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Molecular Profiling of Landraces of Cassava (*Manihot esculenta* Crantz) Using ISSR Markers

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***Abstract*: Six accessions of cassava *viz.*, C1 (Narrow leaved - MNS-13-1), C2 (Devan Kappa - SRA-13-1), C3 (Pullad Kappa - MNS-13-5), C4 (Karutha Malabar), C5 (MNS-AICRP) and C6 (Pullad Kappa) maintained in the field genebank of Central Tuber Crops Research Institute, Sreekariyam, Thiruvananathapuram were subjected for molecular analysis using 5 selected ISSR primers. UBC 808 produced highest number of bands (12) and UBC 817 produced the lowest number of bands (3) while UBC 845 is not amplified. The percentage polymorphism of the ISSR primers studied ranged from 33.3% - 100%. UBC 817 recorded the lowest polymorphism (33.3%) and UBC 808 recorded the highest Polymorphism (100%). The similarity coefficient based on ISSR markers ranged from 0.55 to 0.86. The dendrogram generated using UPGMA cluster analysis separated the 6 accessions of cassava in to 3 major groups with 2 accessions each. The accessions C1 (Narrow leaved - MNS-13-1) and C5 (MNS-AICRP) formed a ball cluster with 86% genetic similarity were found to be highly related. Likewise, C3 (Pullad Kappa - MNS-13-5) and C4 (Karutha Malabar) also formed a ball cluster showing 86% genetic similarity. C3 (Pullad Kappa - MNS-13-5) and C4 (Karutha Malabar) recorded 80% genetic relatedness with C1 (Narrow leaved - MNS-13-1) and C5 (MNS-AICRP). Likewise, C2 (Devan Kappa - SRA-13-1) and C6 (Pullad Kappa) also showed 80% genetic similarity with each other. C2 (Devan Kappa - SRA-13-1) and C6 (Pullad Kappa) recorded only 67% genetic relatedness with C3 (Pullad Kappa - MNS-13-5), C4 (Karutha Malabar), C1 (Narrow leaved - MNS-13-1) and C5 (MNS-AICRP). However, the two accessions C3 and C6 of the landrace Pullad Kappa got separated with only 67% genetic similarity. The dendrogram generated grouped the accessions and species on the basis of Nei genetic distance using unweighted pair group method with arithmetic average (UPGMA) cluster analysis. The results of the study indicated that the primers selected for the present study will be useful for future genetic analysis of cassava germplasm and hence provide breeders with a genetic basis for selection of parent for crop improvement programs.**

***Keywords*: Cassava, Dendrogram, Germplasm, ISSR primers.**

1. Introduction

Roots and tuber crops are second in importance to cereals as global source of carbohydrate yield starchy roots, tuber, corms and stems that are used mainly as food for human, feed for animals and also for manufacturing starch, alcohol and fermented beverages including beer. They provide a substantial part of world's food supply and on a global basis approximately 45% of root and tuber crops production is consumed as food, with the reminder used as animal feed and for industrial processing for products. The consumption of root and tuber crops as food in developed countries is considerably smaller than it is in developing countries, but their use as animal feed is relatively higher. They also provide some minerals and vitamins, although a proportion of minerals and vitamins may be lost during processing. Many tropical tuber crops are used in the preparation of stimulants, tonics, carminatives and expectorants. India holds a rich genetic diversity of tropical root and tuber crops viz., cassava, sweet potato, yams, aroids and several minor tuber crops. Tuber crops constitute a major component of food for indigenous and tribal people throughout the world. Though yams and taro belongs to India, cassava was introduced from South America by the Portuguese during 16th and 17th century only. Sree Visakham Thirunal Rama Varma, the king of Travancore popularized cassava in South Kerala to save his people from the vagaries of recurrent famines.

Cassava is the most widely cultivated tuber crop in tropics and it is cultivated mainly for starchy roots. It is the most important food staple in tropics, where it is the fourth most important source of energy and carbohydrate. Cassava is grown for various end uses such as human food, animal feed and as industrial raw materials. The center of origin of cassava as first reported to be Central America, including Columbia, Venezuela, Guatemala and Southern Mexico, due to the large number of varieties present there (Rogers, 1963). The exact area of origin of cassava as a crop plant is unknown, although several theories have been put forth. Deborah (1998) suggested that cassava was first cultivated in North-Eastern Brazil as he based this theory on the abundance of wild *Manihot* species in that region. South America is known as the native place of cassava, but nowadays cassava is extensively cultivated as an annual crop in many tropical and sub-tropical regions of the world including Africa, India and Indonesia. Among these countries Africa is its largest centre of production.

