Annals of Agric. Sci., Moshtohor **ISSN 1110-0419**
Vol. 55(1) (2017), 73–82 **http://annagricmoshj.com**

Genetic diversity in some foreign Cassava genotypes using SDS-protein electrophoresis and inter simple sequence repeat markers (ISSRs)

[1] H.S. Baiea; [1]M. M. M. Bekhit; M.S. Abdel-Sabour [1] and M.M. El Nagar [2]
[1]Department of Genetics and genetic engineering, Faculty of Agriculture, Benha, Egypt.
[2]Department of Horticulturs, Faculty of Agriculture, Benha, Egypt.

Abstract

Three foreign genotypes of Cassava were used in this study, American Cassava, Brazilian Cassava and Indonesian Cassava genotypes. The electrophoretic banding patterns of proteins extracted from the leaves of the three Cassava genotypes. The results of SDS-PAGE revealed a total number of 15 bands with molecular weights (MW) ranging from about 29 to 220 kDa . Data revealed 13 common bands (monomorphic), while the remaining 2 band were polymorphic with 13% polymorphism. The maximum number of bands (15) appeared in Barazilian while the minimum number (13) was present in Indonisian. Similarity index among the three Cassava genotypes based on protein analysis, carried out using SPSS computer program. The similarity relationships ranged between 93.1% and 96.6%. Dendrogram for the genotypes genetic relationships to Cassava genotypes divided into two clusters. The first cluster contained American and Indonisian , while the second cluster contained Indonisian. Twelve primers for ISSR were successful in generating reproducible and reliable amplicons for the three forigen Cassava genotypes. The total number of bands from twelve primers was 100 bands; distributed as 82 polymorphic bands and 18 monomorphic bands. The nine unique bands were given from interaction among twelve primers ISSR and three Cassava genotypes. The polymorphism level was differed from primer to another reflecting the primer ability to detect the diversity among Cassava genotypes. The HB9-primer revealed higher level of polymorphism than the rest of ISSR primers followed by ISSR3 which produced 90% polymorphism.The lowest polymorphism was produced by ISSR2, SH6 with values 66%. The highest value was similarity between American Cassava and Brazilian Cassava (60.0%) . The lowest similarity value appeared between American Cassava and Indonisian Cassava (45.9%).

Key words: Cassava, *Manihot esculenta Crantz*. Genetic diversity, ISSR marker, Protein electrophoresis.

Introduction

Cassava (*Manihot esculenta Crantz*) belongs to the Euphorbiaceae family and its center of origin is the central region of Brazil.It is growing for its starchy tuberous. Cassava is the fourth most important carbohydrate source for human consumption in the tropics and subtropics after rice and maize **(Bellotti *et al.*, 1999)**. It's believed that cassava originated by hybridization between two wild Manihot species, followed by vegetative reproduction of the hybrid. The center of origin of cassava was first reported to be Central America including Colombia, Venezuela, Guatemala and Southern Mexico due to the large number of genotypes present there (**Sauer, 1952 and Roger, 1965)**. Cassava is the staple food of nearly 700 million people worldwide. Cassava, which is generally propagated vegetatively, is one of the major sources of food in Africa **(Cock, 1982)**. The roots, which are an excellent source of carbohydrates, have a very low protein content**.** Cassava has the advantage of being well adapted to a wide range of environmental stresses. It grows very well in less fertile soil in contrast to many other crops that are highly vulnerable to environmental stresses during critical stages of plant development **(Ugorji, 1998)**. Cassava is generally propagated with stem cuttings, thereby maintaining a genotype. Under natural conditions as well as in plant breeding, propagation by seed is common and farmers in Africa are known to occasional use of spontaneous seedlings for subsequent planting **(Silvestre and Arraudeaus, 1983)**. Since introduction of the crop to Africa from Latin America by Arab and European traders, asexual propagation and human selection for resistance and adaptability to biotic and abiotic constraints are expected to have reduced cassava's genetic diversity (**Fregene et al., 2003**). Cassava has a big role in order to secure food security **(Raji *et al.*, 2009).** Techniques based on molecular marker analysis (i.e. RFLP, RAPD, ISSR-PCR) may provide a more efficient and accurate screening method biochemical genetic analysis. Simple sequence repeats comprise short oligonucleotide sequences, two to six bases long, repeated in tandem array, which occur very frequently in eukaryotic genomes **(Tautz and Renz, 1984; Beckmann and Soller 1990 and Lagercrantz *et al.* 1993)**. The ISSR-PCR technique uses primers that are complementary to a single SSR and anchored at either the 5′ or 3′ end with a one- to three-base degenerate oligonucleotide ('anchor') **(Zietkiewicz *et al.*, 1994)**. The objective of this study was to investigate the genetic diversity and the molecular profile in imported cassava genotypes using SDS-protein electrophoresis and ISSR markers.

Materials and Methods

Plant Materials: Three Cassava genotypes which were obtained from the Horticulture Research Institute (HRI), Egypt and mature leaves were taken from three genotype (table 1).

Table 1. List of Cassava genotype and their origin of production.

