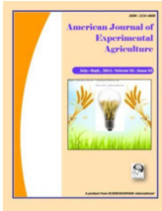

American Journal of Experimental Agriculture
5(4): XX-XX, 2015, Article no.AJEA.2015.032
ISSN: 2231-0606

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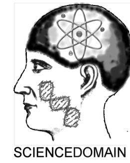

Genetic Diversity of Cassava Mutants, Hybrids and Landraces Using Simple Sequence Repeat Markers

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Authors' contributions

All authors read and approved the final manuscript. This research work was carried out in collaboration between all authors. Author CE designed the study, wrote the protocol and drafted the manuscript. Author AEE managed the analyses of the study. Author KS wrote the protocol and performed the experiment of the study. Author KM and KO designed the experiment and supervised overall experiment. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJEA/2015/7556
Editor(s):
(1).
(2).
Reviewers:
(1).
(2).
(3).
(4).
Complete Peer review History:

Original Research Article

Received 25th October 2013
Accepted 10th January 2014
Published 11th October 2014

Provisional PDF

ABSTRACT

Aims: Cassava (*Manihot esculenta* Crantz) is a crop with largely unexplored and unexplained potentially valuable genetic variability. The knowledge and understanding of the extent of genetic variation of cassava germplasm is important for conservation and improvement.
Objective: The current study was to investigate the genetic divergence of cassava mutants, hybrids and landraces using simple sequence repeat (SSR) markers.
Methodology: Genetic diversity of ten cassava genotypes was determined using 14 SSR markers.
Results: Findings from this study showed that the dendrogram based on UPGMA cluster analysis revealed that SSR data indicated the existence of high divergence among the accessions. The cophenetic matrices obtained showed that SSR data marker type was r = 0.91.
Conclusion: The wider genetic diversity observed using SSR markers would be valuable for efficient management of germplasm and for effective utilization of materials in breeding

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programmes to produce hybrids of desirable characteristics. The extensive genetic diversity is important to coming generations so that it copes with unpredictable environmental changes and human needs.

Keywords: Manihot esculenta; simple sequence repeat (SSR); genetic diversity; germplasm.

1. INTRODUCTION

Cassava (*Manihot esculenta* crantz) is one of the most important food crops grown in the tropics [1]. The crop has significant source of calories for more than 500 million people world-wide [2]. In 2012, FAO estimates the world annual production of cassava starchy roots at 280 million tons [3]. Approximately 71% of cassava products are utilized for human consumption, while the rest is for industrial use as starch or animal feed and others lost as waste [4]. The crop has the ability to adapt well to a variety of climatic conditions; drought tolerant and can be grown in low-nutrient and marginal soils [5].

Breeding programmes in general seek to widen the genetic base, improve crop productivity and maintain its adaptation to specific agro-ecologies. The potential for genetic improvement of cassava has been demonstrated and progress made in increasing yield potential and stability [6]. The existence of genetic variation is a prerequisite for genetic improvement, adaptation and evolution [7,8]. Knowledge of levels of genetic variation is required if farmers continue to use cassava cultivars to form part of the conservation strategy. Sometimes different genotypes may have the same name or same genotype can possess different names [9]. It needs to be linked to how farmers perceive and value diversity, which leads to decision making according to their preferences and needs [10].

Molecular marker technology is an efficient tool for the characterization, management of plant genetic resources, crop improvement programs and evaluation of genetic diversity within and between species as well as plant population [11]. Cassava diversity have been studied using a number of molecular methods such as amplified fragment length polymorphisms (AFLPs), restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), single-nucleotide polymorphisms (SNPs) and single sequence repeat (SSR) markers (also known as microsatellites) [12,13].

SSR markers are a very effective way of assessing genetic diversity since they can be easily adapted for the classification and identification of many organisms and are particularly useful in studying the variation in allelic frequency of unlinked loci. SSRs are widely utilized for genetic diversity and mapping because they have good genome coverage, exhibit high levels of polymorphisms, somatically stable, inherited in a co-dominant Mendelian manner and can be multiplexed on semi-automated systems [12,14] Using SSRs, several studies have shown a high level of heterozygozity in various populations of cassava [15]. The present study aims to measure the variability based on SSR markers to determine the diversity of mutants, hybrids and landraces.

