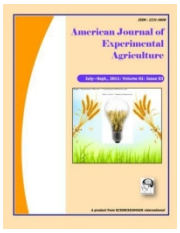

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Application of SSR Markers for Genetic Purity Analysis of Parental Inbred Lines and Some Commercial Hybrid Maize (*Zea mays* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. IOD initiated and supervised the study. JAA and OOO carried out the laboratory studies and prepared the draft manuscript. SAO performed the statistical analysis, while MOA and SOO edited the manuscript. All authors approved the final manuscript.

Research Article

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ABSTRACT

Aims: Morphological evaluation of seeds and growing plants used for certification for purity and variety distinctness in Nigeria is time consuming and expensive. This experiment set to evaluate the usefulness of SSR markers to determine genetic purity of commercial hybrids and their inbred lines.
Place and Duration of Study: Bioscience unit, International Institute of Tropical Agriculture, Nigeria in December, 2011
Methodology: Seedlings of four F1 hybrids and four inbred lines were grown in the screen house of IITA for DNA extraction using Dellaporta method with some modifications. Six Simple Sequence Repeat (SSR) markers were used for Polymerase Chain Reaction (PCR) using Touch-Down PCR profile. The analysis is by fragment analysis as present (1) or

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absent (0) Mathematical equation to determine genetic purity of the genotypes was developed from the genetic distances matrix.
Results: Simple descriptive analysis revealed that average genetic diversity and polymorphism information content (PIC) recorded by the markers was 0.592 and 0.512 respectively. Genetic purity level of inbred lines ranged between 91.3% and 98.7% while the hybrids ranged between 81.3% and 95%.
Conclusion: SSR markers are powerful biotechnological tool capable of detecting genetic purity status of Nigerian maize hybrids therefore inclusion of DNA analysis of seeds using SSR markers to determine genetic purity of maize seed is recommended. However, further research work with larger number of seed samples per variety will be needed to validate reliability.

Keywords: Maize; genetic purity; DNA analysis; SSR markers.

1. INTRODUCTION

Maize accounts for between 30 and 60% of food calories and dietary proteins in developing countries, especially in foods used for weaning infants. The grain is processed into several local dishes and consumed by both young and old (Showemimo et al., 2007). It has made a major contribution to the GDP of Nigeria. Industrially, it is used in the production of flour, livestock feed, processed food (such as cornflakes), sweeteners for soft drinks, pharmaceuticals and malt for brewing beer. Several breeding projects had led to evolution of improved crop varieties in the past five decades in Nigeria. For instance, about 40 inbred lines of maize had been released by public agricultural research institutions to commercial seed companies for hybrid seed production (NACGRAB, 2004). Recent achievements by breeders in the development and release of superior varieties of maize with higher yield potentials and better resistance to insect pests and diseases has played a major role in increasing maize production in Nigeria (Iken and Amusa, 2004). To meet varied and diversified uses of maize, five hybrids and fifty-four open pollinated varieties has also been registered and released into Nigeria seed industry (NACGRAB, 2004).

Hybrid seed production of maize involves crossing two diverse homogeneous inbred lines produced by several generation of selfing to produce heterogeneous hybrids. During hybrid seed production, much effort are always been directed towards managing the process to ensure maximum kernel set and high levels of genetic purity (Fonseca et al., 2003). The genetic quality of seed is influenced by agronomic practices and the ecological characteristics of the site where the seed is planted. In the production of hybrid maize seed, main source of genetic contamination on the field is self-pollination of the female parent due to incomplete removal of its tassel. This contamination reduces the genetic and physiological quality of the seeds that consequently decreases the crop productivity (Kalinka et al., 2006).

Presently in Nigeria, seed certification for purity and variety distinctness is based on morphological evaluation of seeds and growing plants. These evaluation methods often involve field inspection, which are rigorous, resource intensive, time consuming, subject to bias and have very little precision. There are however, good numbers of high precision technologies being used for crop breeding. Among these are electrophoresis (Tkachuk and Mellish, 1980) and use of molecular markers (Perry, 2004; Ali et al., 2008) which saves time and resources.DNA markers seem to be efficient in evaluation and selection of plant material

because, DNA markers segregate as single genes and they are not affected by the environment as morphological markers (Rahman et al., 2006). Also, SSR marker has been proved to be the currently preferred molecular marker for purity identification in some crops (Yashitola et al., 2002) due to its high efficiency and simplicity (Wu et al., 2010).

