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Molecular marker analysis of F_1 progenies and their parents for carotenoids inheritance in African cassava (*Manihot esculenta* Crantz)

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Cassava genotypes were assessed at genomic DNA level to estimate the genetic diversity within and between them using 36 simple sequence repeat markers (SSR). One hundred and forty-seven (147) F_1 progenies derived from crosses amongst the parental genotypes were used to determine the association between three SSR markers and beta-carotene content in cassava. For the diversity study, a total of 131 alleles with an average of 3.7 alleles per locus were found. One yellow fleshed root genotype clustered with a white fleshed root genotype indicating similarity in their genetic background. Three SSR markers were used to screen the parental genotypes and their 147 progenies for a beta-carotene gene. The yellow fleshed root parents and 141 of the F_1 progenies had SSR alleles associated with the presence of beta-carotene gene. The SSR markers identified for beta carotene at CIAT appeared linked to the trait as found in the parents, but evaluation in the progenies indicated that each marker did not account for high phenotypic variance individually. Marker NS 717 (allele 206) accounted for 20% beta carotene content and SSRY 301 (allele 331) accounted for 17%. There are minor QTLs that could probably be involved in beta carotene expression. The markers evaluated therefore do not sufficiently account for beta-carotene expression in the F_1 progenies.

Key words: Cassava, simple sequence repeat (SSR), pro-vitamin A, diversity, validation.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important staple and cash crop in Nigeria; the global leader in the crop's production (Njoku et al., 2011). Cassava production in 2012 was ranked first with 45 million tonnes, followed by yam production at 27 million tonnes, sorghum at 7 million tonnes, millet at 6 million tones and rice at 5 million tonnes (FAOSTAT, 2013). It is also an important staple whose cultivation can provide the nationally required caloric intake for food security which is a minimum of 2400 calories per person per day

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(FAOSTAT, 2013).

Cassava is however, deficient or poor in some important essential food nutrients such as proteins, minerals and vitamins which have affected its utilization as a major food crop. Vitamin A deficiency (VAD) which causes diseases that range from night blindness to *Xerophthalmia* and *Keratomalacia* has been observed as a major health hazard in communities whose nutritional security heavily relies on cassava (Esuma et al., 2012). It is estimated that globally, over 250 million children are at risk of VAD, with 21 of these due to heavy reliance on cassava (WHO, 2009).

The utilization of yellow root cassava in the diets (Sanchez et al., 2006) could offer a possible solution to address this nutritional deficiency. Yellow root cassava is known to have enhanced β-carotene (pro-vitamin A) content (Chavez et al., 2007; Sanchez et al., 2006) and therefore provides sufficient opportunity to sustainably address vitamin A malnutrition through deployment of pro-vitamin A-rich cassava varieties. Global efforts towards breeding cassava for high β-carotene content started in 2002 but progress towards delivery of carotene rich varieties to farmers has been slow probably because of the food quality issues and the apparent negative association between β-carotene and dry matter contents usually, observed in most yellow root cultivars (Akinwale et al., 2010).

Biotechnology tools can enhance efficiency in the breeding process. The selection of target traits can be achieved by indirectly selecting molecular markers that are closely linked to genes for important traits, thus enabling precise identification of genotypes without the confounding effect of the environment (Meuwissen et al., 2001), since selection is based on molecular determination rather than morphological expressions.

QTLs have been identified for mineral concentrations in leaves for phosphorus (Bentsink et al., 2003), caesium (Payne et al., 2004) and potassium (Harada and Leigh, 2006) in *Arabidopsis thaliana* and for zinc in *Thlaspi caerulescens* (Deniau et al., 2006). These studies have demonstrated the presence of allelic variation affecting mineral accumulation, although the identity of the underlying genes remains unknown.

According to Fregene (2006) and Morillo (2009), screening a group of segregating F_1 families from yellow root cassava varieties at CIAT identified two QTLs associated with root colour in cassava in the region between markers SSRY 251 and NS 109 and, SSRY 313 and NS717. The markers were used in screening the parents and progenies for the beta-carotene gene. The objectives of this study were to: 1) examine the relationship among the elite yellow fleshed root and the white fleshed root varieties used in the study using 36 SSR markers commonly used to screen cassava accessions for polymorphism; and 2) to screen parents and F_1 progenies using SSR markers linked to beta-carotene gene expression.

