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Full Length Research Paper

Genetic diversity of some selected Nigeria cowpea using simple sequence repeats (SSR) marker

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Six primers named VM 9, VM 27, VM 36, VM 39, VM 74 and VM 98 were utilized to assess their ability to reveal polymorphisms in 20 cowpea accessions collected from Ibadan, South-western Nigeria using Simple Sequence Repeats (SSR). Reaction products (bands) of the SSR analysis were moderately high and polymorphic. The most distinct accession at agglomerative coefficient of 42 (level of similarity) was NG/SA/07/0320 based on the molecular dendogram. The most informative and reliable primer that was able to distinguish the 20 accessions of cowpea among the six primers was VM 39 with polymorphism information content (PIC) and polymorphic band value of 0.74 and 5 respectively. This information showed that there is sufficient genetic variance to warrant selection for improvement in the cowpea accessions studied.

Key words: DNA, simple sequence repeats (SSR), cowpea, accession, marker and primers.

INTRODUCTION

Cowpea belongs to the genus *Vigna,* (tribe; *Phaseoloideae*, subfamily; *Papilionoideae*). It occupies a unique place as the most widely cultivated and utilized grain legume in Nigeria as well as, one of the cheapest sources of plant protein sources in the diet of Nigerians providing over 57% protein. Its value lies in its high protein content of 23 to 29%, with potential for 35%; and its ability to fix atmospheric nitrogen, which allows it to grow on, and improve poor soils (Steele, 1972).

A number of workers (Paterson et al., 1991; Kumar, 1999) have demonstrated that DNA markers are a promising technique used to differentiate among genotypes at species and sub-species level.

Simple Sequence Repeats (SSRs) markers have been confirmed to be the most informative and appropriate for cassava (Mba et al., 2000). Perera et al. (2000) also supported SSR markers as the most informative for plants. Valuable attributes of all SSR markers are codominance (many alleles are found among closely related individuals), technical simplicity, sensitivity, analytical simplicity (data are unambiguously scored and highly reproducible) and are high abundance (markers are uniformly dispersed throughout genome as frequently as every 10 Kb3 and therefore, are ideal tools for many genetic applications.

Understanding the extent, distribution and nature of the variation within the cowpea landraces would be useful in the development of genotypes with increased yield potential. The main objective of this study is to characterize the 20 cowpea accessions genetically for the best use of the genetic potential of the crop and for a better management of cowpea germplasm.

MATERIALS AND METHODS

The 20 accessions of cowpea used for this study were collected from market places, farmer's fields' in Adamawa, Kano, Zamfara, Jigawa, Ogun, Oyo, Osun, Plateau, Niger states and a breeding line (IFE, BPC) (Table 1), during explorations to various states in Nigeria. These collections have since been maintained in the ex-situ gene bank of the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria. The experiment was carried out at the Biotechnology laboratory of International Institute for Tropical Agriculture (IITA), Ibadan.

A total of 20 cowpea accessions (Table 1) were used for this experiment. The seeds were planted inside polythene pot in the screen house, young leaf samples were collected at fourteen days

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Table 1. The names, origin and seed coat colour of 20 Nigerian cowpea accessions.

S/N	Accession	Symbol	Origin	Seed coat colour
1	NG/SA/07/082	A	Zamfara	White
2	NG/SA/07/075	B	Zamfara	White
3	NG/SA/07/113	C	Katsina	Brown
4	NG/SA/07/089	D	Katsina	White
5	NG/SA/07/132	E	Kano	Brown
6	NG/SA/07/135	F	Kano	Brown
7	NG/SA/07/133	G	Kano	Brown
8	NGB/06/058-1	H	Plateau	Wine
9	NGB/06/058-2	I	Plateau	Peach
10	NGB/06/050	J	Plateau	White
11	NGB/06/044	K	Oyo	Brown
12	NGB/06/052	L	Oyo	White
13	TVU1515	M	Osun	Light brown
14	NGB/06/049	N	Ogun	White
15	IFE BPC	O	IAR&T	Brown
16	NG/SA/07/166	P	Adamawa	Brown
17	NG/SA/07/0308	Q	Niger	Light brown
18	NG/SA/07/0309	R	Niger	Brown
19	NG/SA/07/086	S	Jigawa	White
20	NG/SA/07/320	T	Jigawa	Black

Table 2. List of polymorphic primers.