2. MATERIALS AND METHODS

2.1 Genetic Diversity Assessment

2.1.1 Source of genotypes

Test genotypes comprised of 4 mutants, KME1 (parent of the mutant), hybrid (KMA1) and the two parent of hybrid (HM95/0183 × 990072) selections obtained from Kenya Agricultural Research Institute (KARI) Marigat in Baringo County and two landraces collected from farmer's field one from Chepsigot in Elgeyo Marakwet county and another from Sigor West Pokot county. Mutants were developed from KME1 irradiated in bulk at 15 Gray (Gy) dose rate developed in a previous study, Okwaro H. and Kinyua M. G., in 2008. The mutant's selections were coded KMA2, KMA3, KMA4 and KMA5. Woody stakes containing two to three nodes planted under field conditions were used. DNA extraction was carried in University of Eldoret, Department of Biotechnology laboratory.

2.1.2 DNA extraction

DNA extraction was carried out using modified (15) protocol. Young fresh cassava leaves approximately 0.15g - 0.2g per genotype were collected and weighed. Tissue was ground in a mortar and pestle and placed in a 1.5 millimeter (ml) Eppendorf® tube containing 600µl of 0.1M of Tris-Hydrochloric acid (Tris-HCl) pH 8.0, 0.05M of (w/v) Ethylene-diaminetetraacetate (EDTA),

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0.5M Sodium chloride (NaCl), 1% of Polyvinylpirrodine (PVP), 20% of (w/v) sodium dodecyl sulphate (SDS), 0.07% β-mercaptoethanol extraction buffer. The mixture was incubated at 65ºC for 15 minutes with agitation every 5 minutes. Then the samples were placed at room temperature for 2 minutes followed by incubation with 250µl of ice-cold 5M potassium acetate in ice for 20 minutes. After incubation the samples were centrifuged for 15 minutes at 13,000 revolutions per minutes (rpm) at room temperature, and the supernatant was transferred to another tube where one volume of ice-cold isopropanol was added and mixed gently. The mixture was kept at -20ºC for one hour or overnight and then centrifuged for 15 minutes at 13,000 rpm. The pellet was left to dry at room temperature by inverting the tubes on paper towels until all isopropanol drops disappeared from the walls of the tubes. The supernatant was removed and the pellet was washed with 700µl of 70% ethanol and the pellet dried at room temperature, followed by a brief centrifugation for 5 minutes at 13,000 rpm. Deoxyribonucleic acid (DNA) was air dried and later resuspended in 50µl of Tri-EDTA (TE) 10:1 mM buffer and 4µl of RNAse A (10 mg/ml) and then incubated at 65ºC in a water bath for 10 minutes.

2.1.3 DNA quantification

The purity and quality of the DNA was verified by electrophoresis on a 0.8% (w/v) agarose gel for 30 minutes at 80 volts. Lambda (λ) phage DNA was used as the standard. After electrophoresis, the gel was stained in ethidium bromide (10mg/ml) for 30 minutes and later de-stained in distilled water for 20 minutes before viewing under ultraviolet transilluminator. The concentrations of the samples were determined by comparing band sizes and intensities of the test DNA with those of standard λ DNA. Between 0.5µg and 1µg of high quality DNA was obtained and was diluted to 0.01µg/µl with deionized distilled water for the PCR amplification.

2.1 4 PCR amplification

A subset of 14 SSR primer pairs was selected from a data base of SSRs developed at International Centre for Tropical Agriculture (CIAT) [16,17] because of their high polymorphic content and broad coverage of the cassava genome.