MATERIALS AND METHODS

Plant materials

Six parental varieties and selected progenies were analyzed for beta carotene content. The parents were three varieties with high beta carotene content (pro-vitamin A) and three white storage root (low or no beta carotene) varieties which were crossed to generate segregating F_1 progenies. The white varieties originated from collaborative activities between IITA and NRCRI cassava breeding programs and were released to farmers in 2005 as improved varieties (TMS 98-0002, TMS 98-0505 and TMS 97-0505). The pro-vitamin A cassava varieties TMS 05-0473, TMS 05-1636 and TMS 01-1368 were derived from the IITA breeding collection as elite materials. Pedigree information of the pro-vitamin A parents was not available. There was therefore the need to conduct genetic studies on these varieties to ascertain their relationship. The white varieties were used as female parents while the pro-vitamin A varieties as male parents.

SSR genotyping

Genomic DNA was extracted from young tender leaf tissues from 147 F1 progenies and six parental varieties using the Dellaporta method (Dellaporta et al., 1983). The DNA was checked for quantity and quality using microvolumetric Nanodrop ND-8000 and diluted to 25 ng/100 µl. Three markers were selected as identified in a previous gene tagging study for B-carotene at the International Center for Tropical Agriculture (CIAT), Colombia (Morillo, 2009). Also, subsets of DNA samples of the six parental varieties were assayed with 36 polymorphic SSR markers for diversity study. Amplification was performed in 10 µl reactions containing 50 ng of DNA template, 1 *p*mole of each primer, 1X Taq polymerase buffer, 10 mM $MgCl_2$, 2.5 mM deoxynucleotide phosphates (dNTPs) and 0.075 U Taq polymerase. The PCR profile was run at 95°C for 2 min, followed by 30 cycles of 95°C for 30 s, annealing at 55-57°C for 1 min, extension at 72°C for 1 min and a final extension at 72°C for 30 min.

Based on the fact that amplicons were of different sizes and the forward primers were fluorescently labeled (MWG-Biotech), co-loading of amplicons from the same individual, but at different loci, was therefore possible. Seven co-loading sets were optimized and used for the entire analysis. For each co-loading set, 1 to 2 µl of the different amplicons were mixed and vortexed. Aliquots of 1 µl of the mixture were added to 9 µl of a master mix containing HiDi formamide and GeneScan 500-LIZ size standard (1 ml of HiDi + 12 µl of 500-LIZ). The amplicons were denatured at 95°C for 3 min and subjected to capillary electrophoresis using ABI 3730 DNA sequencer (Applied Biosystems), and allele calls made using GENEMAPPER software version 3.7 (Applied Biosystems).

Screening with markers

One hundred and forty-seven (147) genotypes were derived from crosses involving the six parents and assayed for phenotypic variability and molecular marker genotyping using five amplified SSR markers, but two did not amplified (Table 1).

Quantitative and qualitative analyses

At harvest, three commercial size roots from each plant were selected, washed, peeled, chopped and mixed to obtain a single homogenous sample. The sample was then divided into two sub-samples, one was used for qualitative assessment (colour indicator chart/icheck) and another for quantification of pro-vitamin A

Table 1. The list of three primer sequences used in the SSR marker screening of six parents and 147 selected genotypes.

Name	Left primer	Right primer	Status
SSRY 240	TCGGCTTTTAACATCCTTCG	AGCTAGGAGCAACGCAGTTC	Amplified
SSRY 301	GAACGCTTCAACGGCATAAT	CCAATGCCAACAACACTTCTT	Amplified
NS 717	GCCAAATCGCCAAGGTAATA	GGTGAGTGATAAGGTTACGGC	Amplified
SSRY 215	GTTGATGAGCTGTGGCATTG	CCTAGACGAAGTGGGTCGAA	N/A
NS 313	TGCTGGGGAACTAGTGTGGT	GCAACTTCAAAGGCTGAAGG	N/A

N/A = not amplified.

carotenoids. Carotenoids quantification was by spectrophotometry. The protocol used followed the procedure described in the Harvest-Plus Handbook and in Rodriguez-Amaya and Kimura, (2004). Measurements were made for the six parents and their 147 clones.

