Genetic relationships revealed by simple sequence repeat (SSR) markers among Ghanaian cassava cultivars released by different research groups

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ABSTRACT

Cassava (*Manihot esculenta*) is an important staple crop in Ghana and it is widely cultivated. The crop has diverse industrial applications. Knowledge about the state of cassava genetic structure in Ghana is paramount in any cultivar improvement especially through breeding for superior qualities. The objectives of the study were to assess the SSR allele polymorphism, and the estimation of inter- and intra-population genetic diversity among released cassava cultivars. The results showed high diversity among the studied cultivars with an average of seven alleles per locus. Polymorphic loci varied from 68.6 per cent to 100 per cent with an average of 88.58 per cent. Genetic diversity was observed within populations (HS = 0.552) and, therefore, suggesting a low rate of inter-population gene flow among the individuals constituting the populations.

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Introduction

Cassava (*Manihot esculenta*), belongs to the family Euphorbiaceae containing 28 wild spp. (Raghu *et al*., 2007). It is one of the most important food crops of sub-Saharan Africa and grown throughout the tropics including Asia and Latin America. Cassava plays a famine prevention role wherever it is cultivated widely, because it provides a stable base to the food production system (Romanoff & Lynam, 1992). In Ghana, cassava is produced in all but two regions in the northern part of the country. According to Nweke *et al*. (1999), in collaborative study of cassava in Africa (COSCA), villages that did not experience the famine of 1983 in Ghana were those that cultivated cassava as the most important and dominant staple crop.

Among the many cassava products in Ghana are 'fufu' (made by boiling cassava and pounding them into a glutinous mass using a wooden mortal and pestle), 'gari' (a crispy and crunchy fried cassava paste, 'ag-

belima' (grated fermented cassava paste), 'agbelikaklo' (grated fermented cassava paste fried in oil), 'yakeyake' (steamed cassava paste meal) and 'konkonte' (dried cassava chips).

Cassava is assumed to have evolved from inter-specific hybridization among its wild species. The crop is strongly an out crossing monoecious species but suffers from inbreeding depression, making it difficult to develop appropriate stocks for classical genetic studies (Fregene *et al*., 1996). The issue of inbreeding has led to the use of crossing blocks for inter mating superior individuals so that inbreeding could be minimised (Falahati-Anbaran *et al*.,2007).

Cassava is a diploid plant with 2n = 36 chromosomes and a DNA content of 1.67 pg per cell nucleus (Awoleye *et al*., 1994). This is equivalent to 772 Mbp per haploid genome, and occupying the lower end of the genome size range for higher plants (Bennet, Smith & Heslop-Harrison, 1992). This relatively small genome size of cassava favours development of saturated genetic map, and molecular tag which may contribute to an understanding of the inheritance of many important genetic traits despite the heterozygous nature of cassava (Fregene *et al*., 1996). Considerable amounts of genetic variations in cassava germplasm have been reported by many laboratories (Ragu *et al*., 2007; Acquah, Quain & Twumasi, 2011).

In recent years, there has been increasing interest in the use of DNA-based markers for a more reliable genetic diversity studies compared to the classical morphological characterisation. Morphological descriptors are highly subjective, environmentally influenced and detect little polymorphism. Morphological descriptions have over the years been successfully used in breeding, but remain inadequate for analysis of population genetic structure (Falahati-Anbaran *et al*., 2007). Simple sequence repeat (SSR), a common DNA Marker, has been reliably used to quantify genetic diversity and examine population differentiation in a number of agricultural crops (Morgante *et al*., 1994; Maughan *et al*., 1995; Raghe *et al.*, 2007). SSRs are co-dominant and tend to have multiple alleles per locus so that individuals can be identified as homozygotes or heterozygotes.

An additional benefit of SSR marker is the ability to detect variations in allele frequency at many unlinked loci, which are abundant in plants and the technique adaptable to automation (Donini *et al*., 1998).

The objectives of the study, therefore, were to use SSR markers to assess SSR allele polymorphism, and also to estimate inter and intra-population genetic diversity among 11 released and two cassava germplasms in Ghana.