Cassava, botanically known as *Manihot esculenta* Cants is a perennial shrub belonging to the family *Euphorbiaceous* of class dicotyledons. The *Manihot* genus has about 100 species

among which *Manihot esculenta* is the commercially cultivated one. It is a monoecious species with chromosome number in all species as 2n=36. In spite of this high chromosome number, *Manihot* species behave meiotically as diploid. Cassava is highly heterozygous in nature because of its outcrossing property. Increased genetic viability in cassava is noted because of its increased species variation on wild cassava and also with the traditional farming practices. Cassava cultivars lack many economically important characters such as resistance to insects, diseases, and drought and have low protein content (Nassar and Dorea, 1982; Nassar and Grattapaglia, 1986). This can be attributed to the mode of evolution of the species and modifications of the allogamy system of the plant (Nassar and O'hare, 1985).

The National and international research institutes maintain a very good number of germplasm collections of cassava. ICAR-CTCRI is the only institute in India dealing with the tropical tuber crops research in India. The genetic variability present in these germplasm collections are evaluated by using the morphological descriptors which are published by the International Plant Genetic Resources Institutes (IPGRI) and the ICAR-National Bureau of Plant Resources (ICAR-NBPGR). Morphological descriptors include both the above and below ground plant characters such as plant, stem, leaf, flower, fruit and tuber traits. The characters like plant height, stem diameter, internode length, leaf length and width, petiole length and width, number of tubers, tuber yield per plant, tuber length and weight etc. are collectively called as quantitative characters whereas the plant vigour, growth habit, stem colour, leaf scar, leaf colour, petiole colour, flowering, fruit colour, tuber outer skin, cortex and flesh colour etc. as the qualitative characters. In plant characterization, molecular characterization based mainly on DNA markers has been used to analyse/describe the germplasm to substantiate the morphological characterization and for the development of core groups and in genetic diversity Fregene *et al.* (1997) analysis.

2. Methodology

A. Aim and Objective

1) Aim

To analysis the variability among the 6 accessions of Cassava (*Manihot esculenta* Crantz) using ISSR markers.

2) Objectives

- To isolate DNA from Cassava (*Manihot esculenta* Crantz) germplasm.
- To generate DNA polymorphism data across 6 Cassava accessions using five ISSR markers.
- To estimate the extent of variability among the 6 Cassava accessions by similarity index and dendrogram using the molecular markers polymorphism data.

B. Source of plant materials

Leaf samples of 6 accessions of cassava *viz.*, C1 (Narrow leaved - MNS-13-1), C2 (Devan Kappa - SRA-13-1), C3 (Pullad Kappa - MNS-13-5), C4 (Karutha Malabar), C5 (MNS-AICRP) and C6 (Pullad Kappa) maintained in the field genebank (Figure 1& Plate-1) of the Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram served as the source material.

