locus for 41 tropical maize inbreds determined with 25 SSR markers (Sserumaga *et al.*, 2014); and 9.7 alleles per locus reported for 21 elite West and Central Africa (WCA) normal endosperm maize inbreds genotyped at 18 SSR loci of dinucleotide repeats (Adetimirin *et al.*, 2008). The high value obtained by Adetimirin *et al.* (2008) may be attributable to the exclusive use of dinucleotide repeat SSRs and the diversity of the maize lines used in the study. The efficiency of the allele detection system is often considered in the choice of repeats type, which in turn influences the number of alleles per locus. Dinucleotide SSR makers are known to yield a significantly higher number of alleles per locus than SSRs with longer repeat motif. Their use is, however, limited by the difficulty in accurately sizing alleles (Heckenberger *et al.*, 2002; Choukan *et al.*, 2006; Adetimirin *et al.*, 2008). The average PIC of 0.36 obtained in this study was within the range of 0.30–0.41 reported by Akaogu *et al.* (2013) and Lopes *et al.* (2015) in maize,

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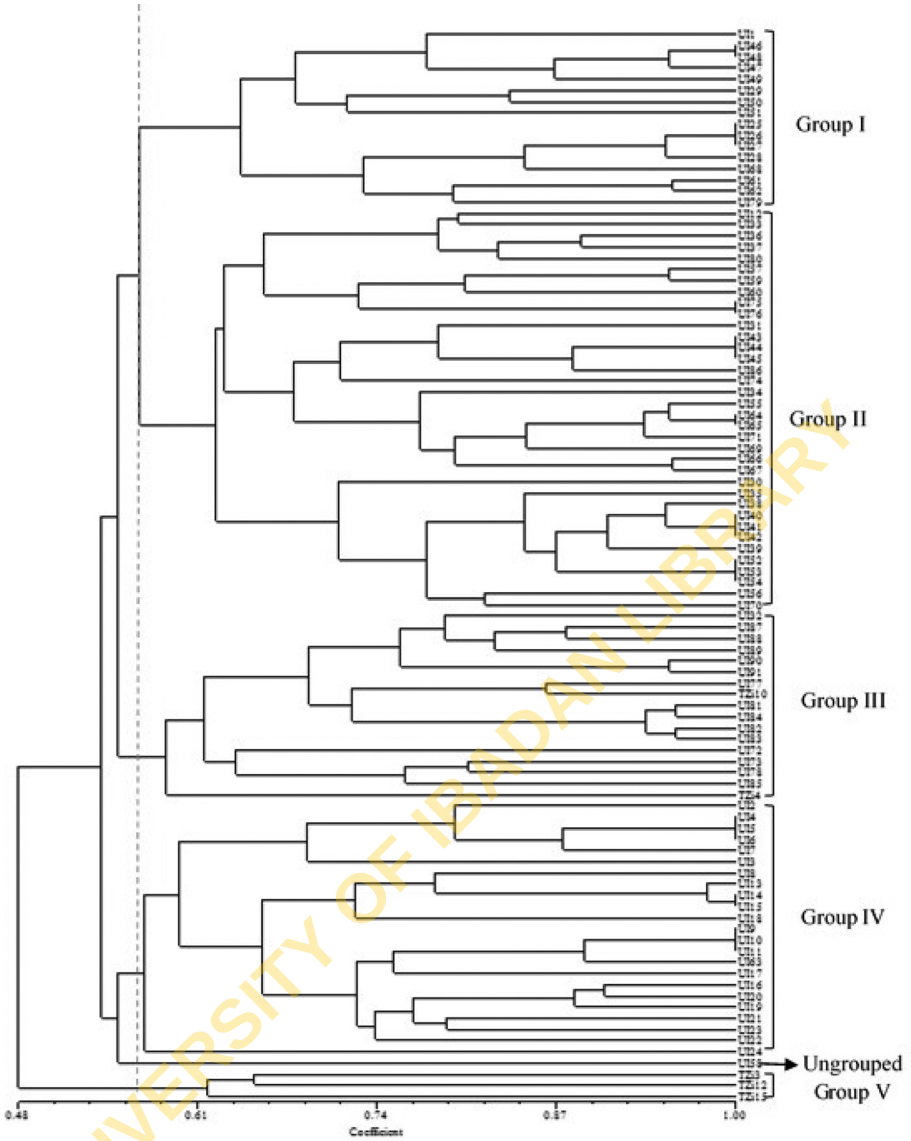

Fig. 1. Dendrogram of the 96 maize inbred lines from 23 SSR loci using Dice genetic similarity coefficients of Nei and Li (1979).

and demonstrates that the SSR markers used were moderately informative, and have the potential for use in other studies to detect differences among inbred lines. Among other studies (Senior *et al.*, 1998; Warburton *et al.*, 2002; Adetimirin *et al.*, 2008) that reported higher PIC values in maize using SSR markers, Mehta *et al.* (2017) obtained an average PIC value of 0.50 in a study of 48 sweet corn inbreds that included *su-1*, *sh-2* and double mutant sweet corn inbreds genotyped at 56 SSR loci.