Data analysis

PowerMarker (version 3.25) software package was used to estimate genetic diversity parameters using the diallelic data generated from the 36 SSR markers. The diversity parameters estimated included percentage of polymorphic loci, mean number of alleles per polymorphic locus, average observed heterozygosity (Ho) and average gene diversity (He). The principle coordinate analysis was performed using GeneAlex6 software. The simple matching Euclidian distance was used to compute the distance matrix and clustering was done using UPGMA algorithm. This analysis was repeatedly done using the software DARwin5. Phenotypic and genotypic data were analyzed using Microsoft Excel. Data capture was done using the genescan software (Applied biosystems) and the resulting fragments analyzed the alleles scored using the Genemapper software version 4.1 (Applied biosystems) (Ezuma et al., 2012).

RESULTS

Diversity study

Genetic diversity parameters were assessed with 36 SSR markers across six cassava genotypes (three white-storage root and three yellow-storage roots), and the results are presented in Table 2. Out of 36 SSRs, 26 SSR markers were polymorphic for the six genotypes. A total of 131 polymorphic alleles were observed. The number of alleles across loci and groups ranged between 1 and 7 with an average number of allele of 3.81. SSRY 4 had the highest number of alleles followed by SSRY 177 and SSRY 69. The average gene diversity or expected heterozygosity (He) averaged across all the groups and loci ranged from 0.15 in SSRY 34 to 0.81 in SSRY 4 with an average of 0.58, while heterozygosity (observed heterozygosity) ranged from 0.16 to 0.83 with an average of 0.65. Polymorphic information content (PIC) of loci across the two groups was highest in SSRY 4 (0.78) and lowest in SSRY 34 (0.14) with an average of 0.54 (Table 2). Also, both gene diversity and heterozygosity average across the six cultivars were high, 0.58 and 0.65, respectively.

Cluster analysis

In addition, a dendrogram was constructed using the Euclidean distance and unweighted pair group with arithmetic mean (UPGMA) grouping method separated the six cultivars into two major clusters (Figure 1). The marker did not fully discriminate the yellow varieties from the white varieties. Group one (I) contained TMS 05-1636 (yellow pulp) and TMS 97-2205 (white pulp). Group two (II) contained four accessions, TMS 01-1368 (yellow pulp), TMS 05-0473 (yellow pulp), TMS 98-0002 (white pulp) and TMS 98-0505 (white pulp), respectively. Clustering of varieties did not reflect colour. However, the dendogram showed strong affinity/relationship between TMS 05-1636 and TMS 97-2205, and between TMS 98-0002 and TMS 98-0505, probably, they have the same pedigree.

Analysis of molecular variance (AMOVA) showed that most of the variation was distributed within individual populations (95%) and the rest (5%) distributed among populations (Figure 2), supporting the genetic diversity results above.

Phenotypic assessment of Beta-carotene content in the parents and F_1 population

The carotene profile of the six cassava parent varieties selected for crosses is shown in Table 3. Total carotene levels varied among the genotypes, while some genotypes had higher total carotene content than the others.

There was variation in the qualitative and quantitative values of total carotene content among the 147 progenies evaluated. The intensity of the root colour among the segregating progenies ranged from white, light cream, cream, light yellow, yellow, deep yellow, orange to pink (Figure 3). The total carotene content among the progenies also varied from 0.71 to 15.41 ug/g.

Also, Figure 3 illustrates the regression of total carotene content and colour intensity in the roots. A moderate degree of association was found between the total carotene content and color of the root (R2 0.58). This result shows that 58% of the observed variability in the beta-carotene content can relatively be explained by

Table 2. Genetic diversity estimates of six cassava parental materials.