Fig. 1. Field genebank of ICAR-CTCRI

C1: Narow leaved

C2: Devan kappa

C3: Pullad Kappa

C4: Karutha malabar

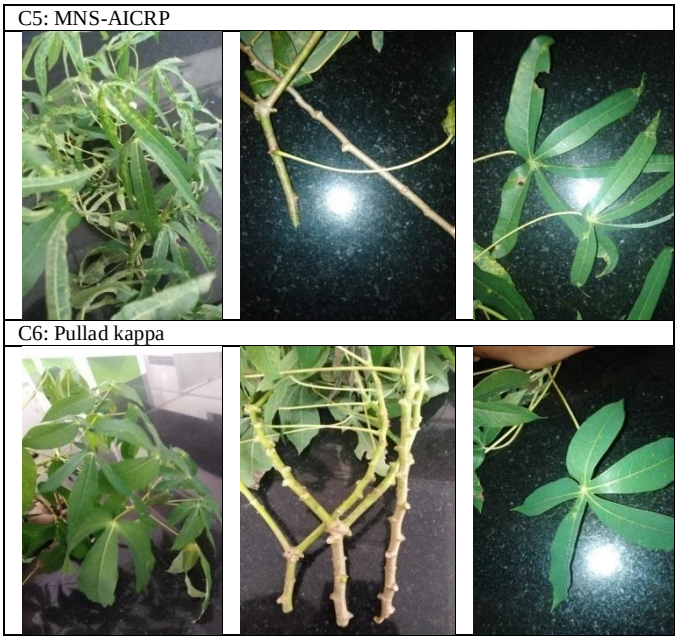

Fig. 2. Plate-1: Landraces selected for the study

C. Molecular Marker Analysis

In order to study the molecular markers in cassava characterization by using ISSR markers were carried out.

1) Sources of Primers

The primers were selected from collected literatures showing high polymorphic values in manihot species, ordered and shipped from TAKARA BIO INC, as lyophilise form. The list of primers used is given in table 1.

Table 1
List of primers used

S. No.	Primer Name	Sequence(5'—3')
1	UBC 811	GAG AGA GAG AGD GAG AC
2	UBC 808	AGA GAG AGA GAG AGA GC
3	UBC 817	CAC ACA CAC ACA CAC AT
4	UBC 836	AGA GAG AGA GAG AGA GYA
5	UBC 845	CTC TCT CTC TCT CTC TRG

D. Chemicals

1) Extraction Buffer

It has elements for the lysis of cell and cell membrane. It contains Tris-HCl, EDTA, CTAB, and NaCl, β-Mercaptoethanol and PVP.

Table 2
List of materials used

S. No.	Materials	Stock	Required concentration	Required volume
1	Tris-HCL(PH 8.0)	1M	100mM	1ml
2	EDTA(PH 8.0)	0.5M	20mM	0.4ml
3	NaCl	5M	2M	4ml
4	CTAB(w/v)		2%	0.2mg
5	PVP		2%	0.2mg
6	Beta-mercaptoethanol		0.2%	0.02ml

E. Isolation of DNA (CTAB Method)

From the sample collected 100mg each samples was weighed and grinded in frozen liquid nitrogen and 1.5ml of CTAB buffer. The grinded samples were taken in centrifuge tubes and were in centrifuge tubes and were heated at 65°C for 30 min. Then, it was centrifuged at 10000rpm for 10min at 4°C. The supernatant was collected and equal volume chloroform isoamyl alcohol (24:1) were added and the mixed by inversion at 37°C for 1hr. To that equal volume of chloroform isoamyl alcohol was added and mixed by inversion. Then, again it was centrifuged at 10000rpm for 10 min at 4°C. To the collected supernatant (aqueous layer) add 0.8ml isopropanol. Then centrifuge again at 10000rpm for 10min at 4°C. Pellet saved and supernatant discarded and washed with 70% ethanol and the pellet was air dried. To the dry DNA pellet, sterile distilled water or 1xTE buffer was added to dissolve the DNA and kept at room temperature for 1hr or till DNA dissolves. Then add 6µl of RNase and incubate at 37°C for 20min. Later this DNA was stored in -20°C till further studies.

F. Quality analysis of isolated DNA using Agarose gel electrophoresis

The quality of DNA isolated from cassava were determined using Agarose gel electrophoresis (AGE). The edges of a clean and dry gel casting tray, which was supplied with electrophoresis apparatus, were sealed with cello tape to make a mould. Agarose (0.5%) was added to 1x TBE buffer and boiled until Agarose melted completely. The solution was cooled and Ethidium bromide (0.5µl) was added. Comb was placed in the mould before pouring the melted Agarose, so that complete wells would be found. After solidification of agarose, the comb was removed and the gel was placed in an electrophoresis unit with wells towards the cathode and submerged in 1× TBE.

Loading the DNA samples in to the wells

4µl of DNA samples were mixed with 1µl of 6x loading dye. The DNA samples were carefully loaded in to the wells of the gel. The gel was run at 90V for 45 min and bands were visualized and documented in gel documentation system (Alpha imager).