The PCR reactions were performed in a Mastercycler (Eppendorf®) using in a final volume of 20µl Bioneer AccuPower® containing 4µl pre-mix (1U Top DNA, 250µM each dNTP, 10mM Tris-HCl pH 9.0, 30mM KCl, 1.5mM $MgCl_2$, stabilizer and tracking dye), 0.0025ng/µl of each forward and reverse primer, 0.5ng of template DNA, and 6µl of double distilled water (ddH2O). The PCR cycles consisted of: 95ºC for 5 minutes, followed by 34 cycles of 30 seconds at 94ºC, 1 minute at 45ºC or 55ºC, 1 minute at 72ºC and a final extension step of 5 minutes at 72ºC. The DNA fragments were separated on 4% agarose gel run at 100 volts (V) for 2 hours (h) using 0.5M TBE buffer. The DNA fragments in gel was visualized by staining in 0.5ug/mg ethidium bromide for 30 minutes and rinsed in distilled water for 20 minutes, visualized and photographed on ultraviolet (UV) transilluminator at 312nm. Allele sizes were scored using a 100 base pair (bp) molecular size ladder.

2.2 Statistical Analysis

A total of 14 SSR markers data were chosen for their di-allelic nature and utilized for gene diversity analysis. Simple sequence repeat primers that showed distinct and scorable DNA bands were considered for analysis. The generated DNA bands were scored based on their band marker sizes. The Power Marker software package [18] was used for the following statistical analysis: percentage of polymorphic loci, mean number of alleles per polymorphic locus, average observed heterozygosity (Ho) and average gene diversity (He) was done [19].

Genetic distance among accessions was estimated using NTSYS-pc, version 2.1 (20). The generated DNA bands were scored based on their allele sizes. The binary data matrix generated was used to construct a dendrogram. The similarity matrix was subjected to UPGMA by selecting the SAHN programme and tree plot analysis. The cophenetic correlation coefficient (r) for the dendrogram was computed. Principal Coordinate Analysis (PCoA) for SSR data was used to construct a matrix plot to show the broad multiple dimension contribution of the genotypes using NTSYS software [20].

3. RESULTS

A total of 11 out of the 14 pairs of primers gave polymorphic bands; the remaining primers failed to amplify any product or were monomorphic and therefore were not considered for further analysis. A total number of 28 alleles were detected with the 11 SSR primer pairs among the

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ten cassava genotypes. The number of alleles ranged from 2 to 4 per locus with a mean of 2 alleles per locus (Table 1) (Plate 1). The polymorphic information content (PIC) values also ranged from 0.09 in SSRY4 to 0.65 in SSRY100 with an average of 0.29. The most polymorphic primers were SSRY100, SSRY106 and SSRY51 based on PIC values. The allele frequency of all the primers was generally below 0.95. Gene diversity was high ranging from 0.1 in SSRY4 to 0.7 in SSRY100 with a mean value of 0.34. The observed heterozygozity (Ho) calculated for each primer ranged from 0.34 (SSRY181) to 0.82 (SSRY9) with the mean of 0.56.

In Fig. 1, the genotypes were clustered in to five groups; (I) consisted of KMA1 and WP, (II) MH95/0183, (III) KMA2, KMA3, KMA5, CHEPS and 990072, (IV) KMA4 and (V) KME1. About 60%, 58% and 39% of the mentioned quantitative, qualitative and combined quantitative and qualitative characteristics, respectively, contribute to differentiate these genotypes. The cophenetic correlation coefficients (r) of the SSR dendrogram were 0.91.

A further molecular analysis using SSR data matrix in Fig. 2 shows the scatter plot that splits the genotypes by the first two coordinates. The principal co-ordinate analysis (PCoA) more closely resembled the dendrogram obtained with SSR data analysis (Fig. 1). The first and second principal components of the 10 cassava cultivars comprised 26.21% and 24.23% of the total variation (50.44%) respectively.

