British Biotechnology Journal
4(5): 551-565, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Molecular Relatedness and Morpho-Agronomic Characteristics of Congolese Accessions of Cassava (*Manihot esculenta* Crantz) for Breeding Purposes

G. Mamba-Mbayi[1,2], K. K. Nkongolo[3*], R. Narendrula[3], P. Tshilenge Djim[1] and A. Kalonji-Mbuyi[1,4]

[1]Unit of Phytopathology, Faculty of Agronomy, University of Kinshasa, P. O. Box 117, Kinshasa XI, DR-Congo.
[2]Unit of Seeds Pathology, National Seeds Testing Laboratory, P.O. Box 15280 Kinshasa I, DR-Congo.
[3]Department of Biology, Faculty of Sciences and Engineering, Laurentian University, Sudbury, Ontario, P3E 2C6, Canada.
[4]Department of Genetic and Plant Breeding, Regional Nuclear Energy Center, Kinshasa, P.O. Box 868, Kinshasa XI, DR-Congo.

Authors' contributions

This work was carried out in collaboration between all authors. Author GMM conducted the field trials, collected and analyzed the data and assisted in writing the first draft of the manuscript. Author KKN supervised the molecular experiments and wrote the manuscript. Author RN performed the molecular analysis and assisted with manuscript formatting. Author PTD supervised the experimental trials and data analysis in the DR-Congo. Author AKM monitored the field experiments in the DR-Congo and reviewed the manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 11th October 2013
Accepted 23rd January 2014
Published 12th May 2014

ABSTRACT

Cassava serves as primary staple food of millions of people in the tropics and subtropics, and is used as a carbohydrate source in animal feed. Knowledge of agro-morphological characteristics and genetic relatedness is essential for an efficient recombination of varieties in a breeding program. The objective of the present study was to determine genetic relatedness and morpho-agronomic differentiation among Congolese cassava

**Corresponding author: Email: knkongolo@laurentian.ca;*

collection for breeding purposes. The morphological and agronomic characters were highly variable among accessions. Every accession could be differentiated from any other one. There were significant genotypes x location interactions for storage root yields. Root weights were positively correlated with the number of roots per plant. In general, all the improved varieties were tolerant or resistant to the Cassava Mosaic Virus (CMV) while the local (non-improved) varieties were susceptible. But the reaction to Cassava Bacterial Blight (CBB) confirmed that genetically improved accessions are susceptible and local varieties are resistant. Molecular analysis revealed that the accessions analyzed were genetically distant with 80% of genetic distance values estimated above 0.5. One local accession was an out-group that was separated from the main groupings with 100% degree of confidence. More importantly, there were no associations between genetic relationships and morphological similarities based on lobe shape, leaf colour, petiole colour, petiole orientation, and stem colour. Although the Congolese cassava genepool is small, there is enough variability to sustain a breeding program without new introductions of germplasms.

Keywords: Morpho-agronomic traits; cassava (Manihot esculenta crantz); inter-simple sequence repeat (issr); DR-Congo.

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a major staple crop in Sub-Saharan Africa [1,2]. Recognized as a plant tolerant to marginal soils and climatic conditions, cassava is usually grown as a subsistence crop by small farmers [2-7]. There are many cassava accessions in several international and national germplasm banks. The largest germplasm collection with over 4700 accessions is located in Colombia at 'Centro International de Agricultura Tropical' (CIAT). This is followed by Embrapa's collection in Cruz das Almas, Bahia, Brazil [5]. In the DR-Congo (DRC), cassava is both a source of food and income for over 70% of population. This crop is grown in more than 50% of arable land for food crops in the DR-Congo [8]. No concerted effort has been made to assess the genetic diversity of this crop in this country. Two main research stations of the Congolese National Institute of Agronomic Studies and Research (INERA) at Mvuazi (Bas Congo) and Gandajika (Eastern Kasai) are responsible for maintaining an official cassava collection.

Evaluating genetic diversity and further characterizing cassava populations is an invaluable asset in the genetic improvement strategies of cassava. An accurate assessment of genetic relatedness can lead to the identification of new combinations with maximum genetic variability, which can then be used for further selection and introgression of desirable genes from diverse germplasm into the available genetic base [9-12].

Morpho-agronomics characteristics are the most widely used information to compare accessions [5]. But such markers are not reliable since they are affected by genotype by environment interactions. A number of molecular methods such as restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNAs (RAPD), amplified fragment length polymorphisms and, most recently (AFLP), single-nucleotide polymorphisms (SNP) and single sequence repeat (SSR) markers (also known as microsatellites) have been used to study cassava diversity [13,14]. Moreover, the complete nucleotide sequence of the cassava (*Manihot esculenta*) chloroplast genome has been established [15]. There is no reported analysis of cassava accessions using inter-simple sequence repeat (ISSR).

Since its introduction in 1994, the ISSR system has grown in popularity and has superseded RAPD method [16]. This molecular marker system is based on the use of a 15-20bp primers designed to be complimentary to microsatellite sequences found throughout the eukaryotic genome, therefore providing information at a number of different loci. It combines the advantages of SSRs and amplified fragment length polymorphism (AFLP) to the universality of random polymorphic DNA (RAPD). Also, as a result of the longer lengths of ISSR primers in comparison to RAPD primers, the required annealing temperatures are higher and as such, non-specific binding is reduced and banding patterns have higher reproducibility [17,18].