G. Quantification of DNA Using Micro Volume Spectrophotometer

The microvolume spectrophotometer was employed for measuring the DNA concentration in the isolated samples of nucleic acid. The key to this advanced spectrophotometer is its unique sample retention technology that overcomes the need for cuvettes when taking measurements and the extremely low quantity of DNA needed for taking a reading. This is accomplished by placing the sample directly on top of detection surface and using the surface tension to create a column between the ends of optical fibers. Thus the measurement optical path is formed. The sensitivity range for DNA detection is between 2 and 3700ng/µl. The spectral range of the device is 220 to750nm and it is possible to scan all of the wavelengths. A single measurement cycles only 10 sec. The instrument is driven by a PC, which allows one to archive a large number of

measurements.

- Pipette 1-2µl sample directly on to the measurement pedestal.
- Lower the sampling arm and initiate a spectral measurement using the software on the PC
- Surface tension is used to hold samples in place between two optical fibers.
- Light from axen on flash lamp passes through the top optical fiber, down through the liquid column and is detected by the internal spectrometer.
- The purity of the DNA was determined by checking the ratio or absorbance. Concentration of DNA was derived using the formula- Concentration of DNA (µg/ml) = OD260 x dilution factor x 50

Dilution of DNA samples:

Samples were diluted to 10ng/µl concentration using nuclease free water irrespective of varying concentration calculated spectrophotometrically.

Table 3
Volume of DNA and SDW taken for the preparation of working stock (10ng/µl)

S. No.	Sample code	Concentration	Vol. of DNA (µl) for 10ng/µl Working stock	Vol. of SDW (µl)
1	C1	3685.8	0.271	99.7
2	C2	5129.6	0.194	99.8
3	C3	2889.01	0.346	99.7
4	C4	2218.6	0.451	99.5
5	C5	4236.4	0.236	99.8
6	C6	3664.3	0.272	99.7

H. Primer dilution

ISSR primers are used as they show high polymorphism. They were centrifuged before first use to avoid loss of DNA pellet. The oligos were dissolved in nuclease free water. Initially freezer stock was made at 100 micro molar concentrations by adding a volume of nucleases free water equal to 10 times the number of Nano moles of DNA present in the tube and stored as main stock. Working stock of 10 micro molar concentration were made by taking 10µl from the main stock and diluted it with 90µl of nucleases free water stored in 20 freezer.

I. PCR (Polymerase Chain Reaction)

Polymerase chain reaction is a laboratory technique for generating millions of copies of a specified DNA. PCR was developed by Kary Mullis in 1984. The reaction is extremely powerful and under perfect conditions could amplify 1 DNA molecule to become 1.07 billion molecules in less than 2 hours. The PCR technique can be used to introduce restriction enzyme sites to ends of DNA molecules, or to mutate particular bases of DNA. PCR has many variations such as reverse transcription PCR (RT-PCR) for amplification of RNA. The success of PCR depends on a number of factors, with its reaction components playing critical roles in amplification. The components used in PCR are given below:

Template DNA:

A PCR template for replication can be of any DNA source, such as genomic DNA (gDNA), complementary DNA (cDNA), and plasmid DNA. Nevertheless, the composition of complexity of the DNA contributes to optimal input amounts for PCR amplification.

DNA Polymerase:

DNA polymerases are critical players in replicating the target DNA. Taq DNA polytnerase is arguably the best-known enzyme used for PCR. Taq DNA polymerase has relatively high thermo stability, with a half-life of approximately 40 min at 95°C.It is as nucleotides at a rate of about 60 bases per second at 70°C and can amplify lengths of about 5 kb, so it is suitable for standard PCR without special requirements.

Primers:

PCR primers are synthetic DNA oligonucleotides of approximately 15-30 bases. PCR primers are designed to bind (via sequence complementarity) to sequences that flank the region of interest in the template DNA. During PCR DNA polymerase extends the primers from their 3'ends. As such, the primers' binding sites must be unique to the vicinity of the target with minimal homology to other sequence of the input DNA to ensure specific amplication of the intended target.

PCR is mainly carried out in three steps and they are:

a. Denaturation

On raising the temperature to about 94°C for about 5 minutes, the DNA gets denatured and the two strands separate.

b. Annealing

In this step, the temperature is lowered to 56.3°C for 1 minute and the added primers pairs with the complementary regions flanking target DNA strands. High concentration of primer ensures annealing between each DNA strand and the primer rather than the two strands of DNA.

c. Extension

This process is carried out in 72°C for 8 minutes. The primers are extended by joining the bases complementary to DNA strands. The synthetic process in PCR is quite comparably to DNA replication of the leading strand.