Table 1. SSR marker, number and frequency of alleles, gene diversity, heterozygozity and polymorphic information content (PIC) values generated from 11 SSR data

SSR marker	Allele number	Allele frequency	Gene diversity	Heterozygosity	PIC
SSRY4	2	0.95	0.10	0.47	0.09
SSRY9	2	0.65	0.46	0.82	0.35
SSRY21	3	0.50	0.50	0.59	0.38
SSRY51	3	0.68	0.48	0.55	0.42
SSRY100	4	0.40	0.70	0.65	0.65
SSRY106	4	0.63	0.56	0.60	0.52
SSRY161	3	0.82	0.31	0.53	0.28
SSRY179	2	0.68	0.43	0.36	0.34
SSRY180	2	0.73	0.40	0.55	0.32
SSRY181	2	0.70	0.42	0.34	0.33
SSRY182	2	0.67	0.44	0.56	0.35
Mean	2	0.74	0.34	0.56	0.29

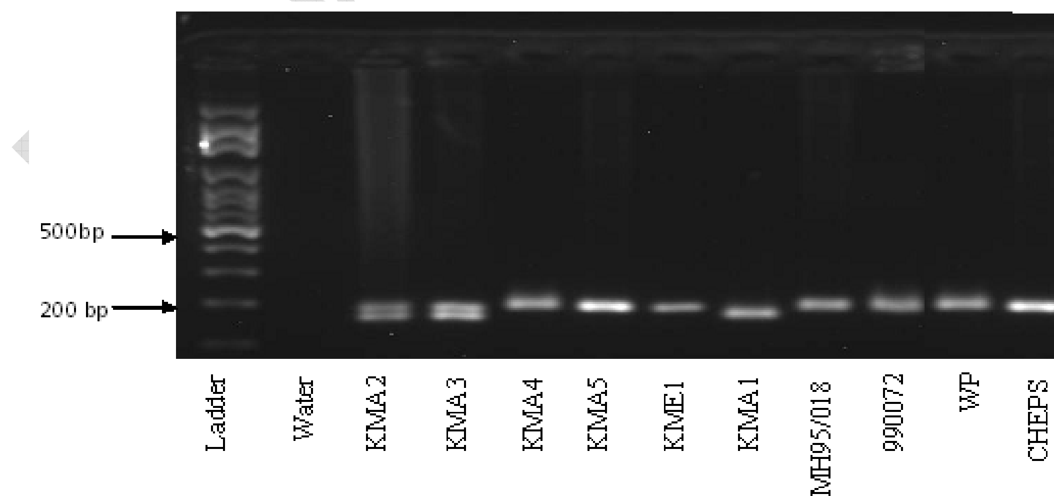

Plate 1. SSR markers profile of 10 cassava genotypes generated by primer SSRY21

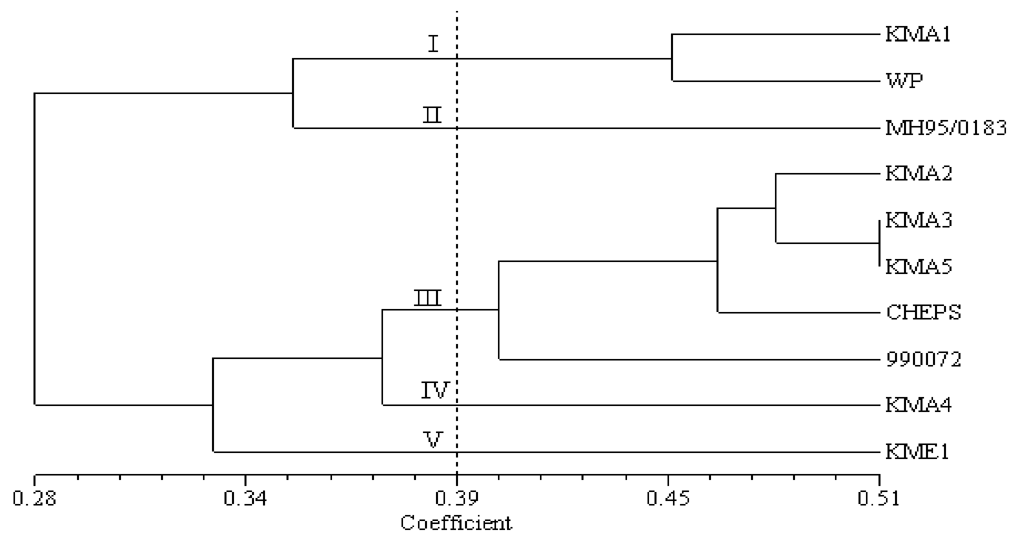

Fig. 1. Dendrogram of 10 cassava genotypes generated by UPGMA clustering based on 11 SSR markers using simple matching coefficient

