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Research Note

Morphological Characterization and SSR-Based DNA Fingerprinting of Cassava (*Manihot esculenta* Crantz) Varieties Released by the National Seed Industry Council (NSIC)

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The genetic variability of 47 cassava varieties released by the National Seed Industry Council (NSIC) was evaluated through morphological characterization and DNA fingerprinting using simple sequence repeat (SSR) markers. Phenotypic similarities based on 34 morphological descriptors were observed in varieties sharing a common parent. Fifty (50) polymorphic SSR markers were used to construct DNA fingerprints. A total of 648 polymorphic alleles and 764 unique banding patterns were observed among the 47 varieties. The polymorphism information content (PIC) values ranged from 0.46 to 0.95, with an average of 0.83. Each variety had a unique banding profile indicating that SSR markers were useful in distinguishing cassava varieties. A wider genetic diversity of cassava varieties was detected in SSR data (70%) compared with morphological data (50%) showing that SSR markers are more effective in determining the extent of variation between genotypes. The DNA fingerprints of cassava varieties have been successfully generated which can be used as a benchmark for identification and authentication of released cassava varieties.

Key Words: cassava, DNA fingerprinting, National Seed Industry Council, PIC, SSR

Abbreviations: NSIC – National Seed Industry Council, PIC – polymorphism information content, SSR – simple sequence repeat, UPGMA – unweighted pair group method with arithmetic mean

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is considered as one of the world's most important crops in the tropics and sub-tropics. It is ranked as the third richest source of carbohydrates in the world, next to rice and maize (FAO 2008). A significant attribute of cassava is its adaptability to a wide range of environmental stresses (Lekha et al. 2011).

In the Philippines, the major institutions involved in cassava breeding and varietal improvement are the Philippine Root Crops Research and Training Center (PhilRootcrops), Visayas State University (VSU), Baybay, Leyte and the Institute of Plant Breeding (IPB), University of the Philippines Los Baños, College, Laguna. The cassava breeding objectives include high yield, high dry matter and starch content, resistance to pests and diseases, tolerance to environmental stresses, and good plant type (Mariscal et al. 2001). In 1992, the National Seed Industry Council (NSIC) was established by virtue of Republic Act 7308 also known as the Seed Industry Development Act with the mandate of implementing the registration of varieties developed by breeders. At present, 47 cassava varieties are registered with the NSIC. Although breeders provide basic morphological characteristics of varieties during the registration or release of these varieties, these traits are not sufficient for the implementation of Intellectual Property Rights (IPR) and the Plant Variety and Farmers Right Protection Act (PVFRPA). Moreover, these morphological traits may change due to interaction between genotype and environment and outcrossing (Molla et al. 2016).

Therefore, a more precise system of identification for varieties is necessary.

With the advent of molecular technology, intensive breeding activities for improvement of different crops have been conducted. One of the most useful and commonly employed molecular techniques in various fields such as forensic, paleontology, archaeology, various fields of biology, medical diagnostics, and agriculture is DNA fingerprinting (Singh 1991). In plant breeding, fingerprinting is helpful in crop improvement programs. Together with molecular markers, DNA fingerprinting allows rapid identification, characterization and assessment of the genetic diversity of varieties, cultivars, and wild species for germplasm establishment (Bhat 2008; Zhu et al. 2012).

Compared with the other molecular markers, microsatellites or simple sequence repeats are codominant markers that have been widely used in various breeding programs due to their characteristics such as reproducibility, reliability and transferability. SSR-marker -based DNA fingerprinting could be used to identify and differentiate close as well as distant relatives of crop species (Acquah et al. 2011). DNA fingerprinting studies using SSR markers have been conducted in several crop species (Taamalli et al. 2008; Rahman et al. 2009; Chinnappareddy et al. 2012; Ahmed et al. 2013). These studies supported the efficiency of SSR markers in distinguishing varieties and the ability to generate distinct profiles for identification and authentication of varieties or accessions. In India, the Central Seed Committee established under the Seed Act of 1996 stipulates the necessity of DNA fingerprint data for the varieties released or proposed to be released (Pujaita et al. 2015). This study was conducted to establish the DNA fingerprints of the 47 NSIC-registered cassava varieties and to assess their genetic diversity using SSR markers and morphological descriptors.

MATERIALS AND METHODS

Morphological Characterization

A total of 47 cassava varieties released by the National Seed Industry Council (NSIC) were assessed using 34 morphological descriptors based on selected morphological and agronomic descriptors for the characterization of cassava by Fukuda et al. (2010). The morphological evaluations were done 3, 6, 9 and 12 mo after planting (Table 1).