PCR Amplification:

The ISSR PCR for the isolated and diluted DNA against respective primers was mixed as mentioned below in well labelled PCR tubes. The thermal cycling was carried out in a Proflex programmable thermal cycler.

PCR using EMERALD Amp GT PCR Master Mix:

EMERALD Amp GT PCR Master mix by TAKARA BIO INC is a 2x premix composed of DNA polymerase, optimized reaction buffer, dNTPs, and a density reagent.

The premix also contains a vivid green dye that will separate into blue and yellow dye fronts when run on an agarose gel. The premix simplifies PCR assembly; simply add primers, template, and water and start reaction. After PCR, the reaction mixture can be applied directly to a gel for analysis. For the sake of convenience and for save time during the PCR mix preparation,

we used Emerald Amp GT PCR master mix for the following set ISSR PCR.

PCR master mix:

Table 4
ISSR reaction mixture

Components	Stock Concentration	Required Concentration	Volume for One Reaction
EMERALD PCR MASTER MIX	2X	1X	7.5 µl
PRIMER	10 µM	0.3 µM	0.5 µl
DNA	10ng/ µl	40ng/ reaction	4.0 µl
SDW	(to a make up the final volume to 15 µl)		3.0 µl
TOTAL			15.0 µl

ISSR PCR Cycling Conditions:

Lid - 105°C
Initial Denaturation - 94°C for 5 Minutes
Denaturation - 94° C for 30 Minutes
Annealing - 56.3° C for 1 Minute
Extension - 72° C for 1 Minute
Final Extension - 72°C for 8 minutes
HOLD - 4°C

Agarose Gel Electrophoresis (AGE):

2% agarose gel electrophoresis was used to separate the amplicons obtained after PCR. Increased percentage of the gel accounts for increasing the sieving effect which is needed for fine resolution of the amplicons obtained. Intercalated ethidium bromide added with the agarose gel help to visualize DNA bands on UV light.

Weighed 1.7gm of agarose in 250ml conical flask, added 85ml 1X TBE (Appendix III) buffer and boiled the in a microwave oven with occasional mixing until agarose was completely dissolved in the buffer. Allowed it to cool to a bearable temperature and added 0.85µl/ml ethidium bromide carefully without spilling. Prepared the gel plates/moulds and kept combs in position. Poured the warm gel to plate and cool for 20 minutes. Filled the horizontal electrophoretic tank with 1X TBE buffer. After gel got solidified removed the comb and placed the gel along with the plate into the tank. Loaded 10uL samples directly on to wells. 3µl 100bp DNA ladder was also loaded on to the first well for reference. Ran the gel at 85V and 220mA for 1 to 1.5 hour. Visualized the band under UV trans-illuminator and documented in a Gel documentation unit (Syngene G: BOX gel documentation system). The images were finally scored to detect polymorphism.

J. Data analysis

The bands obtained by electrophoresis were labelled as present (1) or absent (0). This binary data were scored and statistically analyzed used NTSYS pc Ver.2.2. Pair-wise distance (similarity) matrices were computed using sequential, agglomerative, hierarchical and nested (SAHN) clustering option of the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System, Biostatistic, New York, U.S.A., Software Version 2.02 package). The program also generated dendrogram, which grouped the accessions and species on the basis of Nei genetic distance using unweighted pair group method with arithmetic average (UPGMA) cluster analysis.

3. Experimental Result and Discussions

The result of the study entitled "Molecular profiling of landraces of Cassava (*Manihot esculenta* Crantz) using ISSR markers" was carried out at the Division of Crop improvement, Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala during 2019-20 presented in this section.

A. Isolation of DNA

The isolation of DNA from *D. alata* was done by using manual method. By using this method very good yield of DNA obtained with high purity. Figure 3 shows the 1% Agarose gel electrophoresis image for quality check of the isolated DNA samples.

The concentration of the isolated DNA samples ranged from 2218.6ng/µl to 5127.6ng/µl. The concentrations of different samples assessed using Nano spectrophotometer together with purity ratios are given in Table 5.