Marker	Allele frequency	Number of alleles	Gene diversity	Heterozygosity	PIC
SSR 4	0.3333	7.0	0.8056	0.6667	0.7818
SSR 240	0.5000	4.0	0.6528	0.8333	0.5994
SSR 181	0.7500	3.0	0.4028	0.5000	0.3633
SSR 179	0.2500	5.0	0.7778	0.8333	0.7409
SSR 177	0.3333	6.0	0.7778	0.6000	0.7456
SSR 171	0.4167	4.0	0.6389	0.8333	0.5689
SSR 169	0.6667	4.0	0.5139	0.5000	0.4760
SSR 161	0.5833	3.0	0.5694	0.8333	0.5045
SSR 155	0.5000	3.0	0.6111	0.6667	0.5355
SSR 151	0.4167	4.0	0.6806	0.8333	0.6218
SSR 148	0.5833	3.0	0.5417	0.5000	0.4598
SSR 147	0.8333	2.0	0.2778	0.3333	0.2392
SSR 135	0.3333	4.0	0.7222	0.6667	0.6713
SSR 110	0.8333	3.0	0.2917	0.3333	0.2723
SSR 108	0.6667	3.0	0.4861	0.5000	0.4235
SSR 100	0.3333	5.0	0.7639	0.8333	0.7260
SSR 64	0.5000	3.0	0.6250	0.5000	0.5547
SSR 63	0.3333	4.0	0.7361	0.8333	0.6874
SSR 59	0.4167	5.0	0.7222	0.5000	0.6800
SSR 52	0.4167	4.0	0.6806	0.6667	0.6218
SSR 51	0.4167	4.0	0.7083	0.6667	0.6589
SSR 34	0.9167	2.0	0.1528	0.1667	0.1411
SSR 21	0.8333	2.0	0.1778	0.3333	0.2392
SSR 20	0.5000	5.0	0.6667	0.5000	0.6221
SSR 12	0.4167	4.0	0.6806	0.6667	0.6218
SSR 9	0.6667	3.0	0.4861	0.3333	0.4235
Mean	0.5289	3.8	0.5827	0.6524	0.5377

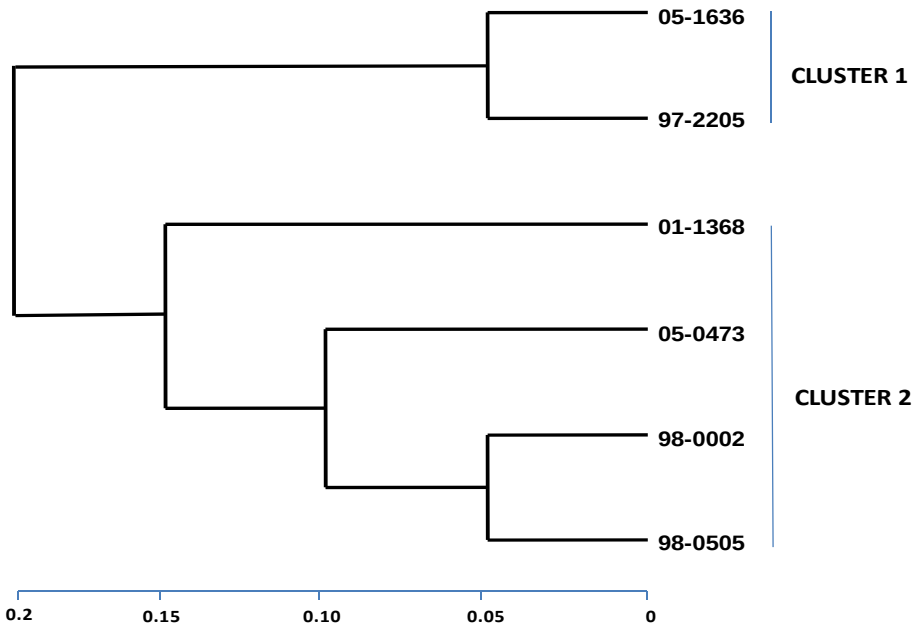

Figure 1. Dendrogram showing cluster analysis of six cassava genotypes based on allelic data at 36 SSR loci using UPGMA.

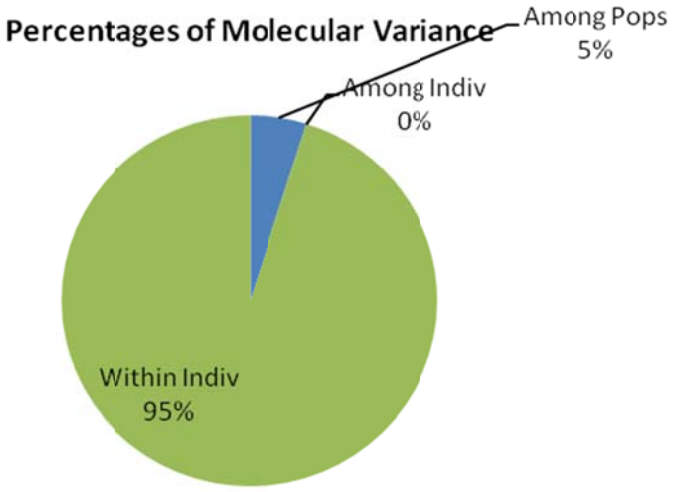

Figure 2. Analysis of molecular variance (AMOVA) of yellow and white fleshed cassava root.