DNA Extraction

Genomic DNA of cassava varieties was extracted from young leaves of cassava using cetyl trimethylammonium bromide (CTAB) method as described by Doyle and Doyle (1987) and Sharma et al. (2008) with some modification. A gram of fresh young leaves was pulverized with liquid nitrogen and PVP using mortar and pestle. Each ground sample was added to 8 mL preheated extraction buffer (0.1 M Tris-HCL pH 8.0, 20 mM EDTA pH 8.0, 2 M NaCl, 2% (w/v) CTAB, 1% (w/v) PVP, and 0.5% (v/v) β-ME) incubated for 1 h at 65 °C and inverted gently every 15 min. Gentle inversion was done twice and then the mixture was allowed to settle for the remaining 30 min. Approximately 600 μL of the mixture were transferred to 1.5 mL microcentrifuge tubes and an equal amount of chloroform-isoamyl alcohol (24:1) was added. The solution was mixed thoroughly and centrifuged at 10, 000 rpm for 10 min. Around 200 μL of 2M NaCl with 4% PEG were added to the sample tubes; the samples were then incubated. After incubation at -4°C for 15 min, the samples were centrifuged at 10,000 rpm for 5min. Around 550 μL of the aqueous phase was transferred to new microcentrifuge tubes and an equal amount of isopropanol was added. Incubation at -20°C was done overnight. After incubation, the tubes were centrifuged at 10, 000 rpm for 5 min and the supernatant was discarded. The resulting pellets were washed twice with 100 μL 70% ethanol and air dried completely. The pellets were dissolved and resuspended in 1x TE buffer with 0.5 μL (1/100 vol) of RNase (10 mg/mL) and incubated at 37°C for 1 h.

SSR Characterization

A total of 57 cassava SSR primers acquired from the studies of De Bang et al. (2010), Siqueira et al. (2009), and Turyagyenda et al. (2012) were screened in this study. Out of these, 50 SSR markers amplified polymorphic bands and were used to analyze the genetic diversity of the 47 cassava varieties.

Screening of SSR primers was done under the following PCR conditions: initial step of denaturation for 3 min at 95°C, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 45–64°C for 30 s, extension at 72°C for 60 s, and final extension at 72°C for 5 min. PCR reactions were carried out in 10 μL volumes containing 60 ng genomic DNA, 4.0 mM $MgCl_2$, 0.3 mM dNTPs, 1 x PCR buffer (10 mM Tris-HCl, pH 8.0, 50 mM KCl), 0.3 μM each of forward and reverse primers and 0.05 U of Taq DNA polymerase. The bands were resolved by means of 6% (>200 bp product size) to 8% (<200 bp product size) polyacrylamide gels electrophoresed using 1x TBE buffer.

Data Analysis

Morphological Data

Gower index was used to determine the distance between each variety. The quantitative data were scaled using R statistical language and environment. The distance

Table 1. Morphological descriptors used to evaluate the 47 cassava (Manihot esculenta Crantz) varieties released by the National Seed Industry Council.

Descriptors	Phenotypic Classes
3 months	
Color of apical leaves	Light green, dark green, green-purple and purple
Pubescence of young leaf	Little, moderate and high
6 months	
Leaf retention	Very poor, less than average, average, better than average and outstanding
Shape of central lobe	Elliptic-lanceolate, obovate-lanceolate, oblong-lanceolate, lanceolate and straight/linear
Lobe margins	Smooth and winding
Petiole color	Yellowish-green, green, reddish-green, greenish-red, red and purple
Distribution of anthocyanin in petiole	Absent, top part, central part and totally pigmented
Petiole orientation	Inclined upwards, horizontal, inclined downwards and irregular
Petiole length	Short (0-10 cm), medium (11-20 cm) and long (21-30 cm)
Leaf color	Light green, dark green, purple green and purple
Leaf vein color	Light green, dark green, purple-green and purple
Number of leaf lobes	Assess on five leaves and take the predominant number of lobes
6 months	
Length of central lobe (cm)	Measure the three leaves from the middle of the plant, measure from the intersection of all lobes to the end of the middle lobe
Width of central lobe (cm)	Measure the three leaves from the middle of the plant, measure from the widest part of the middle lobe
9 months	
Prominence of foliar scars	Semi-prominent and prominent
Color of stem cortex	Orange, light green and dark green
Color of stem epidermis	Cream, light brown, dark brown and orange
Color of stem exterior	Orange, green-yellowish, golden, light brown, silver, gray and dark brown
Distance between leaf scars	Short (1-8cm), medium (8-15cm) and long (>15cm)
Color of end branches of adult plant	Green, green-purple and purple
Length of stipules	Short and long
Stipule margin	Entire and split/forked
Growth habit of the stem	Straight and zigzag
12 months	
Branching habit	Erect, dichotomous and trichotomous
Shape of plant	Compact, open, umbrella and cylindrical
Extent of root peduncle	Sessile, pedunculate and mixed
Root constrictions	Less than three, four to six, more than six
Root shape	Conical, Con-cylindrical, cylindrical and irregular
External color of storage root	Cream, yellow, light brown and dark brown
Color of root pulp	White, cream, yellow, orange and pink
Color of root cortex	White/cream, yellow, pink and purple
Cortex: Ease of peeling	Easy and difficult
Texture of root epidermis	Smooth, intermediate and rough
Levels of branching	Record number of divisions of branching and the most frequent occurrence.