The objective of the present study was to determine genetic relatedness and morpho-agronomic differentiation among Congolese cassava collection for breeding purpose. The usefulness of ISSR markers in assessing genetic variability among cassava accessions was established.

2. MATERIALS AND METHODS

2.1 Field Experimentations

The trial was conducted at two locations in a savannah region in Gandajika in the DR-Congo. Site 1 was located in Mpiana (23° 56'E, 06° 36'S and 685m altitude) and site 2 at INERA research station (23° 57'E, 06° 48'S and 754 m altitude). Gandajika region falls within the Aw4 climate type according to Köppen classification characterized with four months of dry season (from mid-May to august) coupled with eight months of rainy season, sometimes interrupted by a short dry season in January/February. Daily temperature averages 25°C and annual rainfall is close to 1500mm.

In general, the soil of the targeted locations of Eastern Kasaï (Gandajika) is made up of a sandy overlay on loamier sediment that often rests at low depth on an ancient lateritic slab. The adsorbing complex is relatively well saturated and it remains still some alterable minerals. The clay fraction less important seems not only constituted of kaolinite alongside in relation to the depth of loamy sediment. The site topsoil has a high rate of gravels and very few fine elements [19].

The plant material consisted of 12 cassava (*Manihot esculenta* Crantz) accessions that included seven genetically improved (M'vuazi, Nsansi, Gandajika, Butamu, Mbankana, Disanka and Zizila from lumberyards of IITA/PRONAM) and five local cassava cultivars (Tshilobo, Kamana Mabanza, Luenyi, Lac Lomba and Sadisa) widely used by small farmers in the targeted region. Data on lobe shape, petiole orientation, root shape, root taste, and leaf, petiole, and stem colour were collected from experimental plots as well as farmer's fields as described in Alves (5]. For field trials, the experimental design was a randomized complete block design (RCBD) with three replications at both sites 1 and 2.

To test the reaction of the cassava accessions to the cassava bacterial blight (CBB), two field trials were conducted using three artificial inoculations methods. The experimental design was a split plot with three replications, with cassava cultivars as main units and methods of inoculation of culture by the bacterial strain as subunits.

The first inoculation method consisted in the injection of *Xanthomonas axonopodis* pv *manihotis* inoculum with a hypodermic syringe. A 0.5ml of the inoculum suspension with

approximately 10^8 CFU/ml was injected in the stem between the third and the fourth leaf using a hypodermic needle. The second method consisted in cutting leaves with scissors infected with a *Xanthomonas axonopodis* pv *manihotis.* Scissors were dipped in an inoculum suspension (10^8 CFU/ml) and were used to cut cassava leaves. The cuts were made approximately 1cm from the margin of the third or fourth leaves. The leaves were washed with sterile distilled water and kept at 24°C and 70% to 80% humidity level to remove surface microflora prior to inoculation with infected scissors. The last method consisted in spraying the inoculum of cassava leaves. Leaves were sprayed using a 0.5L portable pressure sprayer. Approximately 2.5mL of the inoculum suspension (10^8 to 10^{10} CFU/ml) were sprayed on the surface of the third and the fourth leaves. Mortality rate of cassava plants was recorded once a week for 8 weeks. Only the data at 56 days (or 8 weeks) after infestation (DAI) were used to compare the 12 accessions. The values were transformed with log (x +1) to standardize variances.

To assess storage root diameter, length, number and weight, the cassava accessions were planted in a randomized complete block design (RCBD) with three replications at the two sites (Mpiana and INERA). Each variety represented a treatment. Hardwood stem cuttings of each accession were planted in a plot of 30 plants at spacing of 1mx1m, resulting in a population of 10,000 plants ha^{-1}. Each plot measured 5mx4m. Plots were weeded whenever necessary and no pesticide or fertilizer was applied throughout the period of the study.

Morpho-agronomic data including root weight, diameter, length and the number of roots were subjected to the analysis of variance (ANOVA) using the the R version 2-9-0 and Statistix 8 software. Main effects were separated by least significant differences (LSD) at the P=0.05 level.

2.2 Molecular Analysis

2.2.1 DNA extraction

Genomic DNA was extracted from cassava seedlings derived from the 12 accessions using a modified CTAB extraction protocol described by Nkongolo et al. [20,21] and Mehes et al. [22].The modifications included the addition of 1% polyvinyl pyrrolidone (PVP) and 0.2% beta mercaptanol to the cetyl trimethylammonium bromide (CTAB) buffer solution, two additional chloroform spins prior to the isopropanol spin and no addition of RNAse. After extraction, DNA was stored in a freezer at -20°C.

2.2.2 ISSR analysis

A total of 15 ISSR primers were pre-screened for polymorphism and reproducibility. Of these, seven primers that produced strong and reproducible bands were selected for ISSR analysis. These included 17899A, 17899B, UBC 829, UBC 825, ISSR 5, 7, and 9. PCR amplification was carried out as described by Mehes et al. [22] in a 25µL total volume containing a master mix of 11.4µL distilled water, 2.5µL $MgSO_4$, 2.1µL 10x buffer 0.5µL of dNTPs (equal parts dTTP, dATP, dCTP, dGTP), 0.5µL of ISSR primer, a *Taq* mix of 3.475µL distilled water, 0.4µL 10x buffer and 0.125µL Taq polymerase (Applied Biosystems) and 4µL standardized DNA. Each primer contained a negative control of master mix and Taq mix without any DNA. All samples were covered with one drop of mineral oil to prevent evaporation and amplified with the Eppendorf Mastercycler gradient thermocycler. The program was set to a hot start of 5minutes at 95°C followed by 2minutes at 85°C, then 42 cycles of 1.5minutes at 95°C, 2minutes at 55°C and one minute of 72°C after the addition of

the Taq mix. A final extension of 7minutes at 72°C after which samples were removed from thermocycler and placed in the -20°C freezer until further analysis.