Table 3. Total carotene of parental materials assessed using qualitative and quantitative measurements.

Parent	Pulp colour	Qualitative value	Quantitative value (ug/g)
TMS 01-1368	Deep yellow	6	6.56
TMS 05-1636	Yellow	5	5.51
TMS 05-0473	Light yellow	5	4.19
TMS 97-2205	White	1	1.08
TMS 98-0505	White	1	0.89
TMS 98-0002	White	1	1.12

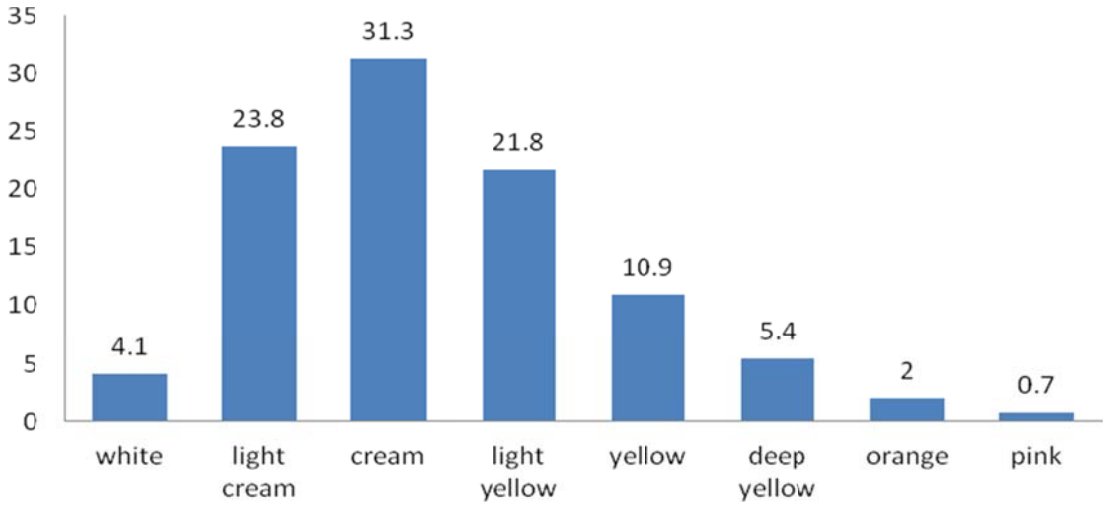

Figure 3. Percentage distribution of frequencies of different root colour classes of F_1 population.

variability in root pulp color, and that any increase in color intensity will result in a proportional increase in the concentration of carotenes (Figure 3).

However, in spite of the apparently good relationship between colour intensity and beta-carotene content as shown in Figure 3, there is still large variation for total