Materials and methods

Plant materials

Five cassava populations including 11 released and two local cultivars were selected (Table 1). They were collected from farms of the CSIR-Crops Research Institute (CRI) at Fumesua in the Ashanti Region. Fresh stem cuttings 20 – 30 cm with 5 – 8 nodes were obtained from disease-free matured cassava plants. They were planted in loamy soil for a period of 5 weeks in plastic pots at the CSIR-Crops Research Institute screen house. The growing conditions used were 30 °C day temperature, 24 °C night temperature, 12 h day light, and 55 per cent relative humidity in December 2008. The

Table 1

Five Populations, "CRI", "Professor Safo Kantanka", "Professor J. P. Tettey", "SARI" Released Materials and Local Cultivars Involved in the Diversity Studies

Population	*Members*	*Constituting cultivars*
Population 1	Crops Research Institute (CRI) released materials	Agbelifia, Essam Bankye, Afisiafi, Doku Duade, Bankye Hemmaa
Population 2	Professor Safo Kantaka released materials	IFAD and Nkabom
Population 3	Professor J. P. Tettey released materials	UCC andBankye Botan
Population 4	Savanna Agriculture Research Institute (SARI) released materials	Filindiiakoh and Nyerikobga
Population 5	Local cultivars	Akosua Tumtum and Debor

experiment was repeated in January 2009 (27 °C average day temperature, 22 °C average night temperature, 12 h average day light and 55 per cent relative humidity). The plants were watered daily with tap water.

DNA isolation, PCR and gel electrophoresis

Genomic DNA was isolated according to Egnin, Mora & Prakash (1998) isolation protocol adopted by the CSIR-CRI molecular laboratory with some modifications. DNA quality was determined on 0.8 per cent agrose gel stained with ethidium bromide. 10 µ1 PCR reaction mixture sample [1.0µ1 of (10×), 0.9 µ1 of MgC12 (25 m*M*), 0.4µ1 of dNTPs (10 m*M*), 0.25 µl of both forward and reverse primer (10 µ*M*), 0.125 µ1 Taq polymerase (5 U), 1.0 µ1 of genomic DNA template (10 ng/µ1) all together with 6.075 µl of nuclease free PCR water] were prepared. Amplification was performed for 30 cycles in MyCycler thermal cycler (Bio-Rad Laboratories Inc.) with heated lid to reduce evaporation. The 36 SSR markers used were in three cycling programmes, namely Yucadiv, MicroBC1 and NewBC1 (Table 2).

Cycling profiles

Yucadiv [95 °C for 2 min (initial denaturation), 30 cycles of the following steps; 94 °C for 30 sec (denaturation), 55 °C for 1 min (primer annealing), 72 °C for 1 min (extension) then 72 °C for 5 min (final extension) and storage at 4 °C]; MicroBC1 [94 °C for 2 min (initial denaturation), 30 cycles of the following steps: 94 °C for 1 min (denaturation), 55 °C for 30 sec (primer annealing), 72 °C for 1 min (final denaturation), 55 °C for 1 min (final primer annealing), 72 °C for 1 min (final extension) and storage at 4 °C].

Amplified DNA fragments (8 µ1) were separated on 6 per cent PAGE gel [12% acrylamide solution (19:1), TBE (12×) 0.4% APS, TBE (12×) 0.4% APS, TEMED and filtered autoclaved distilled water (FADW)] at 200 volts for 35 min in TBE (1×) using a mini-protean 3 cell electrophoretic apparatus. A 100 bp DNA maker (0.05 µg/µ1, 25 µg) (invitrogen) was used as a standard and