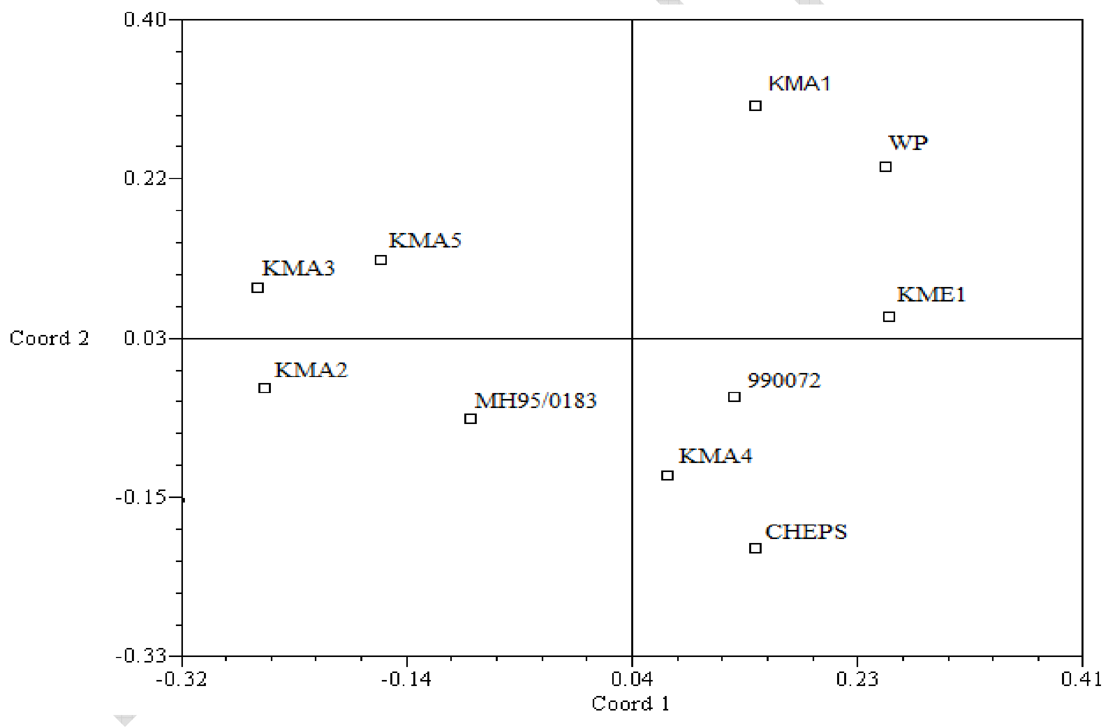

Fig. 2. Principal coordinate analysis of 10 cassava genotypes based on SSR markers with simple matching similarity coefficient

The UPGMA and the PCoA clustering analysis have the same clustering for genotypes 990072, KMA4 and CHEPS, KMA3 and KMA5 while KMA1, WP and KME1, KMA2 and MH95/0183 were found clustering together in the PCoA and not in the UPGMA clustering.

4. DISCUSSION

From the results 11 out of 14 primers used produced a number of alleles that ranged from 2 to 4 with an average of 2.0 alleles per locus, which agrees with an author studying 31

improved cassava cultivars and landraces [21]. An author studying Indian cassava accessions with 15 SSR primers recorded a range of 2 to 6 alleles with mean number of 4 alleles per locus while other obtained a ranged from 2 to 9 with an average of 5.0 alleles per locus among 43 cassava accessions with 36 SSR primers [22,23].

The polymorphic information content (PIC) value was calculated to characterize the capacity of each primer to detect polymorphic loci which ranged from 0.09 to 0.65 in the current study. The result showed that most of the primers were found to be highly informative and can be used to study phylogenetic relationship and genetic diversity studies in future. The allele frequency of all the primers was generally below 0.95 indicating that they were all polymorphic in character. Gene diversity was high ranging from 0.1 to 0.7, comparing favourably with other results [23]. The differences in allele frequencies seen among some genotypes in this study are probably due to genetic drift effects subsequent to mutation. The observed heterozygozity (Ho) mean was 0.56 which suggests a diverse set of considerable genetic heterogeneity among the cassava genotypes that could be useful for improving cassava diversity [16,24].