S/N	SSR Primers	Primer sequence
1	VM 9	5' accgcacccgattatttcat 5' atcagcagacaggcaagacca
2	VM 27	5' gtccaaagcaaatgagtcaa 5' tgaatgacaatgagggtgc
3	VM 36	5' actttctgttttactcgacaactc 5' gtcgctgggggtggcttatt
4	VM 39	5' gatggttgtaatgggagagtc 5' aaaaggatgaaattaggagagca
5	VM 74	5' ctctacaccttccatcattc 5' cctttgctgtgtggtggttt
6	VM 98	5' ggaagcctttggaaattgatg 5' ccctacattgaaggtcaacaa

from all the accessions for DNA isolation and analysis. Extraction of genomic DNA was done according to the procedure reported by Thottappilly et al. (1999) with some modifications. The bands were scored as presence (+) 1 and absence (-) 0. The result of the analysis was utilized to construct a dendogram.

The genetic similarity among accessions based on SSR (Simple Sequence Repeats) marker was presented in form of a dendogram using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) according to Sneath and Soka (1973).

RESULTS AND DISCUSSION

Nucleotide sequence of selected primers with number of amplified products, fragment size (bp) and the PIC value was presented in Table 3. The six primers generated a total of seventeen bands. The number of bands per primer varied from 2 to 5 with an average of 2.83. The primers were able to produce fragments that varied from 134 to 271 in size. Out of the six polymorphic (Table 2), SSR primers used, 4 primers (VM 9, VM 27, VM 36, and VM 98) had two bands each, VM 39 had 5 bands and also had the highest polymorphic index content PIC of 0.742 followed by VM 74 with 4 bands and PIC of 0.59. Other primers had lower PIC of 0.48 and 0.46. However, VM 39 showed the highest diversity among the 20 accessions of Nigerian cowpea used in this study. Therefore, it is the most informative primer among the six primers (Figure 2).

Table 3. Nucleotide sequence of selected primers with the number of polymorphic bands, fragment size range (bp) and PIC value.

SSR primers	Primer sequence	Number of polymorphic bands	Allele size (bp)	PIC value
VM9	5' accgcacccgattatttcat 5' atcagcagacaggcaagacca	2	271	0.46
VM27	5' gtccaaagcaaatgagtcaa 5' tgaatgacaatgagggtgc	2	207	0.48
VM36	5' actttctgttttactcgacaactc 5' gtcgctgggggtggcttatt	2	160	0.48
VM39	5' gatggttgtaatgggagagtc 5' aaaaggatgaaattaggagagca	5	212	0.74
VM74	5' ctctacaccttccatcattc 5' cctttgctgtgtggtggttt	4	134	0.59
VM98	5' ggaagcctttggaaattgatg 5' ccctacattgaaggtcaacaa	2	168	0.48
Total		17		