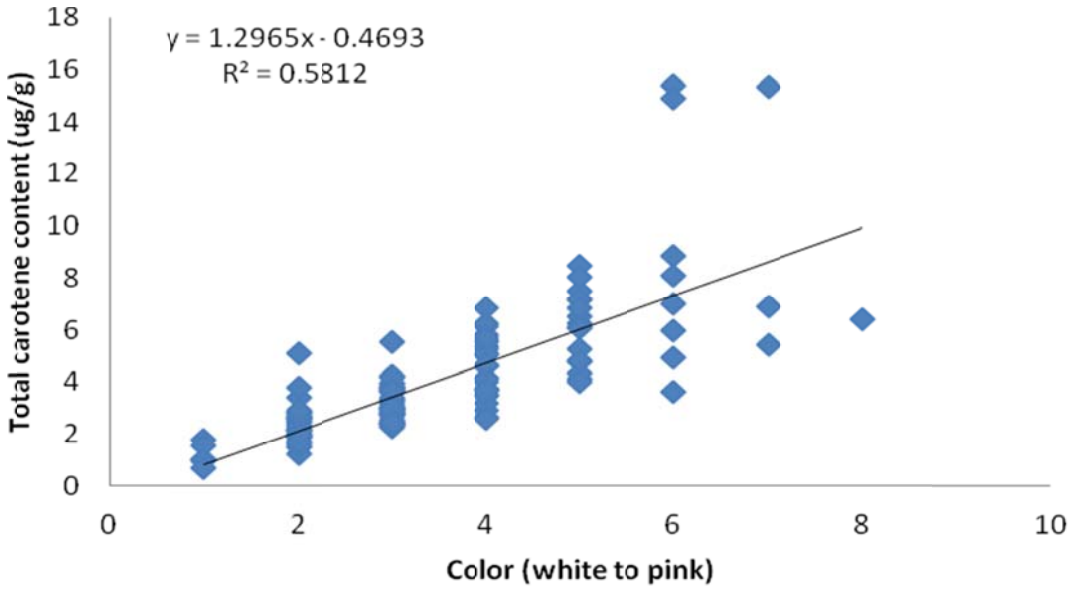

Figure 4. Relationship between colour intensity in the roots (based on visual scale of 1 to 8) and average total carotene content (fresh weight basis) of 147 genotypes.

carotene content as illustrated in Figure 4. For instance, there were several clones on the right side that showed high color intensity but low beta-carotene content. The same thing was observed on the left edge of the figure showing the absence of roots with relatively high content of carotenoids but low or no color intensity in the roots (Figure 3). However, this could be explained if the roots of these clones have other pigments in them different from carotenoids.

Analysis of SSR markers for beta-carotene in parent genotypes

Three markers previously identified (Morillo, 2009) for beta carotene content was analyzed in the parents: (a) Marker NS 717: Three marker alleles were identified (based on the base pairs) at this locus. The marker alleles identified were 196, 206 and 211. Allele 211 was only found in the white pulp parents. The other two marker alleles were found in the yellow pulp parents. In the white pulp parents, allele 211 was found in the homozygous state. For two of the yellow varieties (TMS 01-1368 and TMS 05/1636), allele 206 was also found in the homozygous state. In the third yellow parent (TMS 05/0473), marker NS 717 was heterozygous for alleles 196 and 206; (b) SSRY 301: Three marker alleles were identified as alleles 324, 326 and 331. Allele 324 was only found in the white pulp parents in the heterozygous state. Allele 326 was found in the white and yellow pulp parents while allele 331 was found only in the yellow parents. For two of the yellow varieties (TMS 05-1636 and TMS 01-1368), allele 326 and 331 were also found in the heterozygous state, while in the third yellow parent (TMS 05-0473), allele 326 was found in a homozygous state; (c) SSRY 240: In SSRY 240, two marker alleles were identified. The marker alleles identified were 171 and 181. Allele 181 was only found in the yellow parents in the homozygous state. Allele 171 was found only in the white parent TMS 98-0002 (Table 4).

Molecular screening of F_1 populations for beta-carotene gene

One hundred and forty-seven (147) genotypes were screened with three SSR markers. Results show that 96% of the genotypes had at least one marker allele for a beta-carotene gene. Further analysis of the results indicated 17% of the genotypes had one marker allele associated with beta-carotene gene, 27.9% had two informative marker alleles, 27.9% had three informative marker alleles, and 22.5% had four informative marker alleles linked to beta-carotene. Six genotypes had no marker alleles associated with beta-carotene genes (Figure 3).

Marker - trait correlation

Association with colour of root pulp was determined by simple linear correlation of phenotypic data on marker allele means. Among the markers, alleles 206, 181, 326 and 331 (quantitative) were positively correlated with beta-carotene phenotypic values, while allele 196 was negatively correlated with beta-carotene contents. Marker

Table 4. Summary of SSR alleles identified in six cassava clones varying in beta-carotene.

Clone	Pulp color	SSRY 240		SSRY 301			NS 717		
		Allele		Allele			Allele		
		171	181	324	326	331	196	206	211
TMS 98-0002	White	+	-	+	+	-	-	-	+
TMS 97-2205	White	-	-	+	+	-	-	-	+
TMS 98-0505	White	-	-	+	+	-	-	-	+
TMS 05-0473	Yellow	-	+	-	+	-	+	+	-
TMS 05-1636	Yellow	-	+	-	+	+	-	+	-
TMS 01-1368	Yellow	-	+	-	+	+	-	+	-
Total		1	3	3	6	2	1	3	3
Freq		16.7	50.0	50.0	83.3	33.3	16.7	50.0	50.0

Presence (+) or absence (-) of favourable marker allele for beta-carotene gene.