The genetic relationships observed among the cassava genotypes using cluster analysis revealed differentiation of the genotypes at 45% distance coefficient. The clustering of the some varieties in the dendrogram and PCoA analysis were the same hence complements each other. Mutant varieties KMA2, KMA3, KMA4 and KMA5 were clustered far from the parent material (KME1) in the dendrogram and PCoA analysis, which likely suggests that mutation which is a random phenomenon, might have occurred resulting in variations within the clones [25]. For a useful mutation to be found it requires individual plant evaluation since mutation is a single cell event and that even the useful mutant might be accompanied by many changes in biochemical, morphological and functional properties [4]. Other similar findings have been reported to have a high degree of genetic variability among 94 cassava accessions using PCR-based markers of Brazilian origin [26]. It has also been found that most gene diversity assessed was concentrated within cluster groups of cassava from Africa with SSR markers [27]. The same pattern was observed with sweet potato landraces from the Vale do Ribeira [28].

5. CONCLUSION

There is sufficient genetic diversity among cassava genotypes studied using 14 SSR primers in which 11 SSR primers produced 28 polymorphic alleles. The dendrogram constructed were able to group the cassava genotypes into 5 clusters based on SSR markers at 63% distance coefficient. This indicates that there is sufficient genetic diversity among cassava genotypes which could be exploited for conservation, breeding and selection programmes for improved genotypes for cassava production.

ACKNOWLEDGEMENTS

This work was funded by the Alliance for a Green Revolution in Africa (AGRA), supervised and examined by University of Eldoret supervisors. We are grateful to technicians at University of Eldoret and other scientists whose work aided the achievement of these results.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Phillips T, Taylor D, Sanni L, Akoroda M. A cassava industrial revolution in Nigeria: The potential for a new industrial crop. IFAD, FAO, Rome; 2004.
2. Food Agricultural Organization (FAO). Cassava. Food Outlook. 2011;2:11. Available:http://www.fao.org/docrep/004/x4910e03.htm#P1446_82916 (verified 30 July 2007).
3. FAO. Save and Grow. A policymaker's guide to the sustainable intensification of smallholder crop production. Rome; 2012.
4. Ceballos H, Sánchez T, Denyer K, Tofińo AP, Rosero EA, Dufour D, et al. Induction and identification of a small granule, high-amylose mutant in cassava (*Manihot esculenta* Crantz). Journal of Agriculture and Food Chemistry. 2008;56(16):7215-7222.
5. Gómez LB. Cassava origin: A briefly introduction for breeding. Informaciones agricolasy temas de intes en la Ingenieria Agronómica. In El Agrónomo; 2010.