Amplified DNA products were separated for analysis on a 2% agarose gel and stained in ethidium bromide. Then, 5µL of 2x loading buffer were added to the PCR products and 10µL of this solution were loaded into the wells of the gel. The gel was run at 64V for 120minutes, documented with the Bio-Rad Chemi Doc XRS system and analyzed with Image Lab Software.

These agarose gels were visualized under UV light source, documented with the Bio-Rad ChemiDoc XRS system and analyzed for band presence or absence with the Discovery Series Quantity One 1D Analysis Software. The ISSR bands on each gel were scored as either present (1) or absent (0).

The resulting data matrix of the ISSR phenotype was analyzed using POPGENE software (version 1.32) to estimate genetic diversity parameters [23]. POPGENE is software designed to enable analysis of genetic variation among and within populations using co-dominant and dominant markers and quantitative traits. The program was used to determine the inter-varietal diversity parameters such as percentage of polymorphic loci (P%), Nei's gene diversity (h), and Shannon's information index (I). The genetic distances were calculated using Jaccard's similarity coefficients with the Free Tree Program version 1.50. A Neighbour-Joining dendrogram was produced from the similarity coefficients [24].

3. RESULTS AND DISCUSSION

3.1 Morphological Characterization

The cultivated forms of cassava belong to the species *Manihot esculenta* Crantz, which derive from the wild populations of *Manihot esculenta* subsp. *flabellifolia* [18]. The morphological characteristics of cassava are highly variable, which indicates a high degree of interspecific hybridization. This perennial shrub of 1 to 5m height of the family *Euphorbiaceae*, is cultivated mainly for its starchy roots. The roots are adventitious and develop to a fibrous root system. Some roots bulk and become storage roots, while the remaining ones are involved in water and nutrient absorption [5,25].

The cassava genotypes are usually characterized on the basis of morphological and agronomic descriptors. International Plant Genetic Resources Institute (IPGRI) defined 75 cassava descriptors, 54 being morphological and 21 agronomic [5,26]. In the present study, colour variables were the most salient characters used by farmers to identify varieties. Qualitative characteristics are described in Table 1. There was high variability among cassava varieties used for morphological characters. Leaves from all the accessions analyzed were either dark green or light green. Lobe shape was ovoid, lanceolate, oblong-lanceolate, obovate-lanceolate, and elliptic-lanceolate. Petiole color was red, purple, green-yellow, green-red, and red-green. Stem color were green-yellow, orange, gold or light-brown. Leaf petioles were horizontal, inclined upward, or downward, or irregular. Roots at maturity were cylindrical, conical-cylindrical or irregular.

A subset of the morphological and agronomic characters was used to characterise two groups of accessions that included genetically improved and local varieties. There was no association between morphological characteristics and breeding status of the accessions

(local vs genetically improved) used. The local varieties were in general sweet in taste while the genetically improved were either sweet or bitter. In general, morphological traits such as stem colour, leaf colour, lobe shape have a higher heritability than agronomic such as root length and yield which are more affected by environmental conditions.

3.2 Agronomic Characteristics

Cassava roots are the main storage organ. There was variability in root storage characteristics among the accessions analyzed (Tables 1 and 2). The genetically improved variety Mbankana has the highest storage root yield at site 1 with an average of 10.11kg/root, and Zizila (also an improved variety) at site 2 (INERA) with 21.08kg/root. The local variety Luenyi had the lowest root weight at sites 1 and 2 with 1.87kg/root and 3.7, respectively (Table 2). There were significant genotypes x location interactions for storage root yields. For example, the improved variety Zizila had a low root weigh of 3.58kg/root at site 1 but had the highest root yield at site 2. Likewise, the local accession Gandajika was among the bottom three accessions for root weight at site 1 but was among the top 5 at site 2. The storage root yields for other local varieties (Tshilobo, Lac Lomba and Kamana Mabanza) were statistically similar to those of several improved varieties under trial conditions. Weight of storage roots per plants were positively correlated with number of roots at both sites (Table 3). This is consistent with Ntawuruhunga et al. [27] who identified cassava root number and root weight as the main components of storage root yields.

