Research article

Genetic diversity and population structure analysis of Ghanaian and exotic cassava accessions using simple sequence repeat (SSR) markers

Joseph Adjebeng-Danquah a,⁎, Joseph Manu-Aduening b, Isaac Kwadwo Asante c, Richard Yaw Agyare b, Vernon Gracen a, Samuel Kwame Offei c

a CSIR-Savanna Agricultural Research Institute, P. O. Box TL 52, Tamale, Ghana
b CSIR-Crops Research Institute, Fumesua, P.O. Box 3785, Kumasi, Ghana
c West Africa Centre Crop Improvement (WACCI), University of Ghana, Legon, Accra, Ghana

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ABSTRACT

Genetic diversity is fundamentally important in crop improvement and provides plants with the capacity to meet the demands of changing environments. This work was carried out to assess the diversity and the extent of genetic relatedness among a number of assembled cassava (Manihot esculenta Crantz) accessions. We conducted a microsatellite marker analysis of 89 cassava accessions collected from Ghanaian and exotic sources. These accessions were assayed using 35 simple sequence repeat (SSR) markers. A total of 167 alleles were detected from 35 polymorphic markers with an average of 4.77 alleles per locus. High allelic frequency was detected across the accessions, ranging from 0.32 to 0.99 with an average of 0.62 per marker. Observed heterozygosity ranged from 0.03 - 0.97 across the accessions. Polymorphism information content (PIC) ranged from 0.03 to 0.78 with a mean of 0.45, indicating high level of polymorphism across the accessions. Comparatively, higher number of alleles, gene diversity and observed heterozygosity were detected among the local accessions compared with the exotic accessions indicating rich genetic diversity among them. Population structure analysis based on STRUCTURE identified two subpopulations and a large number of admixtures. Cluster analysis based on the neighbour joining algorithm further separated the collection into seven sub-groupings irrespective of geographical origin. This indicates the possible sharing of common genomic regions occurring across the accessions. High allelic frequency differences and levels of heterozygosity were observed among the germplasm. These findings indicated significant genetic variability in the germplasm to warrant selection.

1. Introduction

Cassava (Manihot esculenta Crantz) is an important staple crop, widely cultivated and consumed in Sub-Saharan Africa. It is mostly grown by smallholder farmers often in marginal ecologies due to its ability to give better and appreciable yields than most staple crops in ecologies of drought and poor soils [1, 2]. The crop is fast gaining popularity as an important industrial raw material in Ghana, leading to its widespread cultivation particularly in the savannah ecologies [3, 4]. However average yields at farm level are low (8t ha⁻¹) [5], compared with a potential yield of 90 t/ha under good agronomic management [2]. This could be due to the use of low yielding varieties that are susceptible to pests and diseases, as well as high sensitivity to harsh environmental conditions [6]. Thus, there is the need to develop new improved varieties that are adapted to these environments.

Progress made in a breeding programme depends on a better understanding of the genetic variability present in the population assembled [7]. In some cases, the genetic base is broadened through hybridisation with wild and related species [8], plant introductions from external sources or locally assembled germplasm [9, 10]. Local germplasm, particularly adapted landraces from farmers’ fields, are valuable genetic resources for crop improvement [11, 12]. Significant genetic variability has been reported across the cassava gene pool for traits associated with tolerance to harsh environments (such as drought) [2, 13] which can be exploited.

Several studies have reported the existence of diverse germplasm of crops on farmers fields as a result of in situ germplasm conservation and farmer to farmer planting materials transfer [14]. Farmers keep a wide range of crop varieties to provide harvest security, yield stability and the possibility to adapt to changing ecological conditions [15, 16]. Through the exchange of planting materials among farmers [17], accessions are

⁎ Corresponding author.
E-mail address: barchus2003@yahoo.com (J. Adjebeng-Danquah).

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Table 1. List of cassava accessions used for the study.

| No. | Accessions     | Source     | No. | Accessions     | Source     |
|-----|----------------|------------|-----|----------------|------------|
| 1   | AFS2000/023    | Local      | 31  | Nkoranza       | Local      |
| 2   | AFS2000/043    | Local      | 32  | SAA 007        | Local      |
| 3   | AFS2000/131    | Local      | 33  | SAA 004        | Local      |
| 4   | ATR002         | Local      | 34  | TAP7/054       | Local      |
| 5   | ATR007         | Local      | 35  | UCC2001/449    | Local      |
| 6   | Bankyebrodie   | Local      | 36  | UCC2001/464    | Local      |
| 7   | BD96/009       | Local      | 37  | Kwasiaberdi    | Local      |
| 8   | BD96/021       | Local      | 38  | 00/0140        | IITA       |
| 9   | BD96/040       | Local      | 39  | 00/0203        | IITA       |
| 10  | BD96/093       | Local      | 40  | 00/0338        | IITA       |
| 11  | BD96/154       | Local      | 41  | 00/0354        | IITA       |
| 12  | TAP7/137       | Local      | 42  | 00/0364        | IITA       |
| 13  | UCC2001/104    | Local      | 43  | 01/0046        | IITA       |
| 14  | UCC2001/111    | Local      | 44  | 01/0069        | IITA       |
| 15  | BAN 001        | Local      | 45  | 01/0093        | IITA       |
| 16  | AWA 004        | Local      | 46  | 01/0114        | IITA       |
| 17  | BIBASSE *      | Local      | 47  | 01/0134        | IITA       |
| 18  | Debor          | Local      | 48  | 01/0169        | IITA       |
| 19  | DMA 005        | Local      | 49  | 01/0220        | IITA       |
| 20  | Esiabaya        | Local      | 50  | 01/1088        | IITA       |
| 21  | KSI2000/092    | Local      | 51  | 01/1412        | IITA       |
| 22  | KSI2000/126    | Local      | 52  | 02/0540        | IITA       |
| 23  | KSI2000/191    | Local      | 53  | 191/02324      | IITA       |
| 24  | KW2000/53      | Local      | 54  | 2000/0388      | IITA       |
| 25  | Kwanwoma       | Local      | 55  | 94/0006        | IITA       |
| 26  | NWA 004        | Local      | 56  | 96/0708        | IITA       |
| 27  | OFF2000/019    | Local      | 57  | 96/1708        | IITA       |
| 28  | OFF2000/023    | Local      | 58  | 96/409         | IITA       |
| 29  | OFF2000/145    | Local      | 59  | 97/1856        | IITA       |
| 30  | Ponti*         | Local      | 60  | 98/2132        | IITA       |