formula is given by:

$$d_{ij} = 1 - s_{ij}, \text{ with } s_{ij} = \frac{\sum_{k=1}^{p} \delta_{ijk} s_{ijk}}{\sum_{k=1}^{p} \delta_{ijk}}$$

The formula used for the quantitative variable was

$$s_{ijk} = 1 - \frac{|x_{ik} - x_{jk}|}{range\ of\ variable\ x_k}$$

where *sijk* refers to the distance between plants i and j in character k. For qualitative data, *sijk* is equal to 1 if i and j agree with respect to variable k and is equal to 0 if they disagree. Indicator *ijk* is equal to 1 if both quantities *xik* and *xjk* are recorded; and is equal to 0 in the opposite situation (Gower 1971).

Molecular Data

The resulting PCR bands were scored based on the presence (1) or absence (0) of DNA fragments. The Jaccard coefficient was used to determine the distance between varieties. The distance formula between plant I and j is given by:

Sij = a / (a + b + c)

Dij = 1 – Sij

where a is the number of DNA band(s) present in both plants i and j, b is the number of DNA band(s) present in i and not in j and c is the number of DNA band(s) present in j and not in i, and D is the distant coefficient. Model-based clustering was performed to determine the genetic structure and the number of clusters in the data set using the R software version 3.6.0 (R Core Team 2012). Based on the distance coefficient, the dendrogram was generated using the unweighted pair group method with arithmetic mean (UPGMA).

Polymorphism Information Content (PIC)

The Polymorphism Information Content (PIC) values for each SSR were estimated by determining the frequency of alleles per locus using the formula:

$$\text{PIC} = 1 - \sum_{i=1}^{h} p_i^2$$

where pi is the frequency of the ith allele out of the total number of (n) alleles at an SSR locus. Markers were classified as informative when PIC is ≥ 0.5 (Nei 1973).

RESULTS AND DISCUSSION

Morphological Characterization of 47 NSIC Cassava Varieties

Similarities in morphological traits in some varieties sharing a common parent were observed. NSIC cv. 25 and NSIC cv. 33 exhibited only 10 similar characteristics which were also found in their common parent, PSB 3, namely: smooth leaf margin, petiole inclining upward, prominent foliar scars, short distance between leaf scars, straight growth habit of the stem, short stipule, forked stipule margin, sessile extent of root peduncle, dark brown external root surface and rough root epidermis.

NSIC cv. 31, NSIC cv. 39, NSIC cv. 40 and NSIC cv. 42 with PSB 2 as the common parent shared red petiole, total distribution of anthocyanin in petiole and rough root epidermis with its offspring except for NSIC cv. 42. Green -purple end branches of adult plant and sessile extent of root peduncle were found to be similar among PSB 2 and its resulting varieties except for NSIC cv. 40. PSB 2 together with its offspring (NSIC cv. 31, NSIC cv. 39, NSIC cv. 40 and NSIC cv. 42) had the same short stipule length.

PSB 6, together with its offspring (NSIC cv. 37 and NSIC cv. 39), showed the same characteristics including moderate pubescence in young leaf, straight shape of central lobe, red petiole, total distribution of anthocyanin in petiole, dark green leaf color, prominent foliar scars, light green stem cortex, straight growth habit, short stipule, dichotomous branching habit, cylindrical plant shape, dark brown external root surface, and rough root epidermis. These findings show that it is possible to identify similarities between parents and offspring through morphological characterization.

Generally, cassava varieties are distinguished from each other by their morphological characteristics. A large amount of variation exists among the cassava leaf, stem and root characteristics (Richardson 2011). Moreover, Richardson (2013) pointed out that leaf retention and branching habit may influence cassava yield. Branching genotypes form a better canopy that can intercept more light than erect genotypes, which results in higher tuberous root yields (De Souza et al. 2016). Tan and Cock (1979) found that cassava with late-branching types with three branches at each branch point are ideal genotypes which could give high yields. On the other hand, leaf retention may present an additional opportunity to increase cassava yield and is also implicated in drought tolerance (Fukuda et al. 2010; Kawuki et al. 2011). Better leaf retention increases total biomass production which accumulates in the roots, leading to higher root yield (Lenis et al. 2006). These characteristics could be incorporated in developing new varieties of cassava. Varieties which exhibit these traits could be included in forming the base population of breeding programs.