With the exception of Sadisa, all the improved varieties were tolerant or resistant to the cassava mosaic virus and the local (non-improved) varieties were susceptible. But the reaction to CBB showed that in general genetically improved accessions were susceptible and local varieties resistant (Fig. 1, Table 1). Resistance to CBB in *M. esculenta* introgressed from a wild relative, *M. glaziovii*, is polygenic and additively inherited [28]. Likewise, CMV resistance is controlled by several genes [29]. Thus, environmental conditions play an important role in the expression and the incidence of these two diseases. The reactions to CMM of the cassava accessions tested are consistent with an earlier report by Muengula et al. [30]. An assessment of the reaction to BCC for the 12 accessions was conducted under an artificial inoculation of *Xanthomonas axonopodis* pv. *manihotis*. Three inoculation methods of *Xanthomonas axonopodis* pv. *manihotis* were used to compare the cassava accessions. They included syringe infiltration, leaf wounding with infected scissors and foliar spraying of inoculum. The first two methods assess the resistance to secondary infection (type 2 resistance) while the last determine the resistance to primary infection (Type 1). Type I resistance is referred to as resistance to penetration, which prevents initial pathogen penetration; Type II is resistance to spread within plant, where spread of the pathogen is limited to the inoculation spot [31]. Mortality rate 56 days after infestation (DAI) was the only criterion used to compare the 12 accessions for resistance to CBB. Kamana Mabanza, Tshilobo and Luenyi, three local varieties were the most resistant to both secondary and primary infections. All the genetically improved varieties showed different levels of susceptibility to CBB.

Table 1. Lobe, leaf, petiole, stem, and root characteristics along with disease resistance of 12 accessions of cassava from the DR-Congo

Cultivars	Lobe shape	Leaf colour	Petiole color	Stem color	Petiole orientation	Root shape	Root taste	Field resistance to CMV	Field resistance to CBB
Genetically Improved									
Butamu	Ovoid	Dark green	Green-Yellow	Orange	Horizontal	Conical-cylindrical	Sweet	Tolerant	Tolerant
Disanka	Oblong-lanceolate	Light green	Green-yellow	Green yellow	Inclined upwrad	Cylindrical	Bitter	Resistant	Resistant
Gandajika	Obovate-lanceolate	Dark green	Green-red	Green yellow	Horizontal	Cylindrical	Bitter	Susceptible	Susceptible
Mbankana	Oblong-lanceolate	Light green	Red	Gold	Irregular	Cylindrical	Bitter	Tolerant	Tolerant
M'vuazi	Ovoid	Dark green	Purple	Light brown	Inclined downward	Cylindrical	Bitter	Resistant	Tolerant
Nsansi	Ovoid	Light green	Green-yellow	Green yellow	Inclined upward	Cylindrical	Sweet	Tolerant	Tolerant
Sadisa	Lanceolate	Dark green	Red-green	Green yellow	Inclined downward	Cylindrical	Bitter	Susceptible	Susceptible
Zizila	Elliptic-lanceolate	Light green	Red	Green yellow	Inclined upwards	Cylindrical	Sweet	Resistant	Tolerant
Local Variety									
Kamana Mabanza	Lanceolate	Light green	Red	Green yellow	Irregular	Irregular	Sweet	Susceptible	Tolerant
Lac Lomba	Lancceolate	Light green	Red	Green yellow	Inclined downward	Irregular	Sweet	Susceptible	Susceptible
Luenyi	Obovate-lanceolate	Light green	Red	Green yellow	Inclined downward	Cylindrical	sweet	Susceptible	Tolerant
Tshilobo	Oblong-lanceolate	Dark green	Red	Green yellow	Irregular	Cylindrical	Sweet	Suscepible	Tolerant

CMV= Cassava mosaic virus; CBB=cassava bacterial blight

Table 2. Weight, diameter, length and number of cassava storage roots

Cassava Accessions	Site 1 (Mpiana)				Site 2 (INERA)			
	Number of roots	Weight of roots (kg)	Diameter of roots (cm)	Length of roots (cm)	Number of roots	Weight of roots (kg)	Diameter of roots (cm)	Length of roots (cm)
Butamu	7.33	6.18	25.37	33.48	6.75	5.51	27.16	37.33
Disanka	6.17	5.08	25.46	24.42	20.00	17.83	21.17	38.17
Gandajika	5.90	2.91	25.02	37.37	21.09	14.00	21.17	44.00
K-Mabanza*	6.25	4.33	23.17	28.00	19.75	12.42	23.75	34.84
Lac Lomba	5.42	6.06	25.09	33.88	5.42	6.06	25.09	33.88
Luenyi	5.42	1.87	17.82	31.48	5.67	3.37	23.79	35.09
Mbankana	11.33	10.11	18.90	30.32	20.09	10.00	23.08	35.50
M'vuazi	7.34	7.04	22.46	38.20	7.34	7.04	22.46	38.18
Nsansi	6.17	5.10	21.50	35.63	8.09	6.27	23.44	38.96
Sadisa	6.50	4.67	24.73	38.93	5.75	3.83	21.81	30.17
Tshilobo	7.17	7.82	22.46	31.61	22.58	17.80	24.84	29.00
Zizila	6.72	3.58	21.05	38.86	42.67	21.08	24.08	53.75
$LSD_{(p=0.05)}$	4.00	5.10	6.60	11.80	8.90	5.70	NS	16.8

** K-Mabanza represents Kamana Mabanza*

Table 3. Spearman correlation coefficients among weight, diameter, length, and number of storage cassava roots at site 1 – Mpiana and site 2 – INERA (in parenthesis) in Gandajika, DR- Congo

Root measures	1	2	3	4
1	0(0)	0.62*(0.86**)	-0.15 (0.15)	0.02(0.54*)
2		0(0)	0.02 (0.13)	-0.04(0.49)
3			0 (0)	0.22(0.37)
4				0(0)