* Farmer preferred varieties, Local = accessions collected locally from farmers’ field and research stations in Ghana, IITA = accessions obtained from International Institute of Tropical Agriculture (IITA), CIAT = International Centre for Tropical Agriculture (CIAT).

given unique names at their new location [18]. The same genetic materials are often given different names resulting in duplicates when germplasm are assembled from such localities [19, 20, 21, 22]. This results in underestimation or overestimation of the actual genetic diversity present in the population [23].

Ability to remove duplicates during characterization of germplasm collections for breeding activities is very important. Characterization of crops can be done using agronomic, phenotypic descriptors or molecular markers [7, 24]. Phenotypic characterization is easy, rapid to score, and provides useful preliminary information for diversity assessment of germplasm [5, 24, 25]. Phenotypic descriptors have been used to effectively characterise several crop genotypes, particularly in stressful environments [5, 26, 27, 28]. However, most phenotypic descriptors particularly quantitative characters, are not very reliable as markers due to strong influence by genotype - environment interaction [29]. For this reason, analysis of diversity at the molecular level is used to validate and/or complement phenotypic characterization [5, 30].

Molecular markers are segments of DNA that can represent different functional classes and have been widely used to estimate genetic variation among different populations [31, 32]. An ideal molecular marker must be highly heritable, applicable to any part of the genome and polymorphic enough to enable the discrimination of closely related genotypes [31, 33]. Molecular markers are easily detectable and stable in plant tissues irrespective of environmental influence [5, 32, 33]. Available molecular markers for assessing genetic diversity include random amplified polymorphic DNA (RAPD) [34]; amplified fragment length polymorphism (AFLP) [33]; allozymes [35], simple sequence repeats (SSRs) [36]; single nucleotide polymorphism markers (SNPs) [37, 38] and diversity array technology markers (DArT) [39]. Specifically, SSRs are the markers of choice for genetic diversity analysis due to their abundance in the genome, highly polymorphism and codominant nature, which make them useful for characterizing heterozygote plants such as cassava [36]. SSR markers have been successfully used to assess genetic diversity between cassava accessions and their wild relatives [5, 40]. The objective of this study was to characterize and assess the extent of genetic diversity among 89 cassava accessions collected from Ghanaian and exotic sources using simple sequence repeat (SSR) markers.

2. Materials and methods

2.1. Plant materials

The study involved 89 cassava accessions obtained mainly from farmers’ field in Ghana, CSIR-Crops Research Institute (Kumasi, Ghana), International Centre for Tropical Agriculture (Cali, Colombia), CSIR-Savanna Agricultural Research Institute (Nyankpala, Ghana) and International Institute of Tropical Agriculture (Ibadan, Nigeria). Names of the various accessions and the place of collection are presented in Table 1.