Cluster Analysis of Morphological Data

Using the morphological data gathered in four evaluation phases, a dendrogram was generated through the R statistical language and environment based on Gower's index coefficient. At 0.5 dissimilarity index, the dendrogram (Fig. 1) was divided into nine clusters (I, II, III, IV, V, VI, VII, VIII and IX). Five clusters (I, II, III, IV, and V) had only one member compared with the remaining four clusters. NSIC cv. 41, NSIC cv. 13, NSIC cv. 15, NSIC cv. 12 and NSIC cv. 30 belong to these five clusters, respectively. Cluster IX had the most number of members with a total of 18 varieties. Out of these 18 members, 11 varieties were developed by UPLB, four by PhilRootcrops, VSU and three by VSU.

Although varieties and their common parent were observed to exhibit similarities in some morphological characters, cluster analysis revealed that most of these genotypes did not group together. Only NSIC cv. 37 and NSIC cv. 39, with PSB 6 as a common parent, grouped together in Cluster VI. This may be explained by the heterozygous nature of cassava. According to Nakabonge et al. (2018) cassava varieties have a high level of morphological variability in which some confer adaptability to different agro-ecological zones. Thus, various genotypes may exhibit variations in morphological characters depending on the environmental conditions.

Morphological descriptors are subjective in nature and can be affected by the environment. Nevertheless, morphological characters are continuously being used in various studies since they are economical, readily available for on-site assessment of genetic variability and can measure the genetic relatedness of accessions (Asare et al. 2011). A study conducted by Laila et al. (2015) found that a genetic diversity level of 49.82% was observed in 181 cassava accessions in Indonesia. The results corroborated with the findings of this present study.

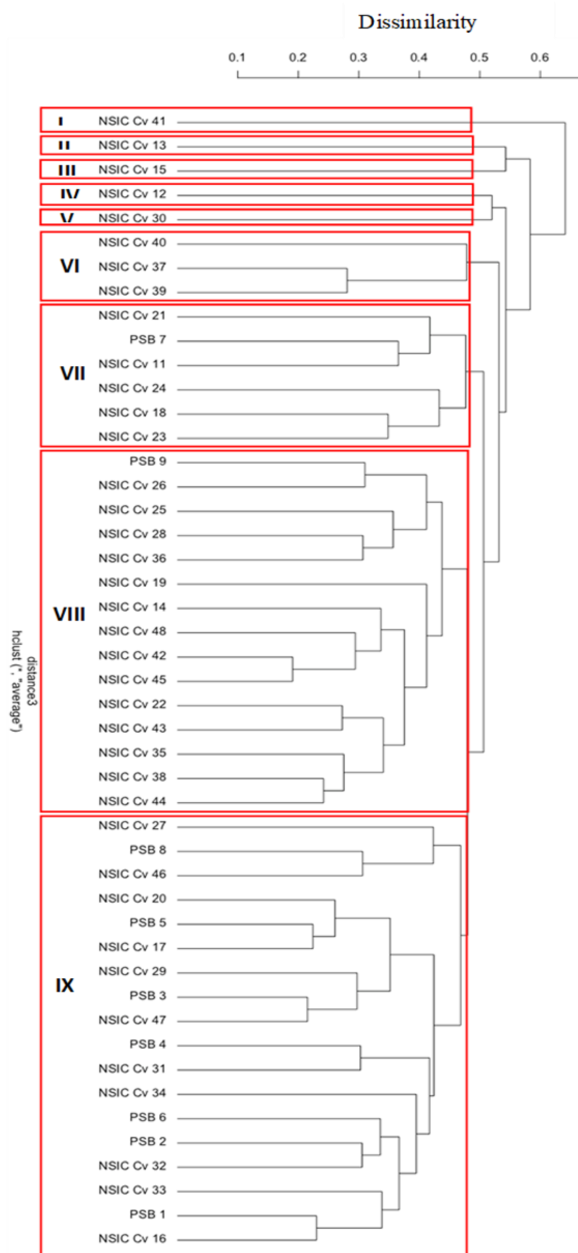

Fig. 1. Dendrogram of 47 NSIC-released cassava (*Manihot esculenta* Crantz) varieties that generated nine clusters at 0.50 dissimilarity coefficient based on morphological data.

These studies were able to determine and evaluate the genetic variability and relatedness among cassava genotypes using morphological traits in which genotypes were grouped into distinct clusters. Thus, morphological characters are useful tools for preliminary evaluation for assessing the extent of diversity (Lekha et al. 2011).

Molecular Characterization

A total of 50 cassava-based SSR primers showed polymorphic amplifications. A profile of 47 cassava varieties run in 8% PAGE gel stained with GelRed™ showing the banding pattern generated by SSR 135 is shown in Figure 2.

DNA Fingerprinting of 47 Cassava Varieties Released by NSIC

Unique banding patterns were observed for each primer. Out of 648 alleles, a total of 764 unique banding patterns were recorded ranging from 0 to 44 for each primer (Table 2). Primer CA 18 had the greatest number of unique banding patterns (44) and as a result was able to discriminate 44 varieties except for PSB 9, NSIC 11 and NSIC 13 which exhibit the same banding pattern. On the other hand, primers MeESSR 2 and MeESSR 23 were not able to give unique banding patterns and thus were not able to differentiate the varieties. This shows that each primer varies in distinguishing capacity at the genetic level.