1 = Number of roots; 2 = Weight of roots (kg); 3 = Diameter of roots (cm) and 4 = Length of roots (cm)
**, **significant at P = 0.05 and 0.01, respectively*

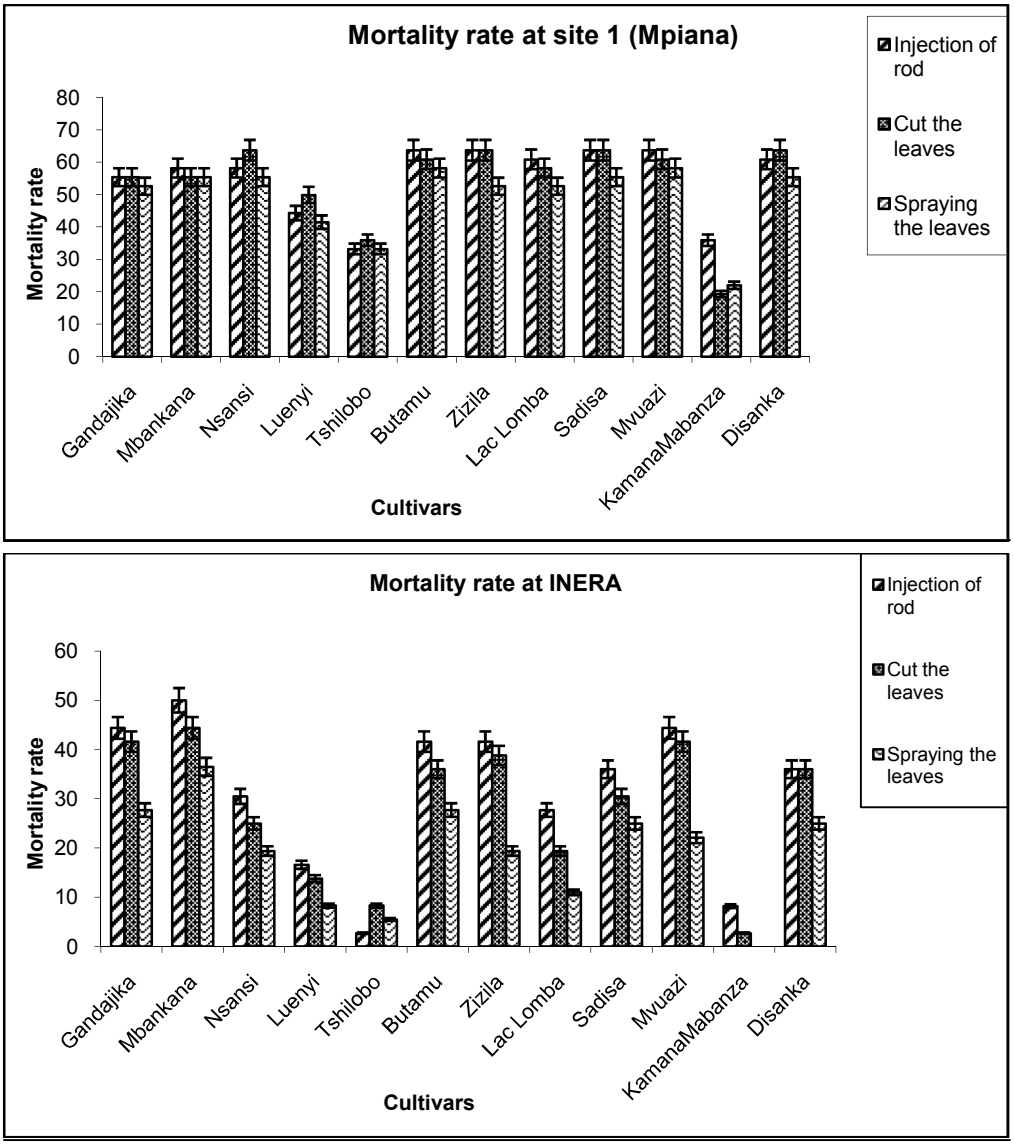

Fig. 1. Mortality rate 56 days after inoculation

Extensive use of closely related cultivars in farmers' fields could result in vulnerability to diseases such as CBB and CMV. The use of diverse parental combinations in breeding may provide a greater supply of allelic variation that can be used to create new favourable gene combinations and increase in the levels of genetic variation [32]. The assessment of genetic relatedness among cassava varieties used in the DR Congo breeding program was conducted using the Inter-Simple Sequence Repeat (ISSR) random marker system.

3.3 Molecular Analysis

Preliminary screenings with fifteen ISSR primers were performed on bulk DNA from each accession and seven most polymorphic and reproducible were chosen to amplify all standardized DNA samples. These included ISSR 5, ISSR 7, ISSR 9, ISSR 178 98B, ISSR 17899 A, ISSR UBC 825, and ISSR 829. These primers are described in Table 4. Fig. 2 depicts PCR products from DNA amplifications using the ISSR primers 7.