2.2. Simple sequence repeat marker analysis

2.2.1. Genomic DNA extraction

DNA extraction was done at the Biotechnology Laboratory of CSIR-Savanna Agricultural Research Institute (SARI), Nyankpala. Genomic
DNA was extracted from the 89 cassava accessions using CTAB method [41] with slight modifications. Leaves were sampled from two weeks old cassava cuttings which were raised in pots for the DNA extractions. About 20 mg of the leaf sample from each plant was taken and ground in 2.0 ml Eppendorf tubes into fine powder with liquid nitrogen. Then 800 μl of 2% CTAB and 0.5 μl mercaptoethanol were added. The samples were incubated in a sand bath at 65°C overnight. Pelleting of nucleic acids was done by centrifuging at 14000 rpm for 15 min. Pellet was washed in 500 μl of washing buffer. The washing buffer contained 1.4 M NaCl, 20 mM Tris-HCl (pH 8.0) and 10 mM EDTA. The washing buffer was decanted and the pellet was washed in 400 μl of 0.1 M Tris-Cl (pH 8.0) and 0.8 M NaCl. The washed pellets were resuspended in 50 μl of TE buffer and then centrifuged at 14000 rpm for 15 min. Equal volumes of the chloroform isoamyl alcohol solution were added to the samples in clean 1.5 ml Eppendorf tubes and centrifuged at 14000 rpm for 15 min. Nucleic acids were precipitated by adding two thirds volume of ice cold isopropanol (100 μl) whilst shaking gently. Precipitation was enhanced by storing the samples at −20°C overnight. The samples were cooled at room temperature after which equal volumes (800 μl) of chloroform isoamyl alcohol (24:1) were added. The tubes were inverted several times to ensure that a thorough mixture was obtained and then centrifuged at 14000 rpm for 15 min. Equal volumes of the chloroform isoamyl alcohol solution were added to the samples in clean 1.5 ml Eppendorf tubes and centrifuged at 14000 rpm for 15 min. Nucleic acids were precipitated by adding two thirds volume of ice cold isopropanol (400 μl) whilst shaking gently. Precipitation was enhanced by storing the samples at −20°C overnight. Pelleting of nucleic acids was done by centrifuging at 14000 rpm for 5 min. The isopropanol was decanted and the pellet was washed with 500 μl of washing buffer. The washing buffer was decanted and the pellet was washed in 400 μl of ethanol (80%) and then centrifuged at 6000 rpm for 4 min. The ethanol was decanted and the pellet was dried. The DNA was suspended in 100 μl of TE buffer and centrifuged at high speed for 30 s and stored at 4°C until ready for use.

DNA of each accession was confirmed by electrophoresis on 2% agarose gel stained with ethidium bromide (3 μl) which revealed positive results.

### 2.2.2. SSR (microsatellite) markers and PCR amplification

A total of 35 simple sequence repeat (SSR) primers, widely distributed across the cassava genome [42], were used for the study (Table 2). The SSR markers were synthesized at Metabion International AG (Germany). Polymerase chain reactions (PCR) were carried out in a Techne Thermo cycler (TC-412) in a 10 μl reaction mixture in 96-well plates. PCR master mix kits (KAPA 2G Fast ReadyMix with dye) procured from KAPA Biosystems (Pty) Ltd (South Africa) were used for the amplification. The kit 2X PCR master mix contained KAPA2 Fast DNA Polymerase (0.2 U per 10 μl reaction), KAPA2 Fast PCR buffer, dNTPs (0.2 mM each at 1X), MgCl2 (1.5 mM at 1X), stabilizers and loading dye. An amount of 1 μl of genomic DNA and 0.5 μl of each of forward and reverse primers were added to the PCR kits for DNA amplification. PCR amplifications were done with the following conditions: initial denaturation at 95°C for 3 min, denaturing at 95°C for 10 s, annealing at X°C (annealing temperatures for each marker, Table 2) for 10 s and extension at 72°C for 10 s. The reaction was repeated for 35 cycles and a final extension at 72°C for 10 min was carried out. The reactions were then held at 4°C until electrophoresis.

### Table 2. Simple sequence repeat (SSR) primers used for the study.

| No | Primer name | Sequence (5’-3’) | Ta (°C) |
|----|-------------|-----------------|--------|
| 1  | NS 189      | TGGGCTTGTGTGTATCCTTA | 54     |
| 2  | NS 376      | TGAAGACCCTCTGCTGTGTTT | 55     |
| 3  | SSRY 4      | TCAGAGGAAACTGGTTCAC  | 55     |
| 4  | SSRY 5      | GGAACCTCGCCACGAAAGAAG | 51     |
| 5  | SSRY 9      | AATGCTCAACACACTCATTGCACGGTT | 53     |
| 6  | SSRY 12     | TCAACGCTATGTCGCTACGC | 54     |
| 7  | SSRY 19     | CCGAAGAACCTGAGATTCAGC  | 54     |
| 8  | SSRY 20     | GTGATACCAACACGGGGAAGCC | 52     |
| 9  | SSRY 21     | GCGTTATCATGAGAAAGGGG  | 52     |
| 10 | SSRY 34     | AGTGAGGAACCTGAGATTTCCG | 51     |
| 11 | SSRY 45     | CTGGATATACGAGGAAAGAC | 52     |
| 12 | SSRY 48     | AAGGAAACTGCTCTCCTGTAACATCA | 51     |
| 13 | SSRY 50     | TCAACGAGAACCTGAGATTTCCG | 54     |
| 14 | SSRY 59     | AGAGCTCAATGGCATGATCAGC | 52     |
| 15 | SSRY 63     | TGACTGACAGCATGCCTTCCA | 52     |
| 16 | SSRY 64     | ACACAACTTACGGCAAGGCAAG | 59     |
| 17 | SSRY 69     | CCTTGGAGAGATTAGTTCAGAAG | 54     |
| 18 | SSRY 72     | GGTAGATCAGTGGATCGAGGAG | 53     |
| 19 | SSRY 82     | GAGTACATGACAGAAGTGTTTCA | 53     |
| 20 | SSRY 101    | TGAATTTGACGAGTACAGTAC  | 50     |
| 21 | SSRY 106    | CTGAGTCCTCCACAAATATGATACAT | 52     |
| 22 | SSRY 120    | CCTGACAGATTTATGGATTGAG | 53     |
| 23 | SSRY 135    | TTAAGCAGCGTTTCCACCATAC | 51     |
| 24 | SSRY 147    | ATAGACCGAGAGTGGGTACGGG | 60     |
| 25 | SSRY 148    | TGAAAGGCCTGAAAAATAGAAG | 56     |
| 26 | SSRY 151    | TGGAATCTACGACGTTTATTT | 52     |
| 27 | SSRY 155    | TGAATATAATGGATGAGCCAAG | 57     |
| 28 | SSRY 161    | CGCCTTACTCCTGTTGCTTC  | 52     |
| 29 | SSRY 164    | GCAATGCTGAGAACCCTCCTT | 55     |
| 30 | SSRY 169    | TCAGATACATGACAGAAGTGTTTCA | 55     |
| 31 | SSRY 175    | GCACAGGTGTATATGATGTATCAGG | 56     |
| 32 | SSRY 177    | CGATCTAGGCTAGTAGCCCAAG | 53     |
| 33 | SSRY 180    | TGCAACAGGTGGTTTTCCCAT | 52     |
| 34 | SSRY 181    | TGTGACATTTTCCATGATGCTCTCA | 55     |
| 35 | SSRY 182    | ACAATTTCTACATGACTGATCACAATC | 53     |