Based on the resulting bands scored from the 50 polymorphic primers, the molecular identity of the 47 cassava varieties released by NSIC was established. The resulting amplification patterns of 50 cassava-based primers were analyzed. Only the unique banding patterns amplified per primer were used to construct the DNA fingerprint of each variety. Table 1 shows the allele banding profile of the 47 cassava varieties released by the NSIC.

DNA fingerprinting is important for identification of cultivars and species, assessment of genetic diversity, detection of somaclonal variations, and selection of parents with wider genetic base (Bhat 2008). With the precise identification of genotypes with minimal environmental influence, DNA fingerprints can be applied for implementation of the Intellectual Property Rights (IPR) and the Plant Variety and Farmers Rights Protection Act (PVFRPA) for various crops. Disputes over the true identity of varieties can be resolved since comparison of fingerprints can be done to distinguish one variety from another. Furthermore, DNA fingerprinting could be applied to show whether or not the variety which the farmer planted would demonstrate its expected performance (Bhat 2008; Shivakumar et al. 2014).

Cluster Analysis using Simple Sequence Repeat (SSR) Primers

The banding profiles of the 50 polymorphic SSR markers were used to generate a dendrogram using UPGMA in R, a language and environment for statistical computing and graphics. The Jaccard coefficient was used to determine the genetic distance between varieties. The dendrogram revealed six clusters at a dissimilarity coefficient of 0.7 (Fig. 3).

At 0.35 dissimilarity, NSIC 23 and NSIC 24 grouped

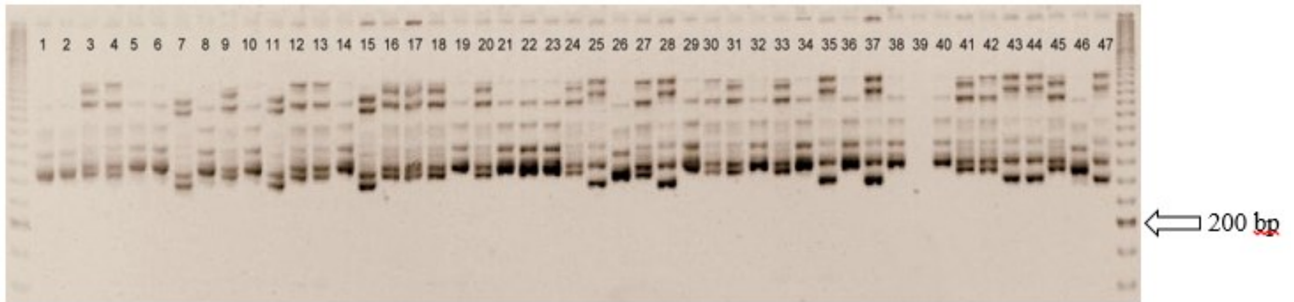

Fig. 2. Representative PAGE gel (8%) stained with GelRed™ showing the banding patterns generated by SSR 135 primer in 47 cassava (*Manihot esculenta* Crantz) varieties released by the NSIC.

together. These varieties were also clustered together using the morphological data at 0.5 dissimilarity. In addition, varieties developed by UPLB, namely, PSB 1, PSB 2, PSB 3 and PSB 6 grouped in Cluster VI. These varieties were also clustered together in the dendrogram based on morphological data.

Thirty-three varieties belonging to clusters XIII and IX of the morphological dendrogram dispersed in the six clusters of the molecular dendrogram. NSIC 42 and NSIC 45 were grouped together at 0.2 dissimilarity in the dendrogram generated through morphological data showing that these varieties were closely related morphologically. However, these two varieties clustered together at 0.7 dissimilarity using molecular data, which shows that the genotypes were distantly related genetically. It indicates that, compared with morphological descriptors, SSR markers show higher sensitivity in detecting variability between genotypes.

Cassava is known to be a highly heterozygous crop (Duputie et al. 2007). Therefore, individuals with the same genetic background may exhibit phenotypic similarities in morphology but differences in genetic composition. The data of this present study can be used in selecting genotypes for the development of a base population to be used in cassava breeding programs.

Studies using SSR markers have been reported in determining the extent of genetic diversity in cassava (Beovides et al. 2015; Lyimo et al. 2012; Raghu et al. 2007; Mendoza et al. 2016). Beovides et al. (2015) found wide diversity in cassava accessions and suggested that this information can be used for genetic diversity conservation and genotype identification studies for the genetic breeding program of cassava. The findings of this study concurred with the studies of Lyimo et al. (2012) and Mendoza et al. (2016); both morphological and molecular data were able to group varieties in distinct clusters. On the other hand, Raghu et al. (2007) pointed out that, even though morphological data were able to group cassava genotypes, SSR markers were able to detect the existence of a wide genetic diversity among cassava accessions compared with morphological data. This study is in agreement with the results of previous studies. Relatively high (70%) dissimilarity between clusters was recorded, indicating high diversity among cassava varieties. This information can be used for future cassava breeding programs. Unrelated accessions with good traits can be used as parentals for hybrid development without exposing lethal recessive traits.