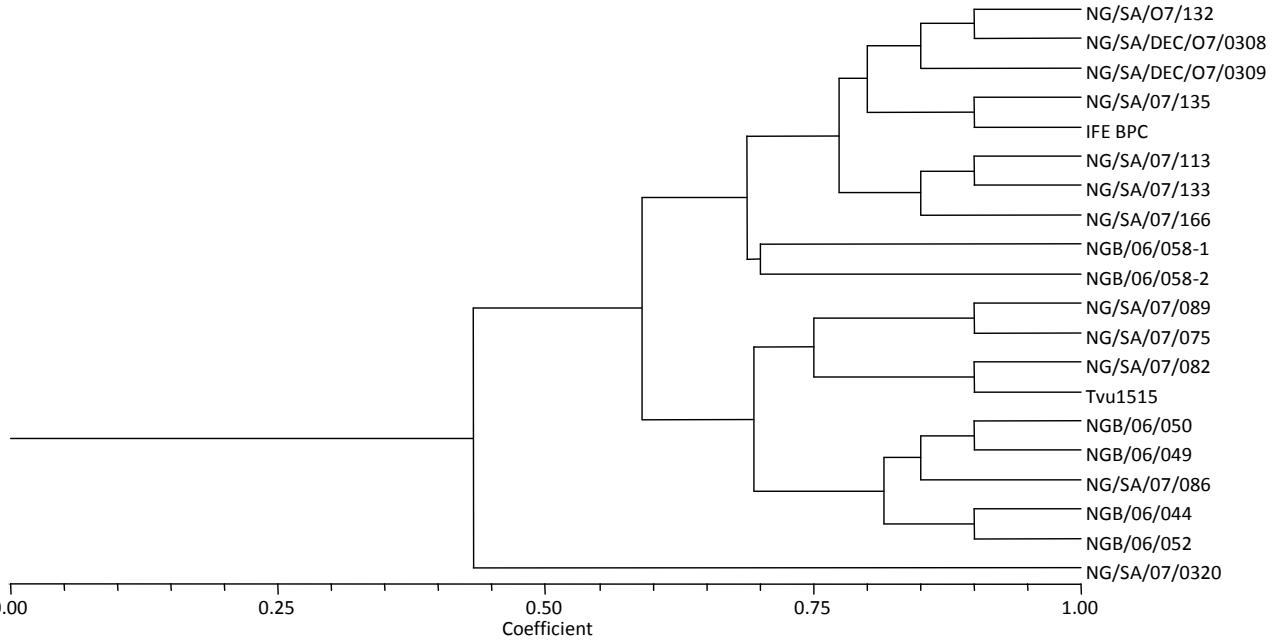

Figure 1. Molecular dendogram showing the genetic similarity among 20 accessions of Nigerian Cowpea revealed by UPGMA cluster analysis based on SSR marker.

The dendogram generated from the presence or absence of DNA fragments is presented in Figure 1. The estimates of distances among the accessions were based on the data of similarity matrix from the base pairs using the Unweighted Pair- Group Method of Arithmetic Averages (UPGMA) and the Neighbour Joining (NJ) method. The molecular dendogram revealed the genetic relatedness of accessions is more than the morphological dendogram. At 100% level of similarity, all the accessions were different from one another while at an agglomerative coefficient of 0.70 (similarity level) on the dendogram, the cowpea accessions were grouped into 5 clusters. Cluster 5 contained the largest number consisting of 8 accessions mainly from Northern part of Nigeria including a breeding line IFE-BPC: 3 from Kano, 2 from Niger, 1 each from Adamawa and Katsina. Cluster 4 consisted of 2 accessions from Plateau state; Cluster 3 had 4 accessions which comprised of 2 from Zamfara, 1 each from Osun and Katsina states; Cluster 2 had 5 accessions mainly (3 accessions) from South-western Nigeria and, one each from Plateau and Jigawa. Lastly, Cluster 1 contained only one accession (NG/SA/07/320) from Jigawa state.