No.	Cultivars	Origin
1	American	USA
2	Brazilian	Brazil
3	Indonesian	Indonisia

SDS-protein electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970), as modified by Studier (1973). Youngest–leaves samples were taken from each genotype of cassava.

Extraction of water–soluble protein fraction from leaves.

One gram leaves of each cultivars was frozen with liquid nitrogen and ground with 2ml water soluble extraction buffer in a mortar and pestle. Samples were transferred to eppendorf tubes, then centrifuged for 20 min at 12000 rpm at 4ºC. Supernatants containing water-soluble protein fraction were transferred to clean tubes and stored at– 20ºC.

Extraction buffer for soluble protein from leaves

Water Soluble buffer :- (0X) Tris (1M, pH8.8) 6 ml , EDTA (0.25M) 800µl, H_2O (dd) up to 100 ml , 2X buffer , SDS(10%) 40 ml , Glycerol 20 ml , Tris (1M pH8.8) 12 ml , 0.25M EDTA 1.6 ml , H_2O (dd) up to 100 ml.

The stock solutions used for protein electrophoresis were as follows:-

1. Acrylamid stocks (kept in dark, 4ºC)

a. For resolving gel Acrylamide 30.0 g , Bis-acrylamid 0.8 g , H_2O (dd) up to 100 ml

b. For stacking gel , Acrylamide 30.0 g Bis–acrylamide 1.0 g , H_2O (dd) up to 100ml

2. Resolving gel buffer (4X Tris, pH 8.4,4ºC) Tris 18.15 g , HCl (conc) 3.5 ml , H_2O (dd) up to 100 ml.

3. Stacking gel buffer (1M Tris- HCl, pH 6.8,4ºC) Tirs 12.11 g , H_2O (dd) up to 100 ml , adjusted pH to 6.8 by HCl.

4. Run buffer , Tris 15.14 g , Glycine 72.07g , 10% SDS 50 ml , H_2O (dd) up to 5 L

Gel preparation

Vertical slab (18x 16 cm) gel electrophoresis apparatus was used as marketed by Biorad. All glass were washed with ethanol 70% Spacers of 1.5 mm were used.

1. Resolving gel (15 % acrylamide) Acrylamide stock (for resolving gel) 31.66 ml,Tris, (4X pH 8.4) 16.25 ml , H_2O (dd) 16.10 ml

This solution was filtered, then the following ingredients were added :

SDS (10%) 750µl , Ammonium persulphate (10%) freshly prepared 500µl TEMED 100µl.

This solution was instantly swirled. Two gels were poured simultaneously to a height of 1.5 below the bottom of the comb. Gels were overlaid with isopropanol and left to polymerize for at least one h. Isopropanol was removed before the stacking gel was poured.

2. Stacking gel : Acrylamide stock 2.66 ml , Tris (pH6,8 2.50 ml , H_2O (dd) 14.70 ml.

This solution was filtered, and then the following ingredients were added:

SDS (10%) 250 µl , A.P.S (10%) 100 µl , TEMED 40 µl

This stacking gel solution was quickly poured over the two resolving gels, and 15 well combs were used. Gels were left to polymerize for 45 min before gels were run.

Application of samples

A volume of 100 µl protein extract for each sample was added to 25%(v/v) volume of water–soluble buffer (2X). mercaptoethanol (10µl) was added to each sample (10%) SDS. Samples were boiled for 5 minutes and 100 µl of each samples was loaded on the gel after adding one drop of loading buffer (bromophenol blue and glycerine).

Gel running and staining

Two liters of the run buffer were poured into the running tank and precooled by flooding cold water (4ºC) through cooling tubes. The run buffer (400 ml) was added to the upper tank just before running. Gels were run at 100 volt for a quarter of an hour then the voltage was raised at 250 volt until the dye reach one inch from the bottom of the gel. Gels were removed from the apparatus and placed in plastic tanks, then covered with staining solution. Gels were agitated gently overnight.

The composition of the staining solution was as following :

Methanol 455 ml, Coomassie Brilliant, BlueR250 1g , Acetic acid (conc) 90 ml , H_2O (dd) 455 ml.

After removing the staining solution, gels were covered with a distaining solution (freshly prepared) of the following composition: Methanol 700 ml , Acetic acid (conc) 200 ml , H_2O up to 3500 ml.

Gels were agitated gently for one hour. After removing the solution, anew one was added. This step was repeated several times until gel background became clear to be photographed.

DNA extraction.