Ta (°C) = Annealing temperature. Sources of SSR markers [40, 42].
The DNA bands were scored based on the fragment length of each allele. The assignment of the fragment was based on its position relative to the 50 and the 100 bp standard molecular marker (DNA Ladder) used. Alleles were scored as present (1) or absent (0). Band sizes for each marker per genotype were scored as a/b where 'a' is the upper band and 'b' is the lower band. PowerMarker version 3.25 [43] was subsequently used to detect allele frequency, allele number per locus, gene diversity, observed heterozygosity and polymorphism information content (PIC) for each marker across all the 89 accessions. Analysis of molecular variance (AMOVA) was performed to distinguish the molecular genetic variance within and among populations using GenAlEx6.502 software [44]. For the population structure analysis, the data from the 35 polymorphic SSR markers was imperiled to population structure analysis based on the admixture model clustering method in the software package STRUCTURE 2.3.4 [45]. This model was run by varying the number of assumed population (K) from 1 to 12 with 5 alterations for each K. A burn-in period of 10 000 and Markov Chain Monte Carlo (MCMC) replications of 20,000 after each burn-in was used. The optimum population (K) which best estimated the structure of the 89 accessions was predicted using the Evanno's method [46] through the online based software STRUCTURE HARVESTER [47]. The model was repeated for the K at maximum ΔK with a burn-in period of 100,000 and an MCMC of 200,000 after each burn-in with one alteration. The accessions were assigned to each subpopulation based on their probability of association of ≥60% to each of the two groups, accessions with probability of association <60% were considered as admixtures. Genotypic associations (cluster analysis) were analysed in DARwin 5 [48] using the simple matching coefficient and neighbour-joining algorithm. To ensure reliability of the results, 10,000 bootstraps were performed in the construction of the dendrogram.

3. Results

3.1. Allelic diversity

A total of 167 alleles were generated by the 35 SSR markers (Table 3). Allele frequency ranged from 0.32 to 0.99 for SSRY-164 and SSRY-48, respectively, with a mean of 0.62. SSRY-169 had the highest number of polymorphic bands (86) whilst SSRY-120 had the lowest (9) among the genotypes. Allele number per locus ranged from 2 to 10 with a mean of 4.77 alleles per locus. Gene diversity varied from 0.03 to 0.81, with primers SSRY-48 and SSRY-164 having the lowest and highest gene diversity, respectively. Primer SSRY-180 had the highest observed heterozygosity of 0.97 while primer SSRY-48 had the lowest of 0.03. Polymorphism Information Content (PIC) ranged from 0.03 to 0.78 with a mean of 0.45. Primer SSRY-164 was the most polymorphic with a PIC value of 0.78.

3.2. Within and between populations diversity

The analysis of molecular variance (AMOVA) based on the molecular data indicated higher within group variation which accounted for 97% of the total variation compared with variation between groups which accounted for only 3% of the total variation (Table 4). Comparatively, the local accessions had the highest PIC (0.44) and were also found to be the

Table 3. Results of the genetic diversity parameters for each of the 35 SSR loci analysed across 89 cassava accessions.