Therefore, the objective of this research work is to explore the use of SSR markers to test the genetic purity of two commercial hybrid maize varieties and their inbred parents used in seed industries in Nigeria.

2. MATERIALS AND METHODS

2.1 Seed Collection

Seeds of two F_1 hybrids and four inbred lines collected from International Institute of Tropical Agriculture (IITA) and two F_1 hybrids provided by Premier Seed Ltd. (a private seed company) were used for this experiment (Table 1).

Table 1. List of the hybrid maize varieties, their inbred parents and sources

Genotypes	Type	Kernel colour
Oba Super 1 IITA)	Experimental hybrid	White
Oba Super 2 (IITA)	Experimental Hybrid	Yellow
Oba Super 1 (Premier Seed Ltd)	Commercial hybrid	White
Oba Super2 (Premier Seed Ltd)	Commercial hybrid.	Yellow
1368 (IITA)	Inbred line of Oba Super 1	White
9071 (IITA)	Inbred line of Oba Super 1	White
4001 (IITA)	Inbred line of Oba Super 2	Yellow
KU 1414SR (IITA)	Inbred line of Oba Super 2	Yellow

Seed source in parenthesis.

2.2 DNA Extraction and Quantification

DNA analysis of the varieties were conducted by randomly selecting five seeds per genotype from the seed lot and planted into a one kg plastic container filled with sieved top soil in the screen house of IITA for young shoots to emerge for DNA extraction. DNA extraction was done according to Dellaporta method (Dellaporta et al., 1983) with some modification stated by Ogunkanmi et al. (2008). Extracted DNA was tested for concentration and quality on 1% w/v agarose gel stained with ethidium bromide. The gel was run at 90V for 30 minutes in 1% running buffer.

2.3 Primer Screening and Polymerase Chain Reaction

Forty one Simple Sequence Repeat (SSR) primer sets provided by IITA were screened with the maize sample DNA before six that showed polymorphic amplifications (Table 2) were used for Polymerase Chain Reaction (PCR). The PCR cycles was carried out with MJR® thermo-cycler (96-wells type) with initial de-naturation at 93ºC for 15 minutes followed by 9 cycles at 93ºC for 15 seconds, 57ºC for 20 seconds, 72ºC for 30 seconds and another 24 cycles at 93ºC for 15 seconds, 55ºC for 20 seconds, 93ºC and 72ºC for 30 seconds each for 15 seconds, Final elongation was done at 72ºC for 5 seconds. This protocol is coded Touchdown Simple Sequence Repeat-1 (TDSSR-1) protocol in IITA. The PCR Products

were electrophoretically separated on 2% agarose gels and visualized after staining with Ethidium bromide using Uvitech gel documentation system.

Table 2. Major alleles, genetic diversity and polymorphism Information Content of six SSR markers tested on F1 hybrids and inbred lines

SSR Markers	Sequence	Major Alleles	Genetic diversity	PIC
phi072	F-ACCGTGCATGATTAATTTCTCCAGCCTT R- GACAGCGCGCAAATGGATTGAACT	0.457	0.642	0.589
phi109642	F-CTCTCTTTCCTTCCGACTTTCC R-GAGCGAGCGAGAGAGATCG	0.500	0.616	0.542
phi123	F-GGAGACGAGGTGCTACTTCTTCAA R-TGTGGCTGAGGCTAGGAATCTC	0.591	0.484	0.367
phi423796	F- CACTACTCGATCTGAACCACCA R- CGCTCTGTGAATTTGCTAGCTC	0.553	0.495	0.372
phi96100	F- AGGAGGACCCCAACTCCTG R- TTGCACGAGCCATCGTAT	0.444	0.673	0.613
umc1136	F- CTCTCGTCTCATCACCTTTCCCT R- CTGCATACAGACATCCAACCAAAG	0.526	0.644	0.597
Mean		0.512	0.592	0.510