DNA was extracted using standard procedures according to **(Dellaporta *et al.* 1983)** with slight

modifications. Freshly harvested young leaf (0.3 g) of each genotype was ground in liquid nitrogen using a pestle and a mortar. The fine powder was transferred to 1.5 ml eppendorf tube using a frozen spatula. Eight hundred microlitres of preheated (65ºC) extractionbuffer and 50 µl of 20% SDS solution were added to each tube and the mixture homogenised for 30 seconds by intermittent inversion. The tubes were incubated at 65ºC for 15 min with intermittent inversions and incubated at room temperature for 5 min. Proteins and polysaccharides were precipitated by adding 250 µl of ice-cold 5M potassium acetate and mixed by inverting the tubes 5-8 times. The tubes were placed on ice for 20 minutes and centrifuged at 13,250 rcf in eppendorf centrifuge 5418 (Germany) for 10 min. The supernatant was transferred to a new eppendorf tube and 500 µl of ice-cold isopropanol added and mixed by inverting gently 8- 10 times to precipitate crude DNA. The mixture was incubated at -20ºC for 30 min and centrifuged at 13,250 rcf for 10 min.

Inter simple sequence repeat markers (ISSRs)

List of the twelve primer names and their nucleotide sequences used in the study for ISSR procedur were illustrated in table 2.

ISSR-PCR was carried out according to **(Williams *et al.*, 1990)**. The primers used were 11 to 18 mer olignucleotide; twelve primers were selected as potentially useful. The codes and sequences of the used primers are shown in Table 2. PCR reactions were optimized and mixtures (25µl total volume) were composed of dNTPs (200µM), MgCl2 (1.5mM),1x buffer, primer(0.2µM), DNA (50ng),Taq DNA polymerase (2 units). Amplification was carried out in a thermo Cycler programmed for 94°C for 3 min (one cycle); followed by 94°C for 30sec, 40°C for 45 sec and 72°C for 1 min (35 cycle), 72°C for 10 min (one cycle) then 4°C (infinitive.(Amplification products (25µl) were mixed with 3µl loading buffer and separated on 1.3%agarose gel and stained with 0.5 µg/ml ethidium bromide, and visualized under ultraviolet light and photographed. DNA fragment sizes were determined by comparisons with the 1kb DNA ladder marker.

Table 2. show ISSR Primer

NO	Name	Sequence
1	814_A	$(CT)^8TG$
2	844_A	$(CT)^8AC$
3	844_B	$(CT)^8GC$
4	17898_A	$(CA)^6AC$
5	HB_9	$(GT)^6GG$
6	HB_{11}	$(GT)^6CC$
7	Issr2(h)	CAC(TCC)5
8	Sh 6(h)	CGC(GATA)4
9	Sh 7(h)	GAC(GATA)4
10	Sh 8(h)	(AGAC)4GC
11	HB_{12}	$(CAC)^3$ GC
12	Issr3(h)	TTT(TCC)5

Data analysis

Clear, unambiguous and reproducible bands were considered for scoring. Each band was considered a single locus. Data were scored as (1) for the presence and (0) for the absence of a given DNA band. Band size was estimated by comparing with 1-kb ladder (Invitrogen, USA) using Totallab, TL120 1D v2009 (nonlinear Dynamics Ltd, USA). The binary data matrices were entered into the NTSYSpc (Ver. 2.1) and analyzed using qualitative routine to generate similarity coefficient and used to construct a dendrogram using unweighted pair group method with arithmetic average (UPGMA) and sequential hierarchical and nested clustering (SHAN) routine **(Nei, 1973 and 1978)**.

Results and Discussion

Molecular fingerprints based on SDS- proteins

The electrophoretic banding patterns of proteins extracted from the leaves of the three cassava genotypes are shown in Figure (1) and their densitometric analysis are illustrated in Table (3). The presence and absence of bands were represented with (1) and (0), respectively. The results of SDS-PAGE revealed a total number of 15 bands with molecular weights (MW) ranging from about 29 to 220 kDa, which were not necessarily present in a 3 genotypes.

Table 3. Densitometric analysis for SDS leaves protein among three cassava genotypes.

MW KDa	American	Brazilian	Indonesian
220	1	1	1
164	1	1	1
112	1	1	1
110	1	1	1
94	1	1	1
91	1	1	1
81	1	1	1
78	1	1	1
74	1	1	1
68	1	1	1
61	1	1	1
55	1	1	1
45	1	1	1
37	1	1	0
29	0	1	0
Total	14	15	13

Data revealed 13 common bands (monomorphic), while the remaining 2 band were polymorphic with 13% polymorphism. The densitometric analysis of the SDS-protein banding patterns of the studied genotypes were found to be useful in varietal identification of the studied cassava genotypes. The three genotypes showed different patterns in the presence of bands. The maximum number of bands (15) appeared in Barazilian genotypes while the

minimum number (13) was present in Indonesian genotypes. However, there are resemblance between genotypes and especially American and Brazilian, Barazilian genotype was characterized by a unique fingerprint. **Pereira et al. (2002)** Investigated Proteins from young unexpanded leaves of seven cassava cultivars (M. esculenta) through SDS-PAGE. Protein patterns, showed a polypeptide subunit present exclusively in Fecula Branca, with a molecular weight of 93.5 kDa. **Souza et al. (2002)** Studied protein population of cassava root layers characterized by SDS-PAGE. It revealed the presence of a protein population in the molecular weight range between 94 and 20 kDa. **Wechkrajang et al. (2006)** Examined protein expression patterns in adventitious and of the commercial cassava cultivars Rayong 1 (R1) and Kasetsart 50 (KU50). Total protein analysed for protein profile using SDS-PAGE. SDS-PAGE revealed unique protein bands in the cassava roots from 35 days after planting on wards.