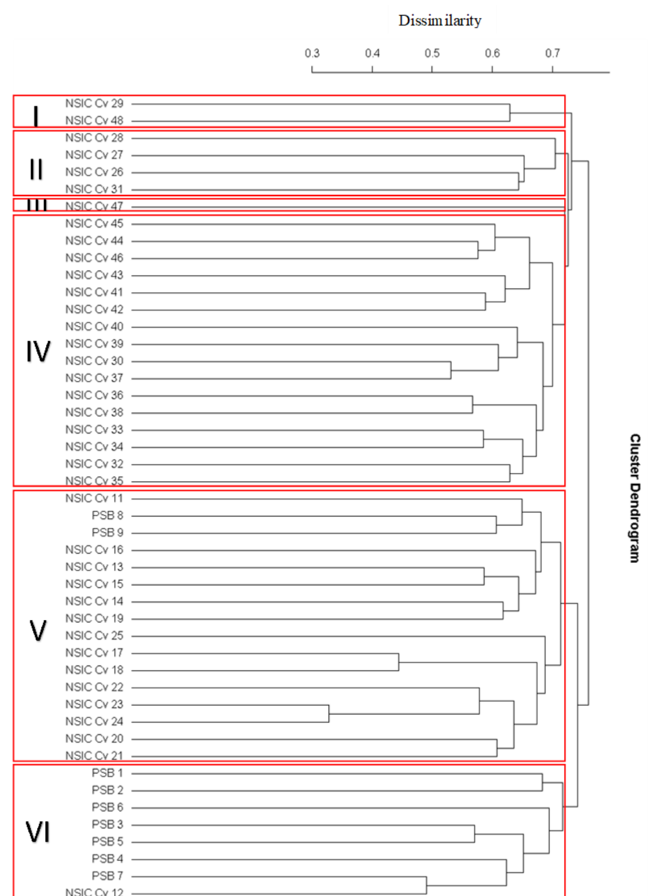

Fig. 3. UPGMA dendrogram of 47 NSIC-released cassava (*Manihot esculenta* Crantz) varieties that generated six clusters at 0.70 dissimilarity coefficient based on molecular data.

Table 2. Allele banding profile of the 47 cassava (*Manihot esculenta* Crantz) varieties released by the National Seed Industry Council (NSIC).

Variety	No. of Primers that Generated Unique Banding Pattern	No. of Amplified Bands per Primer	Total No. of Bands Observed	Range of Amplicon (BP)
PSB1	16	2-6	56	140-560
PSB2	17	1-8	61	80-480
PSB3	17	1-6	53	110-460
PSB4	17	2-6	57	70-420
PSB5	17	1-5	60	110-460
PSB6	22	1-6	74	70-450
PSB7	16	1-7	53	70-540
PSB8	13	2-5	51	130-450
PSB9	14	2-7	56	120-470
NSIC cv. 11	11	3-5	46	100-480
NSIC cv. 12	13	1-5	43	70-410
NSIC cv. 13	16	2-7	62	80-410
NSIC cv. 14	20	1-6	78	140-440
NSIC cv. 15	16	2-9	67	120-450
NSIC cv. 16	26	1-7	110	130-520
NSIC cv. 17	22	1-9	94	120-450
NSIC cv. 18	17	2-8	69	100-450
NSIC cv. 19	14	1-5	51	150-440
NSIC cv. 20	14	1-6	54	130-430
NSIC cv. 21	14	2-6	52	100-410
NSIC cv. 22	14	1-7	58	160-530
NSIC cv. 23	14	2-9	58	150-410
NSIC cv. 24	14	1-8	59	140-440
NSIC cv. 25	15	1-6	59	120-480
NSIC cv. 26	21	1-6	71	120-480
NSIC cv. 27	17	1-6	64	90-480
NSIC cv. 28	23	2-7	88	120-550
NSIC cv. 29	23	2-7	90	100-470
NSIC cv. 30	15	1-8	67	160-480
NSIC cv. 31	15	1-6	50	100-490
NSIC cv. 32	14	1-6	54	130-460
NSIC cv. 33	13	1-7	57	150-470
NSIC cv. 34	11	2-6	44	170-460
NSIC cv. 35	15	1-7	52	130-520
NSIC cv. 36	18	1-7	59	100-470
NSIC cv. 37	14	1-6	61	160-490
NSIC cv. 38	17	3-9	77	120-440
NSIC cv. 39	14	2-7	78	150-460
NSIC cv. 40	14	1-7	77	90-470
NSIC cv. 41	15	2-7	60	100-510
NSIC cv. 42	17	2-8	71	100-510
NSIC cv. 43	16	1-7	58	130-430
NSIC cv. 44	14	1-7	49	130-520
NSIC cv. 45	11	1-5	37	120-380
NSIC cv. 46	14	1-6	48	110-400
NSIC cv. 47	20	1-6	64	130-390
NSIC cv. 48	17	1-6	59	130-510

Polymorphism Information Content (PIC)

To estimate how useful and informative the SSR markers for characterization and variability assessment are, the PIC was computed based on binary data and the resulting SSR banding patterns.