Table 4. Characteristics of ISSR primers used to amplify DNA samples from cassava (*Manihot esculenta*)

Primer identification	Nucleotide sequence (5'→3')	G + C content (%)	Number of fragments generated	Fragment size range (bp)
ISSR Primers				
ISSR 5	ACG ACG ACG ACG AC	27.27	21	270-1111
ISSR 7	AGG AGG AGG AGG AGG GY	64.70	19	170-1765
ISSR 9	GAT CGA TCG ATC GC	57.14	10	492-1523
17899A	CA CA CA CA CA CA AG	50.00	13	380-2570
17898B	CAC ACA CAC ACA GT	50.00	22	270-2384
UBC 825	ACA CAC ACA CAC ACA CT	47.06	15	216-1276
UBC 829	TGT GTG TGT GTG TGT GC	52.94	17	255-2018

Y nucleotide represents a pyrimidine (C or T)

All genetic markers were scored based on the presence or absence of amplification products observed as bands on the agarose gels. The percentage of polymorphic loci within the genepool was 91%. The Nei' gene diversity (h) and the Shannon's information index were 0.33 and 0.49, respectively. This high level of variability is consistent with other reports [5,12,14]. The genetic distances values for the 12 accessions are illustrated in Table 5. The values are based on a scale ranging between 0 (identical) and 1 (different for all criteria). In the present study, the results of the genetic distances ranged between 0.379 (Sadisa and Lac Lombe) and 0.734 (Sadisa and Mbankana). In general, the accessions analyzed were genetically distant with 80% of genetic distance values estimated above 0.5. The distance matrix data was used to construct a dendrogram (Fig. 3) to show the genetic relationships among the accessions. The present study revealed two main clusters with a low degree of confidence, but Kamana Mabanza was an out group that is separate from the two accession groupings with 100% degree of confidence (Fig. 3). Surprisingly, the local variety Luenyi clustered with two genetically improved accessions, Lac Lomba and Sadisa. Likewise, the local variety Tshilobo formed a grouping with genetically improved accessions M'vuazi, Butamu, Mbankana, and Nsansi. More importantly, there were no associations between genetic relationships and morphological similarities based on lobe shape, leaf colour, petiole colour, petiole orientation, and stem colour.

The high level of variability observed in the present study was consistent with other studies that used different molecular marker systems [13,30,33]. In fact, these last authors studied 16 African cassava accessions using SSR and found an average genetic diversity among accessions of 51.4%.

Table 5. Jaccard's Similarity Matrix of 12 Cassava Varieties accessions based on ISSR Data (Free Tree Program)

	Disanka	Zizila	Luenyi	Tshilobo	Lac Lomba	Kamana Mabanza	M'vuazi	Nsansi	Butamu	Gandajika	Mbankana	Sadisa
Disanka	0	0.5676	0.6081	0.6125	0.6081	0.5672	0.6111	0.6216	0.6447	0.6232	0.6026	0.5385
Zizila		0	0.4559	0.5513	0.5211	0.5588	0.5833	0.6316	0.6364	0.5077	0.6296	0.5077
Luenyi			0	0.5714	0.3548	0.5606	0.6800	0.6883	0.6923	0.4590	0.6154	0.4333
Tshilobo				0	0.6250	0.5694	0.4930	0.5467	0.4932	0.5833	0.5128	0.6027
Lac Lomba					0	0.6429	0.7143	0.7375	0.6912	0.5079	0.6988	0.3793
K-Mabanza*						0	0.6269	0.6377	0.7162	0.5738	0.6883	0.5968
M'vuazi							0	0.4688	0.4769	0.6812	0.4328	0.7000
Nsansi								0	0.4219	0.7260	0.3788	0.7260
Butamu									0	0.6944	0.4348	0.6761
Gandajika										0	0.7179	0.5424
Mbankana											0	0.7342
Sadisa												0

**K-Mabanza represents Kamana Mabanza*

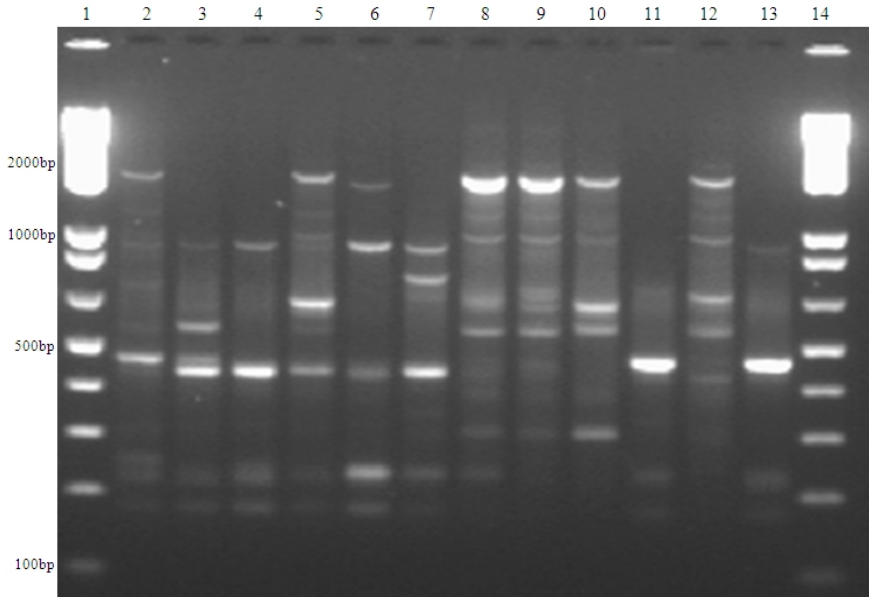

Fig. 2. ISSR amplification of cassava samples with primer ISSR 7. Lane 1 and 14 contains 1Kb^{+} ladder, lane 2 to 13 contain PCR products from DNA amplifications. Lane 2: Disanka, Lane 3: Zizila, Lane 4: Luenyi, Lane 5: Tshilobo, Lane 6: Lac Lomba, Lane 7: Kamana Mabanza, Lane 8: M'vuazi, Lane 9: Nsansi, Lane 10: Butamu, Lane 11: Gandajika, Lane 12: Mbankana and Lane 13: Sadisa