Dendrogram for the genotypes is presented in Figure (2). It showed a degree rapprochement between the American and Brazil while having the spacing between the American and Brazil. American product was closer to Indonesia than the Brazilian.

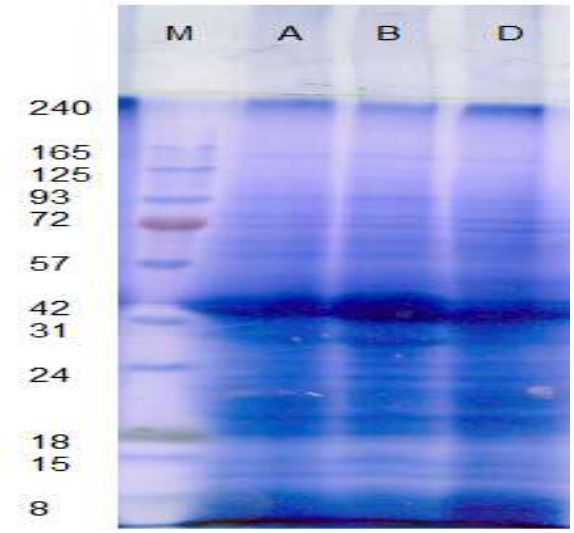

Figure 1. SDS-protein banding patterns among three cassava genotypes.
A- American B- Brazilian D - Indonisian

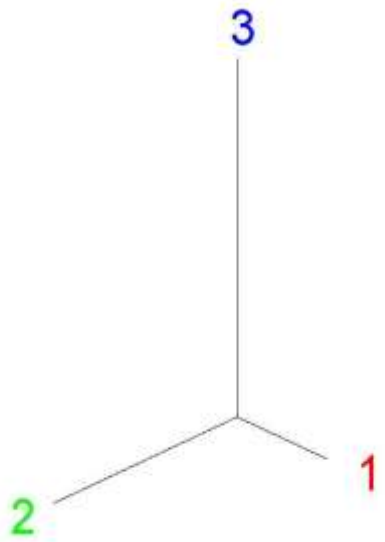

Fig. 2 . Dendrogram of the genetic distances between three Cassava genotypes based on SDS-PAGE
(1 - American 2- Brazilian 3 – Indonesian).

SPSS program was used to analyze the data because of the difficulties in the visual interpretation of SDS-PAGE of leaves protein profiles. The numerical analysis of SDS-PAGE of leaves protein profiles showed that each genotype had slight discriminative protein banding (Table 4), the results obtained from using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique with three cassava genotype showed that the the convergence that exists between the Brazilian and the American because of the the convergence between the genotype source for example American source of the United States and Brazilian exporter from Brazil, both countries are close as Brazil in South America, while the USA in North America , the existence of the spacing between the American and Brazil with Indonesia as the Indonesian from of Indonesia, located in Asia, and thus geographical affect on the degree of the convergence between the genotypes.

Table 4. Genetic similarity matrix among three *Cassava* genotypes. as computed according to Dice's similarity coefficient from SDS protein generated data.

	American	Brazelian	Indonesian
American	100		
Barazelian	96.6	100	
Indonesian	94.7	93.1	100