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6. Kawano K, Narintaraporn K, Narintaraporn P, Sarakarn S, Limsila A, Limsila J, et al. Yield improvement in a multistage breeding program for cassava. Crop Science. 1998;38:325-332.
7. Okogbenin E, Porto MCM, Egesi C, Mba C, Espinosa E, Santos LG, et al. Marker-assisted introgression of resistance to cassava mosaic disease into Latin American germplasm for the genetic improvement of cassava in Africa. Crop Science. 2007;47:1895-1904.
8. Dixon A, Akoroda M, Okechukwu R, Ogbe F, Ilona P, Sanni L, et al. Fast track participatory approach to release of elite cassava genotypes for various uses in Nigeria's cassava economy. Euphytica. 2008;160:1–13.
9. Emperaire L, Peroni N. Traditional management of agrobiodiversity in Brazil: A case study of manioc. Hum. Ecol. 2007;35,761-768.
10. Elias M, Panaud O, Rober T. Assessment of genetic variability in a traditional cassava (*Manihot esculenta* Crantz) farming system, using AFLP markers. Heredity. 2000;85:219-230.
11. Smith GL. Principal component analysis: An introduction. Analytical Proceedings. 1991;28:150-151.
12. Fregene MA, Suarez M, Mkumbira J, Kulembeka H, Ndedya E, Kulaya A, et al. Simple sequence repeat marker diversity in cassava landraces: Genetic diversity and differentiation in an asexually propagated crop. Theoretical and Applied Genetics. 2003;107:1083-1093.
13. Kizito EB, Bua A, Fregene M, Egwang T, Gullberg U, Westerbergh A. The effect of cassava mosaic disease on the genetic diversity of cassava in Uganda. Euphytica. 2005;146:45–54.
14. Varshney RK, Graner A, Sorrells ME. Genic microsatellite markers in plants: Features and applications. Trends Biotechnol. 2005;23:48–55.
15. Dellaporta SL, Wood J, Hicks HBA. Plant DNA minipreparation: Version II. Plant Mol. Bio. Rep. 1983;14,19-21.
16. Chavarriaga-Aguirre P, Maya MM, Bonierbale MW, Kresovich S, Fregene MA, Tohme J, et al. Microsatellite in cassava (*Manihot esculenta* Crantz); discovery, inheritance and variability. Theoretical and Applied Genetics. 1998;97:493-501.
17. Mba REC, Steohenson P, Edwards K, Melzer S, Mkumbira J, Gullgerg U, et al. Simple sequence repeats (SSR) markers survey of cassava (*Manihot esculenta* Crantz) genome: Towards an SSR-based molecular genetics map of cassava. Theoretical and Applied Genetics. 2001;102:21-31.
18. Liu K, Muse SV. Power Marker: Integrated analysis environment for genetic marker data. Bioinformatics. 2005;21(9):2128-2129.
19. Nei M. Estimation of average heterozygozity and genetic distance from a small number of individuals. Genetics. 1978;*89*,583-590.
20. Rohlf. NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software, New York. 2000;98.
21. Moyib OK, Odunola OA, Dixon AGO. SSR markers reveal genetic variation between improved cassava cultivars and landraces within a collection of Nigerian cassava germplasm. Afr. J. Biotechnol. 2007;6(23):2666-2674.
22. Raghu D, Senthil N, Saraswathi T, Raveendran M, Gnanam R, Wnkatachalam R, et al. Morphological and Simple Sequence Repeats (SSR) based Finger printing of South Indian Cassava Germplasm. Int. J. Integrative Biol. 2007;2:141-149.
23. Asare PA, Galyuon IKA, Sarfo JK, Tetteh PJ. Morphological and molecular based diversity studies of some cassava (*Manihot esculenta* crantz) germplasm in Ghana. African Journal of Biotechnology. 2011;10(63)13900-13908.
24. Roa A, Chavarriaga-Aguirre P, Duque M, Maya M, Bonierbale M, Iglesias C, et al. Cross-species amplification of cassava (*Manihot esculenta*) (*Euphorbiaceae*) microsatellites: Allelic polymorphism and degree of relationship. American Journal of Botany. 2000;87:1647-1655.
25. Amenorpe G. Mutation breeding for *in planta* modification of amylose starch in cassava *(Manihot esculenta,* Crantz), PhD Thesis. University of the Free State Bbloemfontein South Africa; 2010.
26. Carvalho LCB, Schaal BA. Assessing genetic diversity in the cassava (*Manihot esculenta*, Crantz) germplasm collection in Brazil using PCR-based markers. Euphytica. 2001;120:133-142.

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27. Lokko Y, Dixon A, Offei S, Danquah E, Fregene M. Assessment of genetic diversity among African cassava *Manihot esculenta* Crantz accessions resistant to the cassava mosaic virus disease using SSR markers. Genet Resour Crop Evol. 2006;53:1441-1453.

28. Veasey EA, Borges A, Rosa MS, Queiroz-Silva JR, Bressan EA, Peroni N. Genetic diversity assessed with microsatellites in Brazilian sweet potato (*Ipomoea batatas* (L.) Lam) landraces. Genet Mol Biol. 2008;31:725-733.

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