2.4 Data Analysis

Each band was considered as a single allele. Alleles were scored as present (1) or absent (0). Polymorphism information content (PIC) of the SSR markers was assessed with the use of Power Marker software while genetic diversity was estimated using PAlaeontologicalSTatistics (PAST) analytical software. To calculate the percentage genetic purity of the genotypes, mathematical equation was developed from the genetic distances matrix as follows:

$$Genetic\ purity(\%) = 100 - \frac{\sum x_1}{n}$$

Where x_1 = values of genetic distances between the tested seeds and n = no of observations.

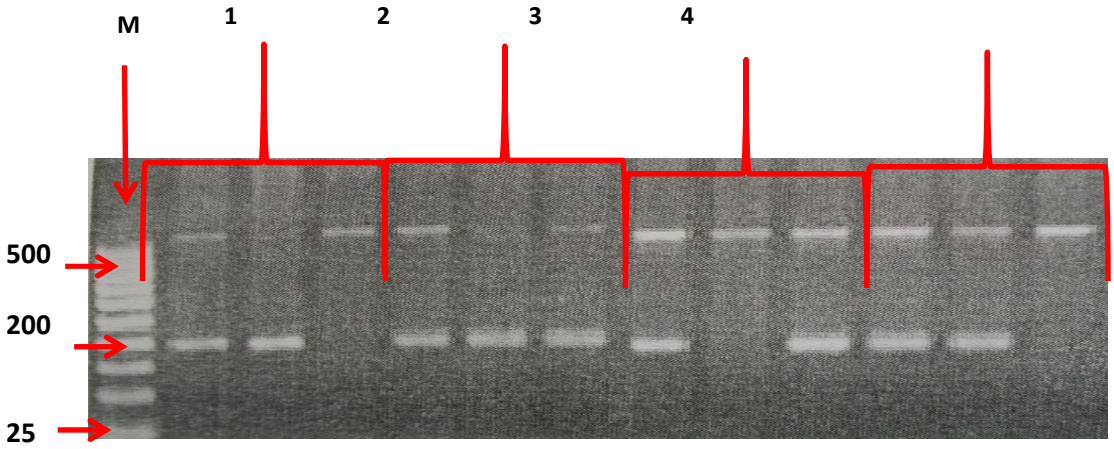
3. RESULTS AND DISCUSSION

3.1 Amplification Profiles and Informative Ability of the SSR Markers

The amplification profile of the maize genotypes using six SSR markers sets showed alleles of different molecular weight. (Figs.1 and 2).All the six SSR markers were able to detect genetic diversity which is defined as the probability that two randomly chosen alleles from the population are different among the maize genotypes (Liu and Muse, 2005).

Information regarding the SSR markers and their informative ability is presented in Table 2. Average genetic diversity and polymorphism information content (PIC) recorded by the six markers was 0.592 and 0.512 respectively. The highest genetic diversity (0.673) and PIC (0.613) was recorded by primer phi96100 while primer phi123 recorded the lowest genetic diversity (0.484) and PIC (0.367). Similar PIC values have been recorded by other researchers in genetic diversity experiments of maize. Legesse et al. (2007) recorded