In this study, the PIC value of the 50 SSR markers ranged from 0.4634 to 0.9513 with an average of 0.8323 (Table 3). The higher the PIC value, the better is the primer. CA 18 had the highest PIC value (0.9513) while the lowest PIC value was observed in MeESSR 23 (0.4634). This result indicated that these primers are useful to determine the genetic differences among the cassava varieties.

CONCLUSION

Both morphological descriptors and SSR primers were able to detect genetic relatedness and variability among cassava varieties. However, SSR markers are more effective in measuring the genetic variability (0.7 dissimilarity) among the cassava varieties than morphological data (0.50 dissimilarity). Nonetheless, morphological characters are useful tools for preliminary evaluation and characterization of genotypes to assess the extent of diversity. In addition, SSR markers used in this study provide a positive assessment of their ability to generate unique DNA fingerprints which act as the molecular identities of cassava varieties. The data obtained can be used for varietal identification and the construction of a data base of released cassava varieties. Moreover, the genetic variability detected can be used for future cassava breeding programs.

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Table 3. Polymorphic Information Content (PIC) of 50 SSR primers that amplified bands in cassava (*Manihot esculenta* Crantz) varieties.

Name	Size (BP)	Primer Sequence	AT (ºC)	Total No. of Alleles	No. of Unique Banding Patterns	PIC
MeESSR2	235-246	F:GTGCGTGGGTTTGTTTTCTT R:TGAAAAGATTGACCTGCGTCT	58	5	0	0.6547
MeESSR126	119-130	F:AGGAAATTGGCAGTGGATTG R:CCAGATGAATCTTTCACCTTATCA	59	5	1	0.7129
MeESSR66	221-230	F:AACACCTCGATTCCTTGTGG R:GCGGTATTATCCCCTCCATT	60	15	12	0.8376
MeESSR60	162-174	F:GCGGAAGAAGAAGGTGACAG R:CCAAACGAAACAAAAATGTGC	61	11	5	0.8554
MeESSR96	239-249	F:AAAAAGAGTCGTCGGCAAAG R:GAAGCAACATTGATTGTACGTCTT	62	16	19	0.9344
MeESSR10	132-159	F:TTTTTCCCTGCTGGCTTAGA R:TTGCAGCACCCATACTGAAG	63	6	3	0.6684
MeESSR31	166-172	F:TCTTTCTGCAACGAAGAGGAA R:TGCAGAGAAACAGGCAAAAA	64	7	8	0.7628
MeESSR26	244-255	F:CGGAAATGACGAAAGAAAGG R:AATTCCAATTCCACCCACAC	65	15	35	0.9128
MeESSR15	150-180	F:TTCGCCTTTCTCATAGCTCAA R:ATGCATCTGCATGCCTATTT	66	8	4	0.7759
MeESSR23	209-216	F:GCTGAGGTTCTGCTGGTTTC R:CGGAGGATTTCACTGAGGAC	67	3	0	0.4634
MeESSR19	202-227	F:TTCTCGTCGGCTCCTTTCTA R:CCCCACTTGATCTGCCTTTA	68	13	17	0.8978
SSRY13	234	F:GCAAGAATTCCACCAGGAAG R:CAATGATGGTAAGATGGTGCAG	55	7	2	0.7894
SSRY19	214	F:TGTAAGGCATTCCAAGAATTATCA R:TCTCCTGTGAAAAGTGCATGA	55	12	25	0.8630
SSRY20	143	F:CATTGGACTTCCTACAAATATGAAT R:TGATGGAAAGTGGTTATGTCCTT	55	13	10	0.7730
SSRY21	192	F:CCTGCCACAATATTGAAATGG R:CAACAATTGGACTAAGCAGCA	55	11	9	0.8766
SSRY38	122	F:GGCTGTTCGTGATCCTTATTAAC R:GTAGTTGAGAAAACTTTGCATGAG	55	7	3	0.7751
SSRY45	228	F:TGAAACTGTTTGCAAATTACGA R:TCCAGTTCACATGTAGTTGGCT	55	16	14	0.8435
SSRY51	298	F:AGGTTGGATGCTTGAAGGAA R:GGATGCAGGAGTGCTCAACT	55	16	16	0.8641
SSRY64	194	F:CGACAAGTCGTATATGTAGTATTCACG R:GCAGAGGTGGCTAACGAGAC	55	17	38	0.9058
SSRY69	239	F:CGATCTCAGTCGATACCCAAG R:CACTCCGTTGCAGGCATTA	55	18	25	0.8899
SSRY100	210	F:ATCCTTGCCTGACATTTTGC R:TTCGCAGAGTCCAATTGTTG	55	17	15	0.9017
SSRY101	213	F:GGAGAATACCACCGACAGGA R:ACAGCAGCAATCACCATTTC	55	9	8	0.7943
SSRY103	272	F:TGAGAAGGAAACTGCTTGCAC R:CAGCAAGACCATCACCAGTTT	55	19	40	0.9255
SSRY135	253	F:CCAGAAACTGAAATGCATCG R:AACATGTGCGACAGTGATTG	45	17	16	0.8995
SSRY148	114	F:GGCTTCATCATGGAAAAACC R:CAATGCTTTACGGAAGAGCC	45	7	3	0.7284
SSRY161	220	F:AAGGAACACCTCTCCTAGAATCA R:CCAGCTGTATGTTGAGTGAGC	55	22	25	0.8969
SSRY171	291	F:ACTGTGCCAAAATAGCCAAATAGT R:TCATGAGTGTGGGATGTTTTTATG	55	20	41	0.9343
SSRY175	136	F:TGACTAGCAGACACGGTTTCA R:GCTAACAGTCCAATAACGATAAGG	55	7	12	0.8169
SSRY181	199	F:GGTAGATCTGGATCGAGGAGG R:CAATCGAAACCGACGATACA	55	15	38	0.8978
GA5	120-130	F:TAATGTCATCGTCGGCTTCG R:GCTGATAGCACAGAACACAG	60	8	5	0.7968
GA12	140-150	F:GATTCCTCTAGCAGTTAAGC R:CGATGATGCTCTTCGGAGGG	57	9	10	0.8507
GA21	110-120	F:GGCTTCATCATGGAAAAACC R:CAATGCTTTACGGAAGAGCC	62	7	3	0.8783