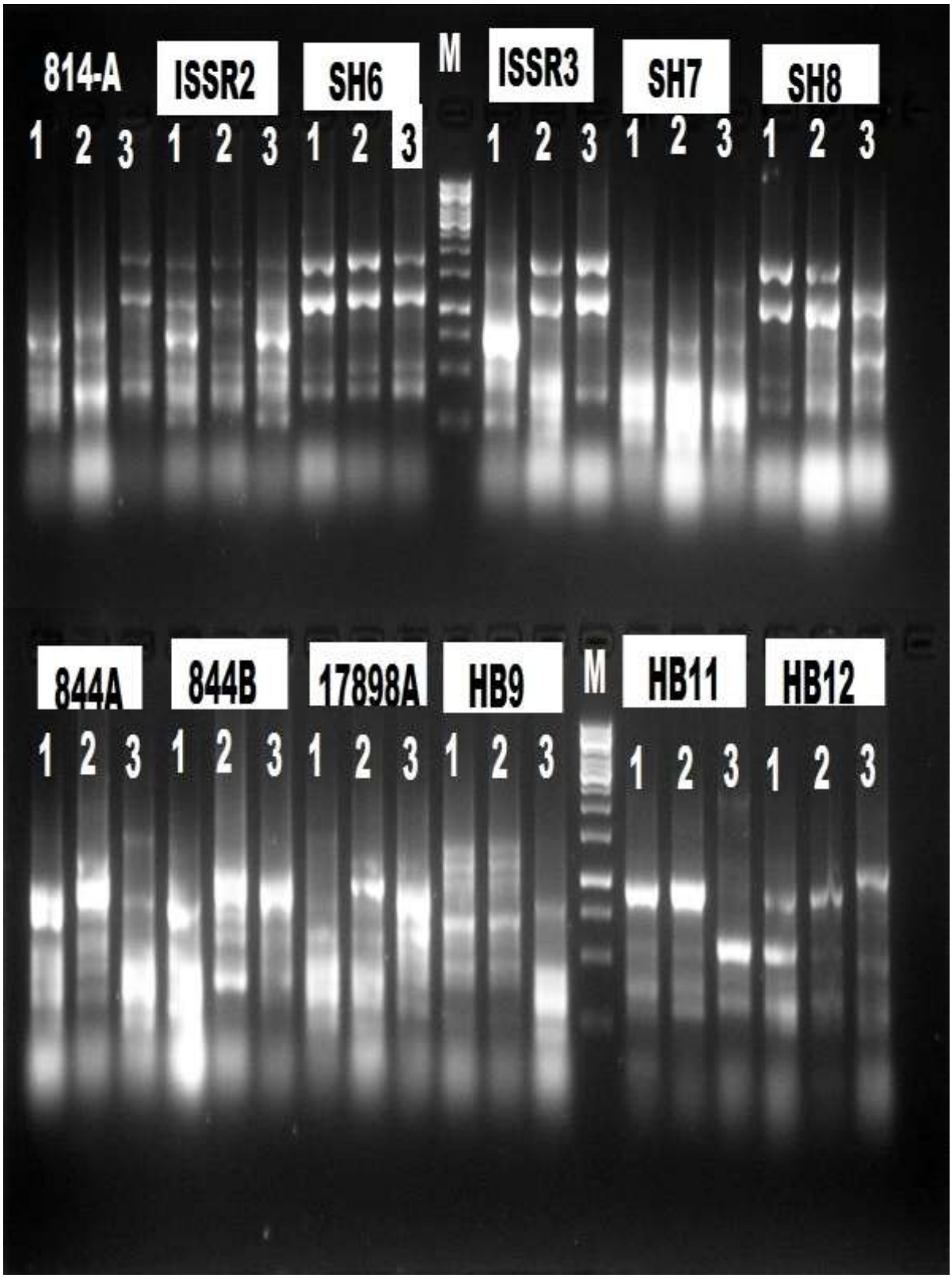

Figure 3. Representative gel showing ISSR marker with three Cassava Genotype (1 = American , 2= Brazilian , 3 = Indonisian)

INTER SIMPLE SEQUENCE REPEAT MARKERS (ISSRs)

Figure (3) shows the DNA banding patterns obtained with twelve ISSR primers for the three genotypes, i.e., American Cassava, Brazilian Cassava and Indonesian Cassava . The total bands from ten primers were 100 bands; these bands were distributed 18 monomorphic bands and 82 polymorphic bands including 53 unique bands Table (5). The fifty three unique bands were given from interaction among twelve primers ISSR and three cassava genotypes. The polymorphism level was differing from one primer to another that reflects the primers ability to detect diversity between cassava genotypes as shown in Table (6.1,6.2).

The HB9 ISSR-primer revealed higher level of polymorphism than the rest of ISSR primers followed by ISSR3 which produced 90% polymorphism. The lowest polymorphism was produced by SH6 and ISSR2 with values 66 Table (6.1,6.2) . Moreover, the primers was differing in the produce bands the primer HB9 and ISSR3 give 10 bands which were highest values in all of them, while, the lowest bands produced is 6 bands by primer 17898A . The unique bands were founding from all ISSR-PCR primers . These results agree with those of **Zayed *et al,* (2013)**. The total number of bands from the ten primers was 79 bands; distributed as 36 monomorphic bands and 43 polymorphic bands including nine unique bands were given from interaction among ten primers ISSR and four cassava genotypes. **Ru *et al.* (2012)** Analyzed the genetic polymorphism of 39 cassava varieties useing Ten pairs of ISSR primers. A total of 70 clear electrophoretic bands were amplified, each pair of primers had amplified 3-9 electrophoretic bands, with an average of 7, and the length of amplified bands ranged from 150 to 2,000 bp

Table 5. Present and absent bands ISSR-PCR products by ISSR-primers in three cassava genotypes. (A=American , B= Brazilian , D = Indonisian).