Table 5. Correlation of quantitative and qualitative values with 3 putative markers in 147 F1 genotypes.

Marker	Allele	R-value	
		Quantitative	Qualitative
NS 717	206	0.19969	0.19464; P<0.001
SSRY 240	181	0.08889	0.03804; P< 0.001
SSRY 301	331	0.13587	0.17219; P<0.001

NS 717 with allele 206 has the highest correlation both quantitatively and qualitatively (19%, P<0.001 and 20%, P< 0.001). This was followed by marker 301 with allele 331 giving a correlation values of 13% (quantitative) and 17% (qualitative) beta-carotene values using qualitative standard colour chart assessment (Table 5).

The proportion of phenotypic variation explained by these individual markers ranged from 0.09 to 0.20 (quantitative) and 0.04 to 0.19% (qualitative). For each trait, markers were identified in which the allelic contribution from each of the parents either had an increased effect or lowered the phenotypic value of the trait.

DISCUSSION

Genetic relationship among three yellow root and three white root cassava accessions was evaluated with 36 SSR markers. The set of 36 unlinked SSR loci used in this study have been identified to be adequate for molecular diversity characterization and good estimation of genetic diversity parameters for cassava accessions. The set of markers used in this study were found to be very highly informative and have good distribution coverage of the cassava genome and thus were highly suitable for genetic diversity evaluation (Fregene et al., 2003). The SSR markers showed high mean PIC (52.3%) which demonstrates their ability to discriminate between individual cultivars. The higher the PIC of a marker, the more informative is the marker (Fregene et al., 2003). The most informative marker from the study was SSRY 4 with mean PIC of 0.78, while SSRY 34 was the least informative marker. These results are in agreement with earlier studies in cassava with SSR markers. Kawuki et al. (2009) reported mean PIC of 55, 57 and 61%, respectively, with accessions collected from Africa landraces, Asia and Latin America.

The mean number of alleles (3.743) detected per locus was within that obtained in previous cassava diversity studies (Esuma et al., 2012). The high number of alleles per locus conforms to the nature of cassava as an outcrossing crop. Previous genetic diversity studies in cassava using different types of DNA molecular markers have shown low to medium genetic diversity.

Both expected heterozygosity and observed heterozygosity averaged across the six cultivars and marker loci were high, 57 and 65% respectively. Gene diversity or expected heterozygosity (He = 57%) detected in this study was higher than the average reported for outcrossing species (He = 20.5%) using isozyme markers. It has been suggested that high levels of polymorphism in microsatellite markers are related to the mechanism of mutations and the high rate at which they occur. Heterozygosity is an indication of the probability that two randomly selected alleles from an accession/cultivar of a germplasm are different is 65%.

Expected heterozygosity which explains the probability that two alleles arbitrarily selected are different was found to be high in this study. The results show that the parent materials have high degree of heterozygosity as expected for cassava. Similar observed and expected heterozygosity in cassava has been reported. Raji et al. (2009), using cassava germplasm from various countries in Africa, reported average values of gene diversity and heterozygosity of 0.630 and 0.730, respectively.

The genetic differences found among the six cultivars resulted in their clustering into distinct groups suggesting that those in the same cluster share similar breeding history or pedigree and hence, higher genetic relatedness. Based on the UPGMA analysis of the parents, two groupings were identified. The cultivar TMS 05/1636 and TMS 97/2205 clustered in the same group 1, and the other four cultivars clustered together in group 2, which reflects their similarity in genetic base.

The AMOVA results found no genetic difference between the yellow and white accessions. As expected, between individual variations were most significant and accounted for the majority of the molecular variance. Similar findings have been reported by several previous studies on genetic diversity of sweet potato germplasm (Gruneberg, et al., 2005). Moreover, it is a clear indication that breeders can form in breeding programs different populations with significant levels of genetic difference, which is a prerequisite to exploit heterosis and improvement of populations.