| Marker   | Allele frequency | No. of polymorphic bands | Allele number per locus | Gene diversity | Hs | PIC       |
|----------|------------------|--------------------------|-------------------------|----------------|----|-----------|
| NS-189   | 0.67             | 41.00                    | 5.00                    | 0.49           | 0.24 | 0.43     |
| NS-376   | 0.66             | 54.00                    | 5.00                    | 0.52           | 0.50 | 0.48     |
| SSRY-4   | 0.68             | 39.00                    | 3.00                    | 0.47           | 0.54 | 0.40     |
| SSRY-5   | 0.45             | 22.00                    | 3.00                    | 0.59           | 0.36 | 0.51     |
| SSRY-9   | 0.33             | 18.00                    | 6.00                    | 0.76           | 0.44 | 0.72     |
| SSRY-12  | 0.52             | 68.00                    | 5.00                    | 0.53           | 0.24 | 0.43     |
| SSRY-19  | 0.34             | 51.00                    | 6.00                    | 0.76           | 0.59 | 0.72     |
| SSRY-20  | 0.70             | 48.00                    | 7.00                    | 0.49           | 0.35 | 0.47     |
| SSRY-21  | 0.60             | 56.00                    | 4.00                    | 0.57           | 0.48 | 0.51     |
| SSRY-34  | 0.92             | 75.00                    | 3.00                    | 0.15           | 0.11 | 0.14     |
| SSRY-45  | 0.57             | 57.00                    | 5.00                    | 0.57           | 0.75 | 0.51     |
| SSRY-48  | 0.99             | 70.00                    | 3.00                    | 0.03           | 0.03 | 0.03     |
| SSRY-50  | 0.60             | 71.00                    | 6.00                    | 0.59           | 0.30 | 0.54     |
| SSRY-59  | 0.47             | 38.00                    | 5.00                    | 0.57           | 0.18 | 0.48     |
| SSRY-63  | 0.75             | 69.00                    | 5.00                    | 0.42           | 0.10 | 0.40     |
| SSRY-64  | 0.58             | 66.00                    | 5.00                    | 0.58           | 0.38 | 0.52     |
| SSRY-69  | 0.42             | 71.00                    | 6.00                    | 0.70           | 0.77 | 0.65     |
| SSRY-78  | 0.69             | 65.00                    | 5.00                    | 0.48           | 0.35 | 0.43     |
| SSRY-82  | 0.61             | 57.00                    | 3.00                    | 0.50           | 0.49 | 0.40     |
| SSRY-103 | 0.53             | 73.00                    | 6.00                    | 0.54           | 0.86 | 0.44     |
| SSRY-106 | 0.66             | 72.00                    | 5.00                    | 0.50           | 0.46 | 0.45     |
| SSRY-120 | 0.67             | 9.00                     | 3.00                    | 0.49           | 0.22 | 0.44     |
| SSRY-135 | 0.76             | 70.00                    | 3.00                    | 0.38           | 0.37 | 0.33     |
| SSRY-147 | 0.95             | 74.00                    | 3.00                    | 0.10           | 0.07 | 0.10     |
| SSRY-148 | 0.93             | 83.00                    | 2.00                    | 0.12           | 0.11 | 0.12     |
| SSRY-151 | 0.45             | 73.00                    | 8.00                    | 0.70           | 0.75 | 0.66     |
| SSRY-155 | 0.78             | 81.00                    | 5.00                    | 0.37           | 0.38 | 0.34     |
| SSRY-161 | 0.59             | 81.00                    | 6.00                    | 0.61           | 0.54 | 0.57     |
| SSRY-164 | 0.32             | 77.00                    | 10.00                   | 0.81           | 0.61 | 0.78     |
| SSRY-169 | 0.88             | 86.00                    | 5.00                    | 0.22           | 0.10 | 0.21     |
| SSRY-175 | 0.45             | 78.00                    | 4.00                    | 0.63           | 0.53 | 0.55     |
| SSRY-177 | 0.49             | 70.00                    | 4.00                    | 0.57           | 0.36 | 0.48     |
| SSRY-180 | 0.51             | 69.00                    | 5.00                    | 0.61           | 0.97 | 0.55     |
| SSRY-181 | 0.63             | 63.00                    | 3.00                    | 0.54           | 0.62 | 0.48     |
| SSRY-182 | 0.57             | 50.00                    | 5.00                    | 0.61           | 0.86 | 0.56     |
| Mean     | 0.62             | 61.29                    | 4.77                    | 0.55           | 0.43 | 0.45     |
| SE       | 0.17             | 13.42                    | 1.61                    | 0.19           | 0.24 | 0.16     |

The bold numbers presented on the row labelled Mean represents the mean values for the various marker details such as Allele frequency, number of polymorphic bands, allele number per locus, gene diversity, etc. This information was used to determine which molecular marker had above average allele frequency among the loc chosen for the study. The S.E. represents the standard error for the various marker details and were used to compare which marker was more informative and whether significant differences existed among the molecular markers. Hs = Observed heterozygosity, PIC = Polymorphism information content, SE = Standard Error.
Table 4. Analysis of molecular variance (AMOVA) of 89 cassava accessions from IITA, CIAT and Local sources.

| Source of variation | DF  | SS    | MS    | EV   | % variation | Stat | Value | Probability |
|---------------------|-----|-------|-------|------|-------------|------|--------|-------------|
| Among population    | 2   | 71.36 | 35.68 | 0.649| 3           |      |        |             |
| Within population   | 6   | 1559.89 | 18.13 | 18.138| 97          |      |        |             |
| Total               | 88  | 1631.25 | 18.788| 100  | PhiPT       | 0.035| 0.001  |             |

DF = Degrees of freedom, SS = Sum of squares, MS = Mean squares, EV = Estimated variance, *, ** = Significant at 5% and 1% probability respectively.

1 The probability is based on permutation across the full data set. PhiPT is a statistic measure for comparison between co-dominant data sets.