TABLE 2

SSR Sequences and their Thermocycler Programmens

SSR Locus	*Left primer sequence*	*Right primer sequence*	*Thermocracycler programme*
SSRY 4	ATAGAGCAGAAGTGCAGGCG	CTAACGCACACGACTACGGA	MicroBCI
SSRY 9	ACAATTCATCATGAGTCATCAACT	CCGTTATTGTTCCTGGTCCT	MicroBC1
SSRY 12	AACTGTCAAACCATTCTACTTGC	GCCAGCAAGGTTTGCTACAT	MicroBC1
SSRY 19	TGTAAGGCATTCCAAGAATTATCA	TCTCCTGTGAAAAGTGCATGA	NewBC1
SSRY 20	CATGGACTTCCTACAAATATGAAT	TGATGGAAAGTGGTTATGTCCTT	MicroBC1
SSRY 21	CCTGCCACAATATTGAAATGG	CAACAATTGGACTAAGCAGCA	MicroBC1
SSRY 34	TTCCAGACCTGTTCCACCAT	ATTGCAGGGATTATTGCTCG	MicroBC1
SSRY 38	GGCTGTTCGTGATCCTTATTAAC	GTAGTTGAGAAAACTTTGCATGAG	MicroBC1/ NewBC1
SSRY 51	AGGTTGGATGCTTGAAGGAA	GGATGCAGGAGTGCTCAACT	MicroBC1/ Yucadiv (Yu)
SSRY 52	GCCAGCAAGGTTTGCTACAT	AACTGTCAAACCATTCTACTTGA	MicroBC1/Yu
SSRY 59	GCAATGCAGTGAACCATCTTT	CGTTTGTCCTTTCTGATGTTC	MicroBC1
SSRY 63	TCCAGAATCATCTACCTTGGCA	AAGACAATCATTTTGTGCTCCA	MicroBC1/Yu
SSRY 64	CGACAAGTCGTATATGTAGTATTCAG	GCAGAGGTGGCTAACGAGAC	MicroBC1/Yu
SSRY 69	AGATCTCAGTCGATACCCAAG	ACATCCGTTGCAGGCATTA	NewBC1(Ne)
SSRY 82	TGTGACAATTTTCAGATAGCTTCA	CACCATCGGCATTAAACTTG	MicroBC1/Yu
SSRY 100	ATCCTTGCCTGACATTTTGC	TTCGCAGAGTCCAATTGTTG	NewBC1
SSRY 102	TTGGCTGCTTTCACTAATGC	TTGAACACGTTGAACAACCA	NewBC1
SSRY 103	TGAGAAGGAAACTGCTTGCAC	CAGCAAGACCATCACCAGTTT	NewBC1
SSRY 105	CAAACATCTGCACTTTTGGC	TCGAGTGGCTTCTGGTCTTC	NewBC1
SSRY 106	GGAAACTGCTTGCACCAAAGA	CAGGCAAGACCATCACCAGTTT	NewBC1
SSRY 108	ACCCTATGATGTCCAAAGGC	CATGCCACATAGTTCGTGCT	MicroBC1/Yu
SSRY 110	TTGAGTGGTGAATGCGAAAG	AGTGCCACCTTGAAAGAGCA	NewBC1
SSRY 135	CCAGAAACTGAAATGCATCG	AACATGTGCGACAGTGATTG	Yucadiv
SSRY 147	GTACATCACCACCAACGGGC	AGAGCGGTGGGCGAAGAGC	Yucadiv
SSRY 148	GGCTTCATCATGGAAAAACC	CAATGCTTTACGGAAGAGCC	Yucadiv
SSRY 151	AGTGGAAATAAGCCATGTGATG	CCCATAATTGATGCCAGGTT	NewBC1
SSRY 155	CGTTGATAAAGTGGAAAGAGCA	ACTCCACTCCCGATGCTCGC	Yucadiv
SSRY 161	AAGGAACACCTCTCCTAGAATCA	CCAGCTGTATGTTGAGTGAGC	Yucadiv
SSRY 164	TCAAACAAGAATTAGCAGAACTGG	TGAGATTTCGTAATATTCATTTCACTT	NewBC1
SSRY 169	ACAGCTTAAAAACTGCAGCC	AACGTAGGCCCTAACTAACCC	Yucadiv
SSRT 171	ACTGTGCCAAAATAGCCAAATAGT	TCATGAGTGTGGGATGTTTTTATG	NewBC1
SSRY 177	ACCACAAACATAGGCACGAG	CACCCAATTCACCAATTACCA	Yucadiv
SSRY 179	CAGGCTCAGGTGAAGTAAAGG	GCGAAAGTAAGTCTACAACTTTTCTAA	`NewBC1
SSRY 180	CCTTGGCAGAGATGAATTAGAG	GGGGCATTCTACATGATCAATAA	NewBC1
SSRY 181	GGTAGATCTGGATGGAGGAGG	CAATCGAAACCGACGTACA	Yucadiv
SSRY 182	GGAATTCTTTGCTTATGATGCC	TTCCTTTACAATTCCTGGACGC	Yucadiv

the DNA amplified fragments were visualised in gel by silver nitrate staining.