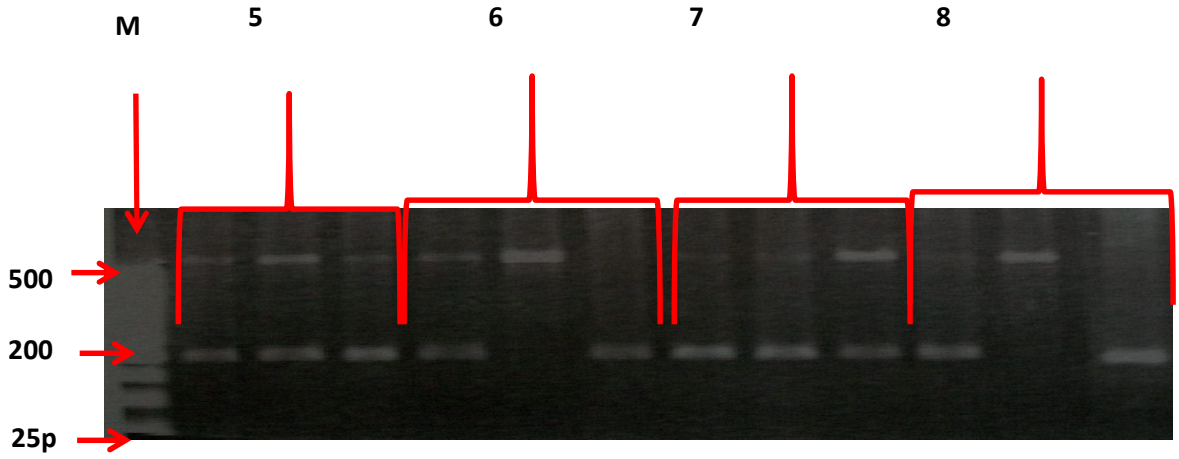
average PIC of 0.58 on 56 inbred lines of maize while Banisetti et al. (2012) reported average PIC value of 0.49 on 22 maize genotypes. Also, Choukan and Warburton (2005) have reported average gene diversity of 0.58 in 36 early maturing Iranian maize inbred lines. Since the genetic diversity and PIC values recorded in these studies are within those recorded for genetic diversity studies in maize, these SSR markers are considered useful for genetic purity analysis of maize varieties.



hi96100

Fig. 1. PCR amplification products generated using phi96100 primer.

Legend: Lane M: Bioline hyper v ladder100lane DNA marker;
1 = 1368; 2 = Oba Super1(IITA); 3=Oba Super 1(Premier); 4= 9071;



phi96100

Fig. 2. PCR amplification products generated using phi96100 primer.

Legend: Lane M: Bioline hyper v ladder100lane DNA marker;
5=4001; 6=Oba Super 2(IITA); 7= Oba super2 (Premier seed); 8=KU1414SR

3.2 Estimates of Genetic Distance among the Inbred Lines and Hybrids

Genetic distance (GD) indices computed by 0/1 method using the six SSR markers are presented in Table 3. Average genetic distances among the seeds of each genotype are rectangular enclosed and ranged from 0% (between seed 2 and 3 of 1368, 9071 and KU1414SR) to 2.6% (between seed 1 and 3 of Oba Super 2 IITA). Highest genetic distance recorded among the genotypes was 2.6%, thus they can be considered genetically similar. Research findings using SSR marker for seed purity are few, but SSR markers used for diversity studies has recorded genetic distance range of 0-53% among 14 genotypes of maize using four SSR markers (Sedaf et al., 2010).

Genetic purity of each genotype calculated from the standard genetic distance indices is presented in Table 4. The result showed that the inbred parents of Oba Super 1 (1368 and 9007) recorded higher purity of 97.3% and 98.7% while inbred parents of Oba Super 2 (4001 and KU-1414SR) purity were 96% and 91.3% respectively. The result clearly confirmed the fact that the purity level of the inbred parents determines the purity of the hybrids because Oba Super 1that has higher purity level of inbred parents' recorded higher purity than Oba Super 2 from both sources despite the fact that the inbred parents used for this experiment was collected from only IITA.

3.3 Cluster Analysis

The clustered dendogram based on genetic distance matrix and obtained with unweighted pair group method arithmetic (UPGMA) average displayed two main groups (Fig. 2).Most of the hybrid seeds collected from IITA were clustered together in group one. Also, one sample each of hybrid seed from Premier Seed Ltd (Oba Super 2) and its inbred parent (KU-1414-SR) clustered into same group.