Table 5. Genetic diversity parameters among three populations of 89 cassava accessions from Ghana, IITA and CIAT.

| Population | Sample size | Allele frequency | No. of polymorphic bands | Allele number per locus | Gene diversity | H_o | PIC |
|------------|-------------|------------------|--------------------------|------------------------|----------------|-----|-----|
| IITA       | 40          | 0.65             | 27.09                    | 3.91                   | 0.47           | 0.42| 0.42|
| CIAT       | 12          | 0.71             | 8.97                     | 2.69                   | 0.40           | 0.37| 0.35|
| Local      | 37          | 0.62             | 25.23                    | 3.97                   | 0.49           | 0.44| 0.44|
| Mean       | 0.65        | 30.65            | 3.83                     | 0.47                   | 0.42           | 0.42| 0.42|

IITA = Accessions from the International Institute of Tropical Agriculture, CIAT = accessions from the International Centre for Tropical Agriculture, Local = accessions obtained from local sources in Ghana, H_o = Observed heterozygosity, PIC = Polymorphism information content.

Figure 1. A: Delta K (ΔK) values for different numbers of populations assumed (K) in the STRUCTURE analysis. B: Population structure of 89 cassava accession showing 2 sub population (Red indicates population 1 and Green population 2), each single line represents an accession. Population ID: 1 = IITA accessions, 2 = Local accessions, 3 = CIAT accessions.
most diverse with the highest observed heterozygosity, allele number per locus and gene diversity (Table 5). Allele frequencies recorded were 0.71, 0.65 and 0.62 for accessions from CIAT, IITA and Local collections, respectively. Allele number per locus observed were 3.97, 3.91 and 2.69 for Local, IITA and CIAT accessions, respectively. Similarly, gene diversity recorded were 0.49, 0.47 and 0.40 respectively for Local, IITA and CIAT accessions.

### 3.3. Population structure analysis

The population structure analysis of the 89 cassava accessions estimated that the optimum number of subpopulation $K$ which best explained the structure of the accessions was $2$ ($K = 2$) using the Evanno method (Figure 1). Majority of the accessions (62) were classified as admixtures at a probability of association of $\geq 60\%$. The rest fell into two subpopulations. Subpopulation one consisted of 14 accessions and was made up of nine accessions from Local sources, four accessions from IITA and one accession from CIAT (Table 6). Subpopulation two on the other hand had 13 accessions which included six local accessions, five from IITA and two from CIAT. The 62 admixtures consisted of 31 accessions from IITA, 22 local accessions and nine accessions from CIAT. The allele frequency divergence observed among the two subpopulations (1 and 2) was 0.0505 with observed heterozygosity of 0.5857 for the more heterogeneous subpopulation one and 0.3849 for subpopulation two. However, subpopulation two had a higher fixation index (0.3772) indicating a high genetic diversity. Subpopulation one on the other hand, showed very low genetic diversity with a fixation index of 0.0008.

Cluster analysis based on simple matching coefficient and neighbour-joining algorithm revealed seven ‘distinct’ clusters or sub-groupings with no apparent connections to the place of origin or collection of accessions (Figure 2). Specifically, cluster one includes 15 accessions, mainly dominated by accessions from IITA with only three local accessions (KW2000/53, Essiabayaa and Debor) and one from CIAT (CTSIA 48). Cluster two included 14 accessions from Local collections, IITA and CIAT. Cluster three included nine accessions: one from CIAT, two from IITA and six from Local collections. Out of the 12 genotypes in cluster four, only one came from IITA (TME 419) with no genotype from CIAT appearing in this cluster. Cluster five had 16 genotypes: three from Local collections, four from IITA and nine from CIAT. Cluster six which had a total of 16 accessions was dominated by accessions from IITA with only one genotype from CIAT. There were seven accessions in cluster seven: one from IITA, three from Local collections and three from the CIAT collections.

### 4. Discussion

The success of any crop breeding programme depends on the amount of genetic variability within the targeted traits for improvement and the extent to which these traits are heritable [49]. For this reason, assessment of genetic variability to aid parental line selections becomes a very important pre-breeding operation. Assessment of diversity at the molecular level can detect variations and/or markers linked to certain genomic regions, which is very reliable due to little or no environmental influence compared to phenotypic markers [50]. The level of genetic diversity is very important for the success or progress in breeding programmes [32]. The results of the current study reveal moderate to high levels of polymorphism across the 89 cassava accessions analysed, as was found for the genus *Manihot* at large [51]. The population structure analysis indicated two subpopulations and a large number of admixtures confirming the heterogeneous nature of the cassava population used.

The average number of primers required to adequately assess genetic diversity in crops differ and depend on the level of out-crossing within
the species [52]. Crop species that are highly inbred require larger number of primers than crops that are naturally heterogeneous, like cassava. The average number of 4.77 alleles per locus detected by the SSR primers (Table 3) is similar to earlier findings on genetic diversity analyses in cassava [40, 53] indicating that the SSR markers used in the current analysis were informative and appeared to be sufficient for the assessment of genetic diversity in the 89 cassava genotypes.