Genetic diversity determination

The PCR amplified DNA fragments were separated on PAGE gel (Fig. 1). The bands were scored as codominant (each separate allele at a locus was given a score that relates to its size), and diploid (where each individual scores at a locus consisted of two

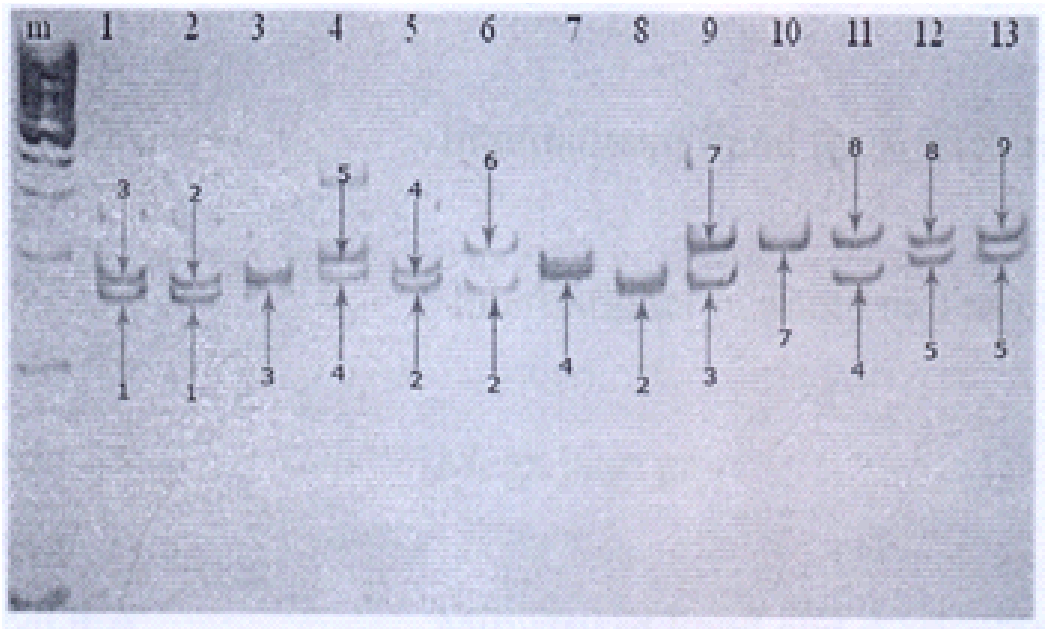

Fig. 1. Six per cent polyacrylamide gel showing silver stained PCR amplified allelic fragments of locus SSRY 59 for 11 released and two local cassava cultivars. The SST amplified bands were scored as diploid (each individual cultivar score at a locus consisted of two digits) and codominant (each separate allele at a locus was given a score that relates to its size) by visual inspection and "climbing ladder" approach. For TFPGA analysis, cultivar eight and 10 (Debor and Afisiafi) for example were scored as 0202 and 0707, respectively. On the other hand cultivar 6 (Essam bankye) was represented as heterozygous for alleles two and six and this was scored as 0206. However, in FSTAT analysis the scoring 0202 and 0206 would be recorded as 22 and 26, respectively.
M(100 bp marker), 1 (UCC), 2 (IFAD), 3 (Agbelefia), 4 (Nyerikobga), 5 (Nkabom), 6 (Esaam Bankye)' 7 (Akosua Tumtum), 8 (Debor)' 9 (Filindiakoh), 10 (Afisiafi), 11 (Doku Duade), 12 (Bankye Hemaa), and 13 Bankye Botan).

digits) by visual inspection, where a digital camera was used to take shots of the gels and images were scored on a computer (using Microsoft word).

The ladder method was employed where bands were scored in sequence of "climbing a ladder", and the genotype of an individual at a locus was represented by a two digit number for both F-statistics (FSTAT) and tools for population genetic analysis (TFPGA) (Fig. 1). The first digit corresponds to an allele seen on a chromosome while the second digit corresponds to the alternate allele, with a higher bp than the first allele observed at that same locus.

The bands scored data was analysed for descriptive statistics parameters [number of alleles per locus, allelic richness, allelic frequency, per cent polymorphic loci, Hardy-Weinberg equilibrium, gene diversity per locus per population and Nei's (1978) estimation of herozygosities, F-statistics and cluster analysis, and Nei's (1978) genetic distance]. With the exception of Hardy-Weinberg equilibrium, cluster analysis, Nei's (1978) genetic distance, and F-statistics which were analysed using TFPGA version 1.3 (Miller, 1997), all other analysis

were carried out with FSTAT version 9.3.2 (Goudet, 1995).