Two deductions from this study are of direct application to cassava breeding. First, the close relationship of TMS 98/0505 and TMS 98/0002, and TMS 05/1636 and TMS 97/2205 cultivars implies that each pair may have been derived from a common source. Second, there is a high genetic diversity among cassava accessions at Umudike which can be exploited for crop improvement.

In addition, the six parents and 147 progenies were phenotyped and genotyped with three SSR markers linked to beta-carotene. The result differentiated eight different distinct phenotypic classes in some of the populations based on eight colour categories. Several phenotypic classes indicate a likely possibility of combination of additive, recessive and/or epistatic effects in the control of beta carotene for the genotypes assessed or more likely variation in root color due to variations in starch content of lines with the same carotene content. Going by these different phenotypic classes, it is obvious that the trait is controlled at least by a few loci, may be about ~2 major genes as was reported in the literature (Grüneberg et al., 2005). None of the loci screened suggest a major dominant gene effects. Marker analyses of the parents indicate that marker allele 181 from SSRY 240 and allele 206 (NS 717) and allele 331 were associated with beta-carotene content. Allele 326 was found in both white and yellow parents. It was found in heterozygous condition in yellow varieties in combination with marker allele 311, indicating that marker allele 311 was dominant to allele 326 and is linked to a beta-carotene gene. The other marker alleles associated with yellow roots were found in heterozygous states also suggesting that they were dominant for beta carotene expression.

The study indicates that 96% of the genotypes showed at least one of the marker alleles associated with a putative beta-carotene gene. In fact, 17% of the genotypes showed one marker allele associated with beta-carotene; 27.9% had two, 27.9% had three informative marker alleles, and 22.5% had four informative marker alleles linked to beta-carotene. Six genotypes had no marker alleles associated with beta-carotene genes, and they were white root genotypes.

There was a good correlation between total carotene content and color intensity in the roots. When qualitative values (color) were correlated with quantitative values (spectrophotometer reading), a relatively high degree of association between beta-carotene content and color values of R^2 = 0.58 was obtained. This means that 58% of the observed variability in the beta-carotene content can be explained by variability in the storage root colour. These results agree with reports by other researchers, using different cassava populations (Chavez et al., 2007; Morillo, 2009).

However, 58% correlation shows that the margin of error in using qualitative measurements to assess beta carotene level is still high. Evaluation and analysis based on color assessments are likely to be misleading. Beyond rapid screening at seedling nursery level when population sizes are big, this may not be very good procedure for accurate and precise determination of beta carotene content in germplasm.

The SSR markers identified for beta carotene at CIAT appeared linked to the trait as found in the parents used here, but evaluation of the progenies indicated that each marker did not account for high phenotypic variance individually. The markers evaluated therefore do not sufficiently account for beta-carotene expression in the F_1 progenies. Allele 206 (Marker NS 717) accounted for 20% beta carotene content and allele 331 (Marker SSRY 301) accounted for 17%. It was not possible to determine whether the alleles found in African germplasm were the same ones discovered in Latin American germplasm. However, major genes have been reported for beta carotene expression from classical genetic studies (Iglesias et al., 1997). It could possibly be that the markers evaluated did not capture the significant region of the genome affecting beta carotene expression in the African germplasm as only few markers were used because the objective was to validate the selected markers for MAS in beta carotene expression.

Conclusion

The study shows that SSR marker analysis is a useful tool in studying genetic diversity for cassava improve-

ment. Analysis of the parental cassava genotypes produced two major divergence groups. However, no single group was observed to be unique with yellow colour. Also, screening of the parental genotypes and 147 progenies with three polymorphic markers (SSRY 301, SSRY 240 and NS 717) revealed four marker alleles linked to beta-carotene gene. Yellow root parental genotypes and 141 progenies showed the presence of these marker alleles while the white root parents and six progenies showed absence of these marker alleles.

Though the correlation between the qualitative (colour indicator value) and quantitative values (spectro-photometer value) was high, the correlation values between genotypic and phenotypic data classes for candidate markers were generally low. The results show that these markers do not sufficiently explain all the phenotypic variance observed; at least, they do not explain a high part of it. Therefore some additional mapping (BSA or QTL approach) to find more markers or genomic regions influencing beta carotene inheritance in Africa cassava germplasm is warranted.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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