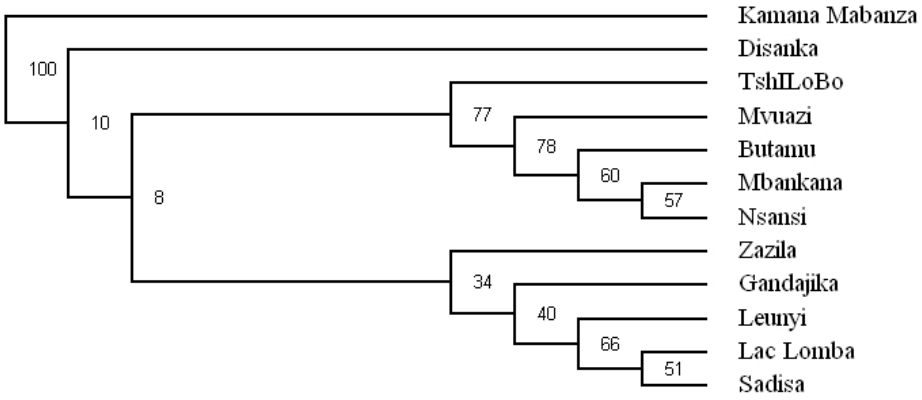

Fig. 3. Dendrogram based on ISSR data for 12 cassava accessions (Free Tree Program)

4. CONCLUSION

In the present study, the Congolese collection of cassava was characterized using morphological, agronomic, and molecular markers. Leaf, petiole, and stem color along with, lobe shape, petiole orientation, root shape, root taste, resistance to cassava mosaic virus, and cassava bacterial blight were the mains traits analyzed. There was variability for leaf, petiole, and stem color as well as for lobe shape and leaf petiole orientation. Roots were cylindrical, conical - cylindrical, or irregular. Weight of storage roots per plants were

positively correlated with number of roots at both sites. Genetically improved varieties were resistant or tolerant to CMV, but susceptible to CBB while the local varieties were susceptible to CMV and resistant to CBB. Molecular analysis revealed that the accessions tested were genetically distant. Thus, the Congolese cassava gene pool is highly variable and any combination of accessions can be efficiently used to sustain a long term breeding program.

ACKNOWLEDGEMENT

We are grateful to the Canadian International Development Agency (CIDA) for the financial support and to “Institut National pour l’Etude et la Recherche Agronomiques” (INERA) at Gandajika and Mvuazi (DR-Congo) for providing the cassava plant materials and the experimental sites.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. De Bruinjn GH, Fresco CO. The importance of cassava in world food production. Neth. J. Agri. Sci. 1989;37:21-34.
2. Nassar NMA, Ortiz R. Cassava improvement: Challenges and impacts. J. Agric. Sc. 2007;145:163-171.
3. Lozano JC. Overview of integrated control of cassava diseases. Fitopatologia Brasileira. 1992;17:18-22.
4. Thresh JM, Otim-Nape GW, Legg JP, Fargette D. African cassava mosaic virus disease: the magnitude of the problem. Afr. J. Root Tuber Crops. 1997;2(1):13-19.
5. Alves AAC. Cassava botany and physiology. In: Hillocks RJ, Thresh JM and Bellotti AC, editors. Cassava: Biology, Production and Utilization. Wallingford, UK: CAB International; 2002.
6. Ntawuruhunga P, Okao-Okuja G, Bembe A, Obambi M, Mvila JCA, Legg JP. Incidence and severity of cassava mosaic disease in the Republic of Congo. Afr. Crop Sci. J. 2001;15(1):1-9.
7. Ntawuruhunga, P, Ssemakula G, Ojulong H, Bua A, Ragama P, Kanobe C, Whyte J. Evaluation of advanced cassava genotypes in Uganda. Afr. Crop Sci. J. 2006;14(1):17-25.
8. Muengula-Manyi M, Nkongolo KK, Bragard C, Tshilenge-Djim P, Winter S, Kalonji-Mbuyi A. Effect of NPK Fertilization on Cassava Mosaic Disease (CMD) Expression in a Sub-Saharan African Region. Am. J. Exp. Agr. 2012;2(3):336-350.
9. Smith J. Genetic variability within U.S. hybrid maize: multivariate analysis of isozyme data. Crop Sci. 1984;24:1041-1046.
10. Cox T, Murphy J, Rodgers D. Changes in genetic diversity in the red winter wheat regions of the United States. Proc. Natl. Acad. Sci USA. 1986;83:5583-5586.
11. Mohammadi S, Prasanna B. Review & interpretation. Analysis of genetic diversity in crop plants. Crop Sci. 2003;43:1235-1248.
12. Montero-Rojas M, Correa AM, Siritunga D. Molecular differentiation and diversity of cassava (*Manihot esculenta*) taken from 162 locations across Puerto Rico and assessed with microsatellite markers. AoB PLANTS; 2011. plr010; doi:10.1093/aobpla/plr010.