No	Base pair	A	B	D	No	Base pair	A	B	D	No	Base pair	A	B	D
844A					HB11					SH6				
1	250	0	0	1	1	220	0	0	1	1	1000	1	0	0
2	190	0	1	0	2	190	0	0	1	2	700	0	0	1
3	160	1	0	1	3	180	1	1	0	3	650	1	1	0
4	140	1	0	0	4	100	1	1	1	4	400	1	1	1
5	110	0	1	0	5	90	0	1	0	5	370	0	0	1
6	90	1	1	0	6	80	1	1	1	6	220	0	0	1
7	80	1	1	1	7	60	1	0	0	7	200	1	1	0
8	70	0	0	1	8	50	0	1	1	8	180	1	1	1
9	50	1	1	0	HB12					9	100	1	1	1
844B					1	200	0	0	1	ISSR3				
1	190	0	1	0	2	170	0	1	0	1	650	0	1	1
2	180	0	0	1	3	160	1	0	0	2	500	1	0	0
3	150	1	0	0	4	150	0	0	1	3	400	0	1	1
4	140	0	0	1	5	100	1	1	0	4	250	0	1	0
5	130	0	1	0	6	90	0	0	1	5	240	1	0	0
6	90	1	0	1	7	80	1	1	1	6	200	1	0	0
7	80	1	1	0	8	50	1	0	1	7	180	0	1	1
8	50	1	1	1	814-A					8	170	1	1	0
17898A					1	650	0	0	1	9	150	0	1	0
1	190	0	1	0	2	400	0	0	1	10	100	1	1	1
2	160	1	0	1	3	300	0	0	1	SH7				
3	130	1	1	1	4	250	0	1	0	1	500	1	0	0
4	90	1	1	0	5	240	1	0	0	2	400	0	0	1
5	80	1	0	0	6	220	0	1	1	3	250	1	0	0
6	50	0	1	1	7	180	1	1	1	4	240	0	1	1
HB9					8	100	1	1	1	5	170	1	0	1
1	240	1	1	0	ISSR2					6	150	0	1	0
2	220	1	1	0	1	650	1	1	1	7	100	1	1	1
3	200	0	1	0	2	400	1	1	0	SH8				
4	190	1	0	0	3	370	0	1	0	1	500	1	1	0
5	150	0	0	1	4	250	1	0	1	2	370	1	1	1
6	140	1	1	0	5	200	0	1	0	3	240	0	1	0
7	90	1	0	0	6	190	0	0	1	4	200	0	0	1
8	80	0	0	1	7	180	1	1	1	5	190	1	0	0
9	60	0	0	1	8	170	0	0	1	6	180	0	1	1
10	50	1	1	1	9	100	1	1	1	7	170	1	0	0
										8	100	1	1	1

Table 6.1. Primer name, total number of bands, monomorphic bands, polymorphic bands,polymorphism ratio, unique bands and genotypes code. (A = American Cassava), (B = Brazilian Cassava) and (D = Indonesian Cassava).

N0	Primer name	Monomorphic band	Polymorphic band	Total band	Polymorphism %	Unique bands		
						No.	Genotype	bp
1	844A	1	8	9	88	5	D	250
							B	190
							A	140
							B	110
							D	70
2	844B	1	7	8	87	5	B	190
							D	180
							A	150
							D	140
							B	130
3	17898A	1	5	6	83	2	B	190
							A	80
4	HB9	0	10	10	100	6	B	200
							A	190
							D	150
							A	90
							D	80
							D	60
5	HB11	2	6	8	75	4	D	220
							D	190
							B	90
							A	60
6	HB12	1	7	8	87	5	D	200
							B	170
							A	160
							D	150
							D	90

Table 6.2. Primer name, total number of bands, monomorphic bands, polymorphic bands,polymorphism ratio, unique bands and genotypes code. (A = American Cassava), (B = Brazilian Cassava) and (D = Indonesian Cassava).

N0	Primer name	Monomorphic ban	Polymorphic band	Total band	Polymorphism %	Unique bands		
						No.	Genotype	bp
7	814-A	2	6	8	75	5	D	650
							D	400
							D	300
							B	250
							A	240
8	ISSR2	3	6	9	66	4	B	370
							B	200
							D	190
							D	170
9	SH6	3	6	9	66	4	A	1000
							D	700
							D	370
							D	220
10	ISSR3	1	9	10	90	5	A	500
							B	250
							A	240
							A	200
							B	150
11	SH7	1	6	7	85	4	A	500
							D	400
							A	250
							B	150
12	SH8	2	6	8	75	4	B	240
							D	200
							A	190
							A	170
Total		18	82	100	82	53		

Similarity and Dissimilarity

Similarity indices among the three cassava varieties based on ISSR analysis revealed that the highest value was between American Cassava and Brazilian Cassava (60.0%) table (7) . followed by Brazilian Cassava and Indonesian (47.7%). The lowest similarity value appeared between American Cassava and Indonesian Cassava (45.9) (Table 7). These results agree with results obtained by **zayed *et al*, (2013).** The genetic distance among cassava was very narrow, with genetic similarity coefficients ranging between 0.750 and 0.823. **Ru *et al*, (2012)** the similarity coefficient of 0.67; in addition, the genetic distance among cassava was very narrow, with genetic similarity coefficients ranging between 0. 80 and 1.00.

Table 7. Similarity indices among the three cassava genotypes based on ISSR analysis.