Overall, the average level of gene diversity detected by the markers among the inbred lines studied (0.43) was sufficiently high for effective management of the lines and their exploitation for cultivar development. Gene diversity values obtained in other studies with SSR in maize were 0.36 using 23 SSR markers for 22 yellow-grain normal endosperm extra-early inbreds adapted to West and Central Africa (Akaogu *et al.*, 2013) and 0.32 for 22 sweet corn cultivars with 30 SSR markers (Lopes *et al.*, 2015). Higher gene diversity values were, however, reported for 20 Ghanaian landraces genotyped at 20 SSR loci (0.54) (Oppong *et al.*, 2014) and 56 Chilean Choclero maize landraces and inbreds using 10 SSR markers (0.51), eight of which were dinucleotide repeats (Salazar *et al.*, 2017). For most of the loci, heterozygosity was lower than 3.2%, in agreement with the theoretical expectation for S_6 lines (Allard, 1999). The unexpected high level of heterozygosity (92.7%) obtained with bnlg1506 was likely due to amplification of similar sequences in different genomic regions. Similarly,

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high level of heterozygosity for homozygous lines has been reported by several authors (Senior *et al.*, 1998; Matsuoka *et al.*, 2002; Adetimirin *et al.*, 2008; Sserumaga *et al.*, 2014). Ten sets of duplicates, each consisting of 2–3 lines, were detected by the 23 markers. The duplicates obtained in the present study confirm the power of SSR markers to identify lines exhibiting phenotypic differences but which are similar at the molecular level (Smith *et al.*, 1997). This has the advantage of reducing redundancy in germplasm management, and improving efficiency in the choice of lines selected for hybridization and field testing.

The dendrogram from the UPGMA clustering algorithm had five groups which in most instances were in accordance with their pedigree data. Gerdes and Tracy (1994) reported that pedigree relationships can be used as a benchmark to test the effectiveness of markers in determining relationships among breeding lines. This observation was similar to the findings by other studies (Senior *et al.*, 1998; Legesse *et al.*, 2007) that SSRs are efficient markers for classifying closely related lines. Our results also indicate the usefulness of SSR markers in clustering lines that may be identical in state but not by descent. Although many of the S_6 *sh2* lines which were derived from the same S_4 plant clustered together, there were a few lines derived from S_4 plants that clustered in different groups, indicating that residual heterozygosity at the S_4 stage of inbreeding yielded plants with considerable genetic differences in later generations.

The clustering of the normal endosperm inbred lines did not follow their established heterotic groupings. However, in a field study carried out by Akintunde (2017), a hybrid formed from UI 48 and UI 35, two lines in Groups I and II in the dendrogram obtained in the present study, showed heterosis, with fresh cob yield >10 t/ha. The author's results provide some justification for exploring heterosis between inbred lines in the two groups. In addition, the two inbred parents of the hybrid would be useful as testers in classifying the *sh-2* inbred lines into heterotic groups based on the field performance of their testcrosses. Thus, the SSR studied have the potential for use in guiding the *sh-2* maize hybrid development process in WA. Choukan *et al.* (2006) reported the usefulness of SSR markers in the establishment of heterotic groupings in late and medium Iranian normal endosperm maize inbred lines. Although not one of the objectives of the present study, it was interesting to observe that the clustering obtained in the present study distinguished lines tolerant of *Striga hermonthica* in Group V (TZi 3, TZi 12 and TZi 15) from those that are susceptible to the parasitic weed in Group III (TZi 4 and TZi 10). These lines have been tested extensively and their reactions under *S. hermonthica* were reported in previous studies (Kim *et al.*, 1987, 2000; Adetimirin *et al.*, 2000). The devastation of maize and other cereals by *S. hermonthica* in sub-Saharan Africa results in losses running into billions of dollars (Gasura *et al.*, 2019). The SSR markers used in the present study deserve further investigation for their usefulness as a resource in the screening of maize genotypes for their reaction to *S. hermonthica*.

This study is the first on the genetic diversity of the first generation of *sh-2* supersweet maize inbred lines developed for the WA sub-region. The genetic diversity among the *sh-2* and normal endosperm maize inbred lines studied was higher than for many other germplasm collections and could be exploited for hybrid cultivar development. Although the clustering for the normal endosperm inbred lines did not follow their established heterotic groups, the SSR markers largely separated the *sh-2* maize inbred lines into groups consistent with their pedigree records and also identified duplicate genotypes; it can thus help to reduce redundancy in germplasm management and improve efficiency in the choice of lines selected for hybrid cultivar development. Research is currently underway using the findings from the present study to fast-track the development of supersweet maize hybrids of diverse agronomic traits for WA.

Acknowledgements

This study was made possible with the molecular biology equipment donated by Kirkhouse Trust, UK and a scholarship awarded to the first author by the Alliance for a Green Revolution in Africa (AGRA).

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