Results

A total of 35 out of the 36 primers, representing 97.2 per cent, produced clear and scorable bands in electrophoretic gels. Amplification at locus SSRY 177 failed in all the triplicate reactions. 'Afisiafi' generated no products at locus SSRY 38, 106 and 164. Similarly, at locus SSRY 179 and 180 no PCR products were observed for 'Agbelifia' cultivar. Again, there were no successful PCR products in 'Nkabom' cultivar at locus SSRY 52. For a total of 13 cultivars analysed, all the 35 microsatellite loci were found to be polymorphic.

The number of alleles observed per locus at all the 35 SSR loci analysed varied from three to 11 alleles with a mean of seven alleles per locus. The number of alles per polymorphic population recordes a range of 66 – 163 with an average of 101.4, whereas the percentage of polymorphic loci population also varied from 68.6 per cent to 100 per cent and averaging at 88.58 per cent (Table 3).

Nei's diversity indices estimation is shown in Table 4. A low value of 0.497 was obtained for the observed heterozygosity (Ho), whereas the expected heterozygosity, (He), on the other hand, recorded a high value of 0.769. However, a high total heterozygosity (Ht) of 0.814 was observed in all the 13 cassava cultivars. The results also established inter population gene diversity (Dst) of 0.044 and coefficient of genetic differentiation (Gst) value of 0.054.

A heterozgote deficit of 0.37 was observed. Hardy-Weinberg equilibrium also recorded 0.273. The unbiased heterozygosities of the individual population, which recorded values ranging from 0.4810 to 0.800 (Table 5). The local cultivars recorded the lowest heterozygosity while Prof. Tettey released materials recorded the highest. Nei's (1978) genetic distance ranged from 0.1440 to 1.0057 (Table 6), whereas the genetic diversity revealed a high genetic variability range of 0.500 – 0.943 (Table 7).

Table 3

Number of Alleles, Percentage Polymorphic Loci

Population	*Members*	*Total number of alleles per population*	*Percentage of polymorphic loci per population*
Population 1	Crops Research Institute (CRI) released materials	163	100
Population 2	Professor Safo Kantaka released materials	86	80.0
Population 3	Professor J. P. Tettey released materials	99	100
Population 4	Savanna Agriculture Research Institute (SARI) released materials	93	94.3
Population 5	Local cultivars	66	68.6
Mean		101.4	88.58

Table 4

Nei's Estimation of Heterozygosity

Observed heterozygosity (Ho)	*Expected heterozygosit (He)*	*Total heterozygosity (Ht)*	*Interpopulation gene diversity (Dst)*	*Coefficient of genetic differentiation (Gst)*
0.497	0.769	0.814	0.044	0.054

Table 5

Unbiased Heterozygosity of Individual Populations

Pop.1	0.795
Pop. 2	0.643
Pop. 3	0.800
Pop. 4	0.729
Pop. 5	0.481n
Mean	0.699

Table 7

Gene Diversity per Locus and Population

Pop1	*Pop 2*	*Pop 3*	*Pop 4*	*Pop 5*
0.835	0.676	0.943	0.850	0.500

Table 6

Nei (1978) Unbiased Distance

Population	*1*	*2*	*3*	*4*	*5*
1	*****				
2	0.5748	*****			
3	0.2732	.5541			
4	0.1440	0.3408	0.4053	*****	
5	0.6477	0.3408	1.0057	0.5197	*****

Table 8 shows results obtained for the Wright' F-statistics analysis. The results established a high correlation of genes within individuals (Fit), heterozgote deficit among individuals within each population (Fis), and a high occurrence of cross pollination (C) at 0.404, 0.372 and 0.663, respectively. The correlation of genes of different individuals in the same population (Fst), and the fixation index coefficient (F) obtained were,

Table 8

Wright's F-statistics

Fit	*Fst*	*Fis*	*F*	*Nm*	*C*
0.404	0.052	0.372	0.373	1.028	0.663

Means obtained for T-statistics parameters.
The pararameters analysed were correlation of genes within individuals overall population (Fit), in the same population (Fst), heterozgote deficit observed in individuals with each population (Fis), fixation index coefficient (F), estimation of gene flow (Nm) and frequency of cross pollination (C).

however, low at 0.052 and 0.373. The analysis also shows a low occurrence (1.028) of inter population gene flow (Nm).