13. Fregene M, Okogbenin E, Mba C, Angel F, Suarez MC, Guitierez J, Chavarriaga P, Roca W, Bonierbale M, Tohme J. Genome mapping in cassava improvement: Challenges, achievements and opportunities. Euphytica. 2001;120:159-165.
14. Fregene MA, Suarez M, Mkumbira J, Kulembeka H, Ndedya E, Kulaya A, Mitchel S, Gullberg U, Rosling H, Dixon AGO, Dean R, Kresovich S. Simple sequence repeat marker diversity in cassava landraces: Genetic diversity and differentiation in an asexually propagated crop. Theor. Appl. Genet. 2003;107:1083-1093.
15. Daniell H, Wurdack KJ, Kanagaraj A, Lee SB, Saski C, Jansen RK. The complete nucleotide sequence of the cassava (*Manihot esculenta*) chloroplast genome and the evolution of atpF in Malpighiales: RNA editing and multiple losses of a group II intron. Theor. Appl. Genet. 2008;116:723-737.
16. Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (ssr)-anchored polymerase chain-reaction amplification. Genomics. 1994;20:176-183.
17. Qian W, Ge S, Hong DY. Genetic variation within and among populations of a wide Oryza granulate from China detected by RAPD and ISSR markers. Theor. Appl. Genet. 2001;102:440-449.
18. Reddy PM, Sarla N, Siddiq EA. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica. 2002;128(1):9-17.
19. Muyayabantu GM, Kadiata BK, Nkongolo KK. Evaluation of Biological Soil Fertility Management Practices for Corn Production in Oxisols. Am. J. Plant Sci. 2012;3:1654-1660.
20. Nkongolo K, Deck A, Michael P. Molecular and cytological analyses of *Deschampsia cespitosa* populations from Northern Ontario (Canada). Genome. 2001;44:818-825.
21. Nkongolo KK, Michael P, Demers T. Application of ISSR, RAPD, and cytological markers to the certification of *Picea mariana*, *P. glauca*, and *P. engelmannii* trees and their putative hybrids. Genome. 2005;48:302-311.
22. Mehes MS, Nkongolo KK, Michael P. Genetic analysis of *Pinus strobus* and *Pinus monticola* populations from canada using ISSR and RAPD markers: Development of genome-specific SCAR markers. Plant Syst. Evol. 2007;267:47-63.
23. Yeh FC, Boyle TJB. Population genetic analysis of co-dominant and dominant markers and quantitative traits, Belgian J. Bot. 1997;129:157.
24. Saitou N, Nei M. The Neighbor-joining Method: A New Method for Reconstructing Phylogenetic Trees. Mol. Biol. Evol. 1987;4(4):406-425.
25. Olsen KM, Schaal BA. Evidence on the origin of cassava: phylogeography of *Manihot esculenta*. Proc. Natl. Acad. Sci USA. 1999;96:5586-5591.
26. Fukuda WMG, Guevara CL, Kawuki R, Ferguson ME. Selected morphological and agronomic descriptors for the characterization of cassava. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 2010. Date accessed: 13 September 2013. Available:http://www.iita.org/c/document_library/get_file?uuid=4530a72e-917d-4801-9239 cb0ee3a4dd4e&groupId=25357.
27. Ntawuruhunga P, Rubaihayo PR, Whyte JBA, Dixon AGO, Osiru DSO. Inter-relanships among traits and path analysis for yield components of cassava: A serach for storage root yield indicators. Afr. Crop Sci. J. 2001;9(4):599-606.
28. Sanchez G, Restrepo S, Duque MC, Fregene M, Bonierbale M, Verdier V. AFLP assessment of genetic variability in cassava accessions (*Manihot esculenta*) resistant and susceptible to the cassava bacterial blight (CBB). Genome. 1999;42:163-172.
29. Lokko Y, Danquah EY, Offei SK, Dixon AGO, Gedil MA. Molecular markers associated with a new source of resistance to the cassava mosaic disease. Afr. J. Biotechnol. 2005;4:873-881.

30. Muengula-Manyi M, Mukwa L, Nkongolo KK, Tshilenge-Djim P, Winter S, Bragard C, Kalonji-Mbuyi A. Assessing reactions of genetically improved and local cassava varieties to Cassava Mosaic Disease (CMD) infection in a savannah region of the DR-Congo. Am. J. Plant Sci. 2013;4:824-837.
31. Nkongolo KK, Dostaler D, Couture L. Study on the wheat infection and deoxynivalenol accumulation relative to the *Fusarium graminearum* inoculation technique. Can. J. Plant Pathol. 1994;16(1):37-42.
32. Lokko Y, Dixon A, Offei S, Danquah E, Fregene M. Assessment of genetic diversity among African cassava *Manihot esculenta* Grantz accessions resistant to the Cassava mosaic virus disease using SSR markers. Genet. Resour. Crop Ev. 2006;53:1441-1453.
33. Beeching JR, Marmey P, Gavalda MC, Noirot M, Haysom HR, Hughes MA, Charrier A. An Assessment of Genetic Diversity within a Collection of Cassava (*Manihot esculenta* Crantz) Germplasm Using Molecular Markers. Ann. Bot. 1993;72(6):515-520.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=494&id=11&aid=4528