Relationships among seed samples clustered into group two is clearly sub-divided into another five sub groups (A-E). Each cluster formed several small groups and revealed the genetic divergence within the genotypes and among the seed samples. All the tested samples of Oba Super-1 (Premier), 4001 and 1368 were clustered as group A, B and C respectively indicating close relationship and high purity level. Similarly, samples of 9071 were clustered together in group D with one of the samples of Oba Super-1 (IITA). The last group comprises of two samples each of Oba Super-2 from Premier Seed Ltd and its inbred parent (KU1414SR).

This result revealed a clear demarcation between most of the genotype indicating differentiation of the inbred lines into groups due to genetic diversity and also the hybrids according to their source. The broad genetic diversity detected within and among the seed samples demonstrates the genetic purity that exists among the tested samples as well as the potentials of using SSR markers for genetic purity analysis in maize.

Table 3. Standard genetic distances indices among and between the inbred parents and the hybrids

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
1	0	0.04	0.04	0.09	0.11	0.19	0.13	0.11	0.11	0.07	0.09	0.09	0.09	0.07	0.09	0.13	0.15	0.17	0.11	0.20	0.11	0.13	0.11	0.11
2		0.00	**0.00**	0.09	0.11	0.22	0.09	0.11	0.07	0.07	0.09	0.09	0.09	0.11	0.06	0.13	0.15	0.20	0.11	0.24	0.07	0.17	0.07	0.07
3			0.00	0.09	0.11	0.22	0.09	0.11	0.07	0.07	0.09	0.09	0.09	0.11	0.06	0.13	0.15	0.20	0.11	0.24	0.07	0.17	0.07	0.07
4				0.00	0.06	0.17	0.15	0.13	0.13	0.09	0.11	0.11	0.11	0.13	0.11	0.11	0.13	0.15	0.13	0.19	0.17	0.15	0.17	0.17
5					0.00	0.11	0.13	0.11	0.15	0.15	0.17	0.17	0.09	0.11	0.09	0.17	0.11	0.09	0.19	0.13	0.19	0.09	0.19	0.19
6						0.00	0.20	0.15	0.22	0.22	0.24	0.24	0.17	0.15	0.17	0.24	0.11	0.06	0.26	0.02	0.26	0.13	0.26	0.26
7							0.00	0.06	0.02	0.09	0.11	0.11	0.11	0.13	0.07	0.11	0.20	0.22	0.13	0.22	0.09	0.15	0.09	0.09
8								0.00	0.07	0.11	0.13	0.13	0.09	0.11	0.09	0.13	0.15	0.17	0.11	0.17	0.11	0.13	0.11	0.11
9									0.00	0.07	0.09	0.09	0.09	0.11	0.06	0.09	0.19	0.24	0.11	0.24	0.07	0.17	0.07	0.07
10										0.00	0.02	0.02	0.09	0.11	0.09	0.06	0.19	0.24	0.07	0.24	0.11	0.17	0.11	0.11
11											0.00	**0.00**	0.07	0.09	0.11	0.04	0.20	0.26	0.09	0.26	0.13	0.19	0.13	0.13
12												0.00	0.07	0.09	0.11	0.04	0.20	0.26	0.09	0.26	0.13	0.19	0.13	0.13
13													0.00	0.02	0.04	0.11	0.13	0.19	0.17	0.19	0.17	0.15	0.17	0.17
14														0.00	0.06	0.13	0.15	0.17	0.19	0.17	0.19	0.13	0.19	0.19
15															0.00	0.15	0.13	0.19	0.17	0.19	0.13	0.15	0.13	0.13
16																0.00	0.24	**0.26**	0.09	0.26	0.13	0.19	0.13	0.13
17																	0.00	0.06	0.15	0.09	0.15	0.13	0.15	0.15
18																		0.00	0.20	0.04	0.20	0.07	0.20	0.20
19																			0.00	0.24	0.04	0.13	0.04	0.04
20																				0.00	0.24	0.11	0.24	0.24
21																					0.00	0.13	0.00	0.00
22																						0.00	0.13	0.13
23																							0.00	**0.00**
24																								0.00

Legend: 1-3=1368;4-6=Oba Super 1(IITA);7-9=Oba Super1(Premier seed);10-12=9071;13-15=4001;16-18=Oba Super 2(IITA);19-21=Oba super-2(Premier seed); 22-24=KU-1414STR