	American	Brazelian	Indinesian
American	100		
Barazelian	60	100	
Indinesian	45.9	47.7	100

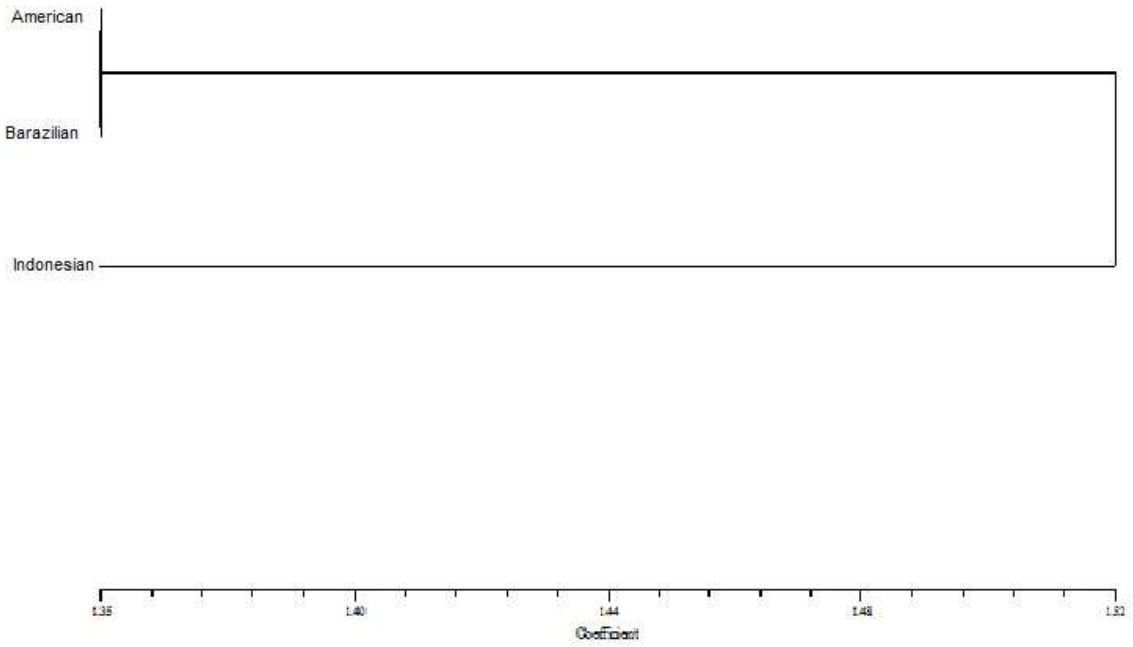

Fig. 4 . Dendrogram of the genetic distances between three Cassava genotypes based on twelve ISSR-PCR primers

Dendrogram and cluster analysis

As shown in Fig. (4) the cassava genotypes were distributed in two clusters. The cluster had Indonesian Cassava only while the second cluster included two American and Barazelian. Therefore, the genetic distance between both the American and Barazelian is very low as shown in Table (4) and the far genetic distance was observed between American Cassava and Indonesian Cassava. The origin of the genotypes may be explain the causes of genetic distance. These results agree with results obtained by **zayed *et al,* (2013).**

References

Beckmann,J.S. and Soller, M. (1990): Towarda unified approach to genetic mapping of eukaryotesbased on sequence tagged microsatellitesites. Bioflechnology, 8, 930-932.

Bellotti, A.C.; Smith, L. and Lapointe, S.L .(1999) : Recent advances in cassava pest management. Annu. Rev. Entomol., Palo Alto, v. 44, : 343-370.

Cock J. H. (1982): Cassava: A basic energy source in the tropics. Science, 218: 755-762.

Dellaporta, S.L.; Word, J and Hicks, J.B (1983) : A plant DNA preparation, Plant Molecular Biology 4: 19-21.

Fregene, M.A.; Suarez, M.; Mkumbira, J.; Kulembeka, H.; Ndedya, A. Kulaya, A. Mitchel, S.; Gullberg, U.; Rosling, H. Dixon, A.G.O.; Dean, R. and Kresovich, S. (2003) : Simple sequence repeat marker diversity in cassava landraces: genetic diversity and differentiation in an asexually propagated crop. Theoretical and Applied Genetics 107:1083-1093.

Lagercrantz U, H. Ellegren and L. Andersson, (1993): The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Research 21:1111–1115.

Laemmli, U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London), 227: 680-685.

Nei, M. (1973): Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci,; USA 70:3321–3323.

Nei, M. (1978): Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Pereira, A. J.; Collet, S. A. de O., Vidigal Filho, P. S. and Machado, M. de F. P. da S.(2002): SDS-PAGE detection of a specific marker in 'Fecula Branca' cassava cultivar, *Manihot esculenta Crantz* (Euphorbiaceae). Acta Scientiarum; 24(2):615-618.

Raji, A.A.J.; Fawole, I.; Gedil, M. and Dixon, A.G.O. (2009): Genetic differentiation analysis of African cassava (*Manihot esculenta*) landraces and elite germplasm using amplified fragment length polymorphism and simple sequence repeat markers. Annals of Applied Biology 155:187-199.

Roger, D.J.(1965): Some botanical and etnological considerations of Manihot escuelnta. Economic Botany,19: 369-377.

Ru, J. P.; Yong, G. Z.; Ping, L.; Qiang, H. ; Chun,Y. J.; Rong, S. L.; and Tian, F. H. (2012) : A study of genetic polymorphism of cassava germplasms and ISSR molecular markers. Agricultural Biotechnology; 1(1):8-11.