The observed heterozygosity levels (0.03–0.97, mean = 0.43) were higher than those found for 64 accessions of cassava with 26 SSR markers, where the heterozygosity levels ranged from 0.47 to 0.66 with a mean of 0.57 [40]. These lower levels of heterozygosity reported could be due to the lower numbers of both accessions and SSR markers used as compared to this study. The high level of heterozygosity observed within the accessions here could be attributed to three reasons. Firstly, the diverse geographical background (place of collection) of the accessions used in the current study. Specifically, the collections obtained from IITA could likely be made up of accessions originating from various geographical areas (countries) in West Africa. Likewise, the accessions obtained from CIAT are more likely to be originated from diverse geographical origins (countries) within the Latin American region. Secondly, there could be ‘secondary’ mixing of the gene pool from the CIAT and IITA collections through exchange of genetic materials and

### Table 6. Inferred subpopulation of the accessions showing their probabilities of association to each sub population based on fixation index.

| Accessions | Pop ID | Populations | Inferred subpopulation | Accessions | Pop ID | Populations | Inferred subpopulation |
|------------|--------|-------------|------------------------|------------|--------|-------------|------------------------|
| 00/0354    | 1      | 0.694       | 0.396                  | 1          | BIABASSE | 2           | 0.420                  |
| 96/1708    | 1      | 0.637       | 0.363                  | 1          | 96/409  | 2           | 0.447                  |
| DMA 005    | 2      | 0.653       | 0.347                  | 1          | BD96/093 | 2           | 0.444                  |
| CTSIA 48   | 3      | 0.654       | 0.346                  | 1          | Debor   | 2           | 0.448                  |
| 99/0240    | 1      | 0.658       | 0.342                  | 1          | Essiabaya | 2          | 0.420                  |
| BD 96/040  | 2      | 0.710       | 0.290                  | 1          | 00/0200 | 1           | 0.503                  |
| KW 2000/53 | 2      | 0.627       | 0.373                  | 1          | 00/0336 | 1           | 0.518                  |
| Kwanwoma   | 2      | 0.606       | 0.394                  | 1          | 01/0069 | 1           | 0.458                  |
| Nkoranza   | 2      | 0.616       | 0.384                  | 1          | 01/0093 | 1           | 0.537                  |
| SAA 004    | 2      | 0.659       | 0.341                  | 1          | TME435  | 1           | 0.540                  |
| OFP2000/019| 2      | 0.607       | 0.393                  | 1          | 01/0114 | 1           | 0.495                  |
| Kwasiabedi | 2      | 0.633       | 0.367                  | 1          | 01/0134 | 1           | 0.517                  |
| TME593     | 1      | 0.709       | 0.291                  | 1          | 01/0220 | 1           | 0.508                  |
| UCC2001/111| 2      | 0.605       | 0.395                  | 1          | 2000/0388| 1          | 0.529                  |
| 01/0046    | 1      | 0.381       | 0.619                  | 2          | 96/0067 | 1           | 0.471                  |
| 191/02224  | 1      | 0.318       | 0.682                  | 2          | 96/1642 | 1           | 0.468                  |
| 94/0006    | 1      | 0.317       | 0.683                  | 2          | 97/1856 | 1           | 0.507                  |
| 96/0708    | 1      | 0.325       | 0.675                  | 2          | 97/4769 | 1           | 0.497                  |
| 98/2226    | 1      | 0.397       | 0.603                  | 2          | 98/0505 | 1           | 0.541                  |
| AFS2000/131| 2      | 0.369       | 0.631                  | 2          | 99/0554 | 1           | 0.515                  |
| ATR002     | 2      | 0.347       | 0.653                  | 2          | AFS2000/023 | 2      | 0.469               |
| Bankyebrodie| 2     | 0.392       | 0.608                  | 2          | ATR007  | 2           | 0.478                  |
| BD96/009   | 2      | 0.340       | 0.660                  | 2          | SAA007  | 2           | 0.532                  |
| BD96/021   | 2      | 0.398       | 0.602                  | 2          | CTSIA1  | 3           | 0.460                  |
| BD96/154   | 2      | 0.390       | 0.610                  | 2          | CTSIA110| 3           | 0.509                  |
| CTSIA72    | 3      | 0.354       | 0.646                  | 2          | CTSIA112| 3           | 0.511                  |
| CTSIA8     | 3      | 0.360       | 0.640                  | 2          | CTSIA131| 3           | 0.527                  |
| 00/0093    | 1      | 0.555       | 0.445                  | 2          | CTSIA133| 3           | 0.496                  |
| 01/1088    | 1      | 0.577       | 0.423                  | 2          | CTSIA65 | 3           | 0.538                  |
| 01/1412    | 1      | 0.578       | 0.422                  | 2          | CTSIA230| 3           | 0.457                  |
| 97/0879    | 1      | 0.552       | 0.448                  | 2          | 97/0783 | 1           | 0.498                  |
| CTSIA 45   | 3      | 0.589       | 0.411                  | 2          | CTSIA162| 3           | 0.489                  |
| 01/0169    | 1      | 0.569       | 0.431                  | 2          | NWA004  | 2           | 0.490                  |
| OFP2000/023| 2      | 0.592       | 0.408                  | 2          | B91934  | 1           | 0.471                  |
| TA97/137   | 2      | 0.584       | 0.416                  | 2          | KS2000/092| 2      | 0.504               |
| UCC2001/104| 2      | 0.581       | 0.419                  | 2          | KS2000/126| 2      | 0.482               |
| UCC2001/449| 2      | 0.571       | 0.429                  | 2          | KS2000/191| 2      | 0.520               |
| UCC2001/464| 2      | 0.562       | 0.438                  | 2          | MM96/JW1 | 1           | 0.461                  |
| 00/0140    | 1      | 0.421       | 0.579                  | 2          | BAN 001 | 2           | 0.539                  |
| 00/0364    | 1      | 0.447       | 0.553                  | 2          | OFF2000/145| 2      | 0.497               |
| MM96/1751  | 1      | 0.423       | 0.577                  | 2          | Ponti   | 2           | 0.484                  |
| 02/0540    | 1      | 0.401       | 0.599                  | 2          | TA97/054| 2           | 0.498                  |
| 98/0581    | 1      | 0.434       | 0.566                  | 2          | TME419  | 1           | 0.481                  |
| 98/2132    | 1      | 0.404       | 0.596                  | 2          | AW004   | 2           | 0.542                  |
| AFS2000/043| 2      | 0.430       | 0.570                  | 2           | Admixture |            |                      |