Table 4. Genetic purity (%) calculated from the genetic distance indices

Genotype	Genetic distance (%) between			Average genetic distance between the genotypes ($\frac{\sum a+b+c}{3}$) (d)	Calculated genetic purity (%) ($\frac{100}{3} - \frac{\sum a+b+c}{3}$)
	Seed 1 and 2 (a)	Seed 1 and 3 (b)	Seed 2 and 3 (c)		
1368	4	4	0	2.67	97.3
Oba Super 1 (IITA)	6	17	11	11.33	88.7
Oba Super 1 (Premier Seed)	6	2	7	5.00	95.0
9007	2	2	0	1.33	98.7
4001	2	4	6	4.00	96.0
Oba Super 2 (IITA)	24	26	6	18.67	81.3
Oba Super 2 (Premier Seed)	24	4	24	17.33	82.7
KU 1414SR	13	13	0	8.67	91.3

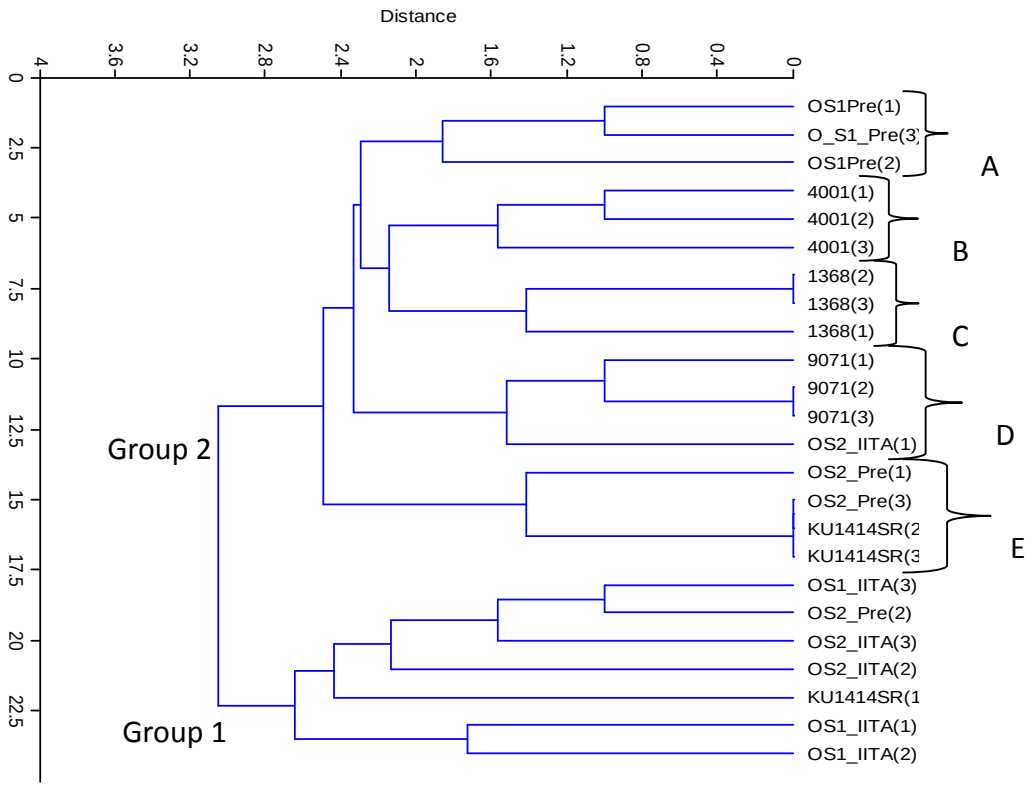


Fig. 3. Dendogram of the hybrids along with their inbred parents developed from SSR data using PAST software

4. CONCLUSION

This study has shown that SSR markers are powerful biotechnological tool capable of detecting genetic purity status of Nigerian maize hybrids. It can be concluded that the inclusion of DNA analysis using SSR markers to determine genetic purity of maize seed is recommended, though more numbers of seed will be required for more precision.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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