Table 3. Continuation ….

Name	Size (BP)	Primer Sequence	AT (ºC)	Total No. of Alleles	No. of Unique Banding Patterns	PIC
GA126	170-210	F:AGTGGAAATAAGCCATGTGATG R:CCCATAATTGATGCCAGGTT	57	21	31	0.8984
GA127	210-235	F:CTCTAGCTATGGATTAGATCT R:GTAGCTTCGAGTCGTGGGAGA	59	12	14	0.8984
GA131	95-140	F:TTCCAGAAAGACTTCCGTTCA R:CTCAACTACTGCACTGCACTC	54	7	5	0.7318
GA134	195-310	F:ACAATGTCCCAATTGGAGGA R:ACCATGGATAGAGCTCACCG	52	15	15	0.8458
GA136	145-165	F:CGTTGATAAAGTGGAAAGAGCA R:ACTCCACTCCCGATGCTCGC	64	9	14	0.8424
GA140	150-165	F:TTCAAGGAAGCCTTCAGCTC R:GAGCCACATCTACTCGACACC	62	4	2	0.5974
R16(III)F2/R1		F: AAGAGTGGAAAAACTCCC R: TCCGAACTGAGATTGA	55	29	23	0.9357
SSRY28		R: TCCGAACTGAGATTGA R: GCTGCGTGCAAAACTAAAAT	55	12	15	0.8511
SSRY63		F: AAGACAATCATTTTGTGCTCCA R: TCAGAATCATCTACCTTGGCA	55	11	12	0.8369
SSRY106		F: CAGCAAGACCATCACCAGTTT R: GGAAACTGCTTGCACAAAGA	55	18	30	0.8228
SSRNS158		F: GTGCGAAATGGAAATCAATG R:TGAAATAGTGATACATGCAAAAGGA	55	7	11	0.8194
SSRNS169		F: GTGCGAAATGGAAATCAATG R: GCCTTCTCAGCATATGGAGC	55	21	21	0.9092
SSRY239		F: TTTCTCAATAGACAGACGAGCA R: TGCATTTCCCTGGGTGTAAG	55	7	6	0.7972
SSRY250		F: AATTGGAAGGGAAAGCCAAA R: GATCGGATGTCTGAGGAGGA	55	11	5	0.8152
UV-AL3		F: TACACATGCCTCRAATCCTG R: CTCCGCCACAAACTTACGTT	55	23	20	0.9357
CA18		F: AATTGTAGCTGTTGCCCCAC R: TATGGCAATGGGAGGTCATT	55	36	44	0.9513
CA141		F: GTTGTCGTTGAGGCCTTGTT R: TGACAAAATCACCCATCCCT	55	19	27	0.8977
CA227		F: GCTGGAAAATTTGGATTGGA R: TGCAACCCAACCACTTATGA	55	8	7	0.7534

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