Population ID: 1 = IITA accessions, 2 = Local accessions, 3 = CIAT accessions.
hybridization and thirdly, the materials collected from the farmers’ field could be part of introductions by research and extension staff through on-farm demonstrations. The genetic similarity among most of the groups could be a reflection of the exchange of genetic materials among farmers, resulting in the generation of duplicates [37, 54]. Accessions collected from farmers fields need to be characterized to remove duplicates for effective breeding.

Cassava is naturally highly heterozygous and diploid (2n = 36) with some few rare cases of polyplody (2n = 3x = 54 and 2n = 108) being recorded [55]. But varieties are cloned and although heterozygous, they are uniform. The results from the analysis of molecular variance indicated greater genetic variation within the populations than among populations which is similar to earlier studies on within and between population genetic variation in cassava genotypes from different sources [40, 56, 57]. The lower level of variation between populations compared with within population variation could be due to selection by farmers for similar traits at the different collection sources. However, within a particular germplasm collection, farmers keep diverse genotypes. Among the accessions from the different sources, the Local accessions had the highest number of alleles per locus, gene diversity observed heterozygosity and PIC, suggesting that farmers normally keep mixtures of cassava varieties for diverse utilisation purposes and to guard against total crop failure. The implication of this to cassava breeding in Ghana is that, ‘farmer held accessions’ remain valuable sources of novel traits/genes for the genetic improvement of cassava in the country. Local genetic materials serve as rich sources of genetic diversity which can be used to complement and broaden the gene pool of advanced accessions [58]. The detected genetic diversity in the landrace population if phenotypically linked with traits of economic importance can be exploited for further enhancement of the germplasm [11, 12]. Notwithstanding, the population structure analysis indicated that a large proportion of the accessions from IITA were admixtures (Table 6), indicating the possibility of sharing common ancestry with most of the accessions from the other sources.

Precise identification of phylogenetic relationship and divergence of germplasm population(s) gives very useful information which helps decision making (especially choice of parental lines for hybridization schemes) in breeding programmes [59]. The results of the current study separated the 89 cassava accessions into two broad subpopulations and admixtures according to the STRUCTURE analysis and, further, into seven clusters based on the neighbour-joining algorithm. The clustering of the 89 cassava accessions into sub-groups or clusters apparently had little or no connection to the place of collection (geographical origin) of the accessions. Apart from cluster four (Figure 2), all clusters contained at least one or more accessions from the Local, IITA or CIAT collections. This grouping of genotypes into clusters irrespective of geographical origins is similar to an earlier study of cassava accessions [5]. The apparent lack of impact of the place of origin on the population structure in the current study, suggests the possibility that most or all of the accessions in the current study have synegetic relations (presence of common alleles across the accessions) [57, 60]. As discussed earlier, this relationship could include factors such as movement of improved varieties to farmer’s fields during participatory breeding programs, germplasm collection from farmers’ fields, and accessions which are progenies of ‘secondary’ hybridization of parents from different sources [57].

The presence of a large proportion of admixtures among the accessions confirms the heterogenous nature of cassava, being predominantly an outcrossing crop. However, larger proportion of admixtures was found among the IITA accessions compared with the Local collections and those from CIAT, which indicates that most of the accessions collected from IITA might share ancestors in earlier collections from several parts of Africa including Ghana. The population structure observed in the current study indicates the existence of similarities among accessions from different origins suggesting that it will be more useful to select parents based on genetic relatedness rather than based on their origins alone.

5. Conclusion

The simple sequence repeat markers used were informative enough and could detect genetic diversity among the cassava accessions to warrant selection. Greater genetic variation was detected within population than among populations. The Local accessions were more genetically diverse compared to those obtained from IITA and CIAT. The population structure of the cassava accessions did not show any apparent link to geographical origin (place of collection). Farmer-held germplasm accessions of cassava remain important sources of rich genetic resources for cassava improvement.

Declarations

Author contribution statement

Joseph Adjebeng-Danquah: Conceived and designed the experiments; performed the experiment; contributed reagents, materials, analysis tools or data; analysed and interpreted the data; wrote the paper.

Joseph Manu-Aduening: Conceived and designed the experiments; analysed and interpreted the data; wrote the paper.

Isaac Kwadwo Asante: Conceived and designed the experiments; wrote the paper.

Richard Yaw Agyare: Contributed reagents, materials, analysis tools or data; wrote the paper.

Vernon Gracen: Conceived and designed the experiments; wrote the paper.

Samuel Kwame Offei: Conceived and designed the experiments; wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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