The SSR analysis showed that all the accessions

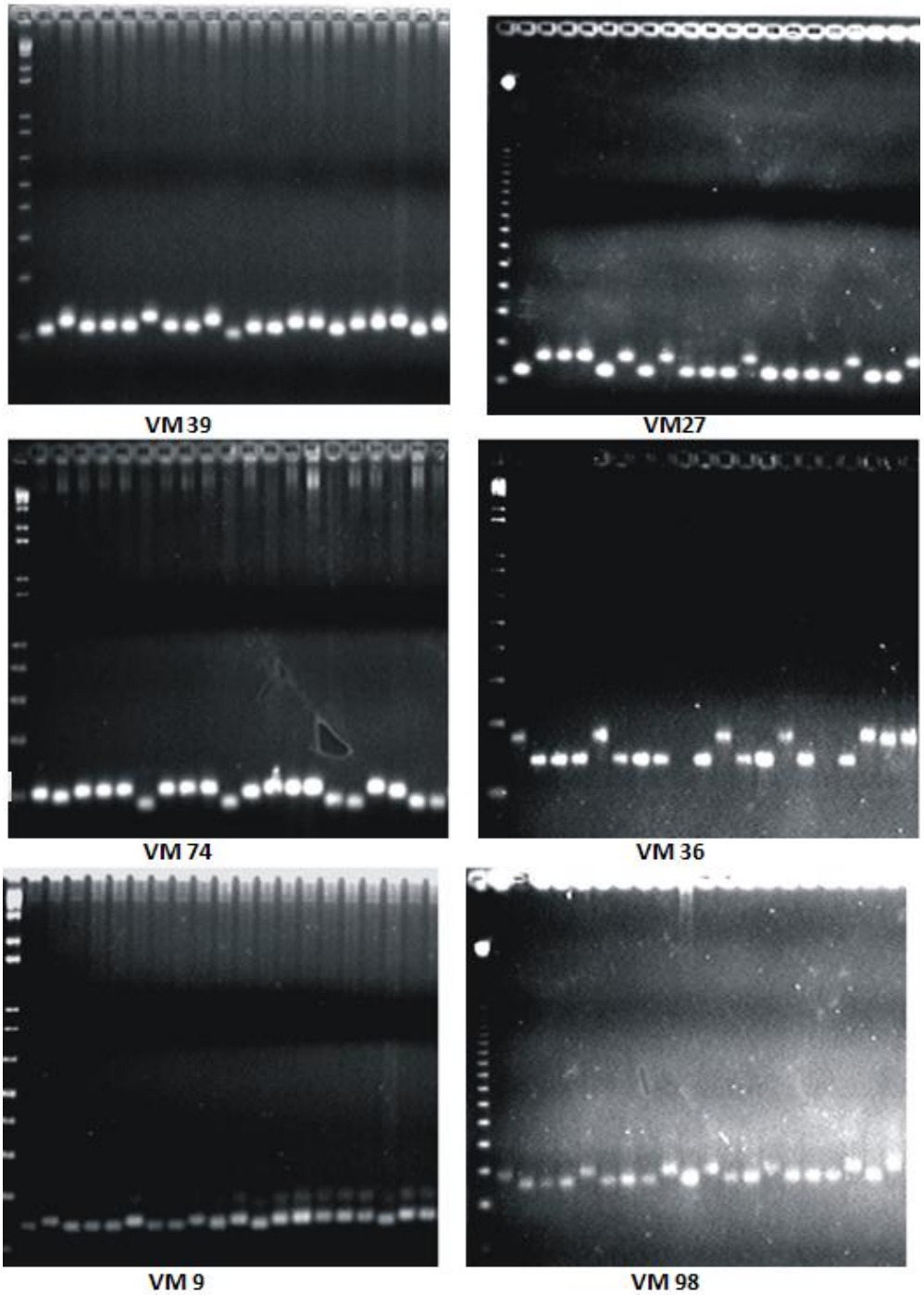

Figure 2. PCR reaction for different primer used.

analyzed belonged to the cultivar group *unguiculata*. The classification of accessions into different groups is independent of collection zones, agro-ecozones and market places. The classifications of accessions into different characters based on seed coat colour are very close and are more than the morphological characters according to the molecular dendogram constructed based on the presence or absence of amplified DNA fragments of a particular size and this supported the result obtained by Zannou (2008). The genetic base of the 20 cowpea accessions was moderately high according to the molecular dendogram and this information will reduce the overall time required in screening large populations of potential parents in identifying breeding stock. Compared to some cereals like soybean, maize e.t.c. cowpea accessions showed a low level of microsatellite marker

polymorphism, indicating its narrow genetic diversity, the same result was obtained by Li et al. (2001), though, opposite results was observed in Malawian landraces by Nkongolo (2003). In general, the dendogram of accessions and level of polymorphism detected in this study supported the established view that genetic diversity in cowpea is moderately high (Zannou et al., 2008).

Conclusions

This study shows the presence of genetic variability among the Nigerian cowpea germplasm which can be used to broaden the genetic basis of the crop for better use of its genetic potential and management.

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