Sauer, C. O. (1952): Agriculture origin and Dispersals Series Two, The American geographic Society, New York.

Silvestre, P. and M. Arraudeaus, (1983): Le manioc. Techniques Agricoles et productions Tropicales Collection. Maisonneuve and Larose Agence de Cooperation Culturalle et Technique, Paris. pp.262.

Souza, C. R. B. de; Carvalho, L. J. C. B.; Almeida, E. R. P. de and Gander, E. S. (2002): Towards the identification of cassava root protein genes. Plant Foods for Human Nutrition; 57(3/4):353-363.

Studier, F.W. (1973): Analysis of bacteriophage T7 early RNAs and proteins of slab gels. J. Mol. Biol., 79: 237-248.

Tautz, D. and Renz, M. (1984) : Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucl. Acids Res. 12,4127-4138.

Ugorji, N (1998): Genetic characterization of cassava cultivars in Nigeria: Morphological and molecular markers. MSc Dissertation, University of Ibadan, Ibadan, Nigeria

Wechkrajang, S.; Vichukit, V.; Toonkool, P. and Nakasathien, S. (2006) : Anatomical characterization and protein profiles in adventitious and storage roots of two commercial thai cassava varieties. Kasetsart Journal, Natural Sciences; 40(2):297-305.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V.(1990): DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531- 6535.

Zayed, E. M.; Shams, A. S. and Kamel, A. S. (2013): Genetic diversity in introduced cassava using inter simple sequence repeat markers (ISSRs). Gene Conserve; 12(47):23-33.

Zietkiewicz, E, A. Rafalski and D. Labuda, (1994): Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20:176-183.

قياس التنوع الوراثى فى بعض أصناف الكسافا الأجنبية بإستخدام تقنيتى SDS و ISSR

[1]حسام سعد محمد بعية ؛ [1]مخلوف محمد محمود بخيت، [1]محمد سراج الدين عبد الصبور ، [2]مهران مختار محمد النجار

[1]قسم الوراثة والهندسة الوراثية، كلية الزراعة، جامعة بنها، مصر

[2]قسم البساتين، كلية الزراعة، جامعة بنها، مصر

تم دراسة درجة التشابه الوراثية بين ثلاث أصناف من الكسافا وهى الكسافا الأمريكية ، الكسافا البرازيلية ، الكسافا الأندونيسيه وذلك عن طريق التفريد الكهربائى للبروتين بطريقة الصوديوم دوديسيل سلفات وكذلك استعمال تقنية من تقنيات الواسمات الجزيئية التى تعتمد على تفاعل البلمرة المتسلسل وهى الـ ISSR .أظهرت النتائج المتحصل عليها عن طريق التفريد الكهربائى للبروتين بطريقة الصوديوم دوديسيل سلفات لثلاث اصناف من الكسافا، أن العدد الكلى للحزم 15 عند وزن جزيئي يتراوح ما بين 29 إلي 220 كيلو دالتون (13 حزم وحيدة المظهر و 1 حزمة متعددة المظاهر و 1 حزمة متفرده . كان هناك تباين بين عدد الحزم فيما بين الأصناف وبعضها حيث كان أعلى عدد هو 15 حزمة أظهره الصنف البرازيلى بينما أقل عدد كان 13 حزمة أظهره الصنف الاندونيسى . أظهرت النتائج أن التشابة الوراثى بين الثلاث أصناف من الكسافا كانت تتراوح ما بين 93.1 إلي96.6%، حيث كانت أعلى نسبة 96.6 بين الصنف الامريكى والصنف البرازيلى بينما كانت أقل نسبة 93.1% بين الصنف الامريكى والصنف الاندونيسى .قسمت الدندوجرامات الـ 3 أصناف من الكسافا إلي مجموعتين المجموعة الاولى تتضمن الصنف الأمريكى والبرازيلى بينما المجموعة الثانية تضمنت الصنف الاندونيسى فقط . تم الحصول على عدد 100 حزمة جزيئية من حزم الـ DNA من الـ 12 بادئ منها 45 حزمة polymorphic وبنسبة تباين قدرها 82% و 18 حزمة monomorphic كما كان بين الحزم 53 حزمة فريدة من التفاعل بين ISSR ذو الـ 12 بادئ والثلاثة تراكيب وراثية من الكسافا. وقد أظهر البادئ الجزيئى HB9 أعلى معدل من التباين (100%) يليله البادئ الجزيئي ISSR3 الذى أظهر نسبة تباين (90 %) ، كما أظهر البادئ الجزيئى SH6, ISSR2 أقل معدل من التباين (66%) ، أظهرت النتائج أن أعلى تشابه وراثي بين الصنف الامريكى والصنف البرازيلى قيمته (60%) وأن أقل تشابه وراثى قيمته (45,9) بين الصنف الأمريكى والصنف الأندونيسى .

الكلمات الرئيسية: الكسافا ، الأختلافات الوراثية ، تفريد البروتين كهربائيا ، دلائل الISSR