Discussion

In the study, high genetic variability was detected in all the five cassava populations, with an average of 7.11 alleles per locus. The study showed a higher genetic variability among the cultivars compared to that reported by Okai *et al*. (2003). In cassava diversity studies involving 320 landraces in Ghana and using 33 SSR loci, reported variability was too low. Muhlen *et al*. (2000), Faraldo *et al.* (2000), Mkumbira, Martins & Ando (2003), Elias *et al.* (2004) and Lokko *et al.* (2006) also observed a maximum of five allele per locus which was much lower than the reported value of 7.11. The high variability of trait observed may be attributed to high tendency of variation in response to environmental and genetic influences (Wagner, 1995). This is, however, very important for enrichment of biodiversity.

The high number of alleles per locus and its corresponding high number of alleles per population averaging at 101.4, contributed to the high polymorphic loci observed (88.58%). A collection of cassava cultivars with such high values is excepted to have wide genetic distances between their constituting cultivars. Nei (1978) genetic distance of 0.1440 – 1.0057 was, however, expected. This wide genetic distance implies that the cassava varieties from the five populations used in the study possess very diverse genetic backgrounds. Extensive diversity in their progenitor is most likely source of genetic variations in the collection considering the young age of the varieties. The large genetic distances observed support effective breeding of new varieties from the existing ones. They have very high potential to be used as progenitors in breeding programmes in Ghana.

The study also established that the probability of any two randomly selected alleles from any given population could be different, (He) 0.769, was 76.9 per cent. This value was observed to be higher than what was obtained in other related works on cassava (Lokko *et al*., 2006; Fregene *et al.*, 2003), and again far higher than what have been observed in outcrossing species of dicots (0.159) and all other plant species (0.205) (Hamrick & Godt, 1997), and substantiate both the outcrossing breeding system and the higher heterozygous nature of cassava due to its vegetative mode of reproduction. These, notwithstanding, Nei's estimation of observed heterozygosity, recorded a smaller value of 49.7 per cent resulting in a heterozygote deficit of 27.2 per cent among the cultivars. The F-statistics analysis, Fis 0.372, Fit 0.404 and F at 0.373 confirmed the observed deficit (Gehring & Lindhart, 1992; Dolan *et al*., 1999). However, the deviation from Hardy-Weinberg equilibrium (0.273), indicating excess of homozygosity and existence of a non-random mating among the cultivars, explained the low observed heterozygosity which resulted in the 27.2 per cent loss of genetic diversity. This could be as a result of the fact that there is no known genetic self-incompatibility system in cassava, although inflorescences are metandrous, whereas the mechanism of seed dispersal in cassava by explosive mechanism does not promote long-distance gene flow. Both of these reasons could favour inbreeding.

This observation is, however, a threat to cassava breeding in Ghana because of the

possibility of narrowing the Ghanaian cassava genetic base. A narrow genetic base restricts the progress of cultivar improvement by compounding the difficulty involved in choosing parental materials for breeding. This observation could also be of great concern to breeders as a narrow genetic base is undesirable for breeding, because a certain level of parental divergence is needed to create productive hybrids.

Though Nei (1978) genetic diversity revealed high genetic variability (0.500 – 0.94.3) among the populations, a large intra population diversity was, however, recorded (HS = 0.552) as compared to inter population diversity (low Gst 0.054, FST 0.052 and Dst 0.044). This suggests that the populations studied have a low rate of inter population gene flow through seed and pollen. Faraldo *et al*. (2000), Mühlen *et al*. (2000), Asante & Offei (2003), Peroni, Kageyama & Begossi (2007) and Lokko *et al.* (2006) also observed similar results and recorded a higher intra genetic variability in their studies on cassava. The same pattern was also observed with sweet potato landraces from the Vale do Ribeira (Veasey *et al.*, 2008). On the contrary, the high genetic variation within Ghanaian released cassava populations indicates the usefulness of each population as a valuable genetic resource for the selection of superior genotypes, as seen in the case of CRI released materials, Prof. J. P. Tettey released materials and Prof. Safo Kantanka released materials showing 83.5, 85.0 and 94.3 per cent genetic diversity, respectively.

Conclusion

The study of five cassava populations, made up of 11 released and two local cultivars, using SSR makers, established the presence of high allele per locus (7.11 alleles), high alleles per population (101.4) and high polymorphic loci (88.58%), indicating the presence of high SSR allele polymorphism in the cassava populations. The estimated genetic diversity revealed an intra-population diversity as indicated by the large intra population diversity (HS = 0.552) value obtained.

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