Table 5
The concentration and primity ratio of different DNA samples isolated

S. No.	Sample No.	Concentration	OD AT 260/280
1	C1	3685.834	2.30
2	C2	5129.579	2.38
3	C3	2889.016	2.33
4	C4	2218.593	2.26
5	C5	4236.363	2.33
6	C6	3664.309	2.29

B. Molecular characterization using ISSR primers

PCR amplification of the above mentioned cassava accessions was done using five selected ISSR primers and the amplicons obtained were resolved in 2% agarose gel (Figure 4).

Molecular variability characterized among the 6 cassava accessions using five selected ISSR markers were given in the Table 6.

Table 6
Number of amplified bands and its polymorphism (%) per primer

S. No.	Primer	Total number of bands	Number of polymorphic bands	Polymorphism (%)
1	UBC 811	7	7	100
2	UBC 808	12	5	41.67
3	UBC 817	3	1	33.3
4	UBC 835	7	4	57.13

UBC 808 produced highest number of bands (12) and UBC 817 produced the lowest number of bands (3) while UBC 845 is not amplified. The percentage polymorphism of the ISSR primers studied ranged from 33.3% - 100%. UBC 817 recorded

the lowest polymorphism (33.3%) and UBC 808 recorded the highest Polymorphism (100%).

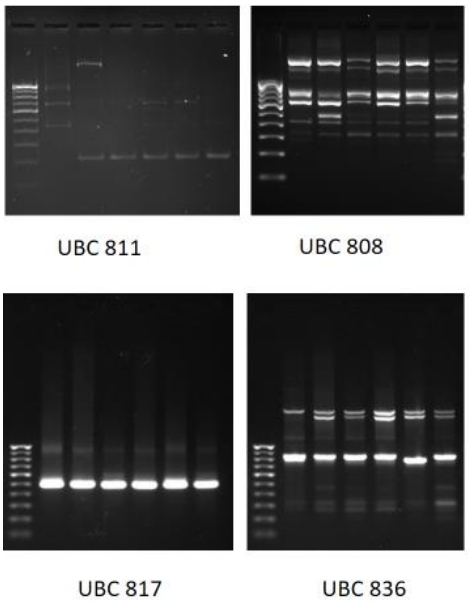

Fig. 3. Agarose gel electrophoresis for the quality check of the isolation DNA samples

C. *Data analysis*

The bands obtained by electrophoresis were labelled as present (1) or absent (0). This binary data were scored and statistically analyzed used NTSYS pc Ver.2.2. Pair-wise distance (similarity) matrices were computed using sequential, agglomerative, hierarchical and nested (SAHN) clustering option of the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System, Biostatistic, New York, U.S.A., Software Version 2.02 package). The program also generated dendrogram, which grouped the accessions and species on the basis of Nei genetic distance using unweighted pair group method with arithmetic average (UPGMA) cluster analysis.

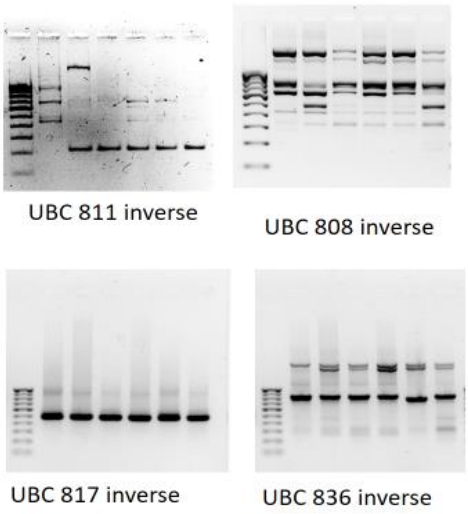

Fig. 4. PCR amplification of cassava accessions using four selected ISSR marker

D. *Similarity index*

The similarity index values obtained among the 6 genotypes based on ISSR marker data is given in the Table 7. The similarity coefficient based on ISSR markers ranged from 0.55 to 0.86.

Table 7
Similarity matrix based on Jaccard's coefficient for 6 cassava genotypes based on 4 ISSR markers

	C1	C2	C3	C4	C5	C6
C1	1.00					
C2	0.62	1.00				
C3	0.76	0.79	1.00			
C4	0.76	0.72	0.86	1.00		
C5	0.86	0.69	0.83	0.83	1.00	
C6	0.55	0.79	0.72	0.66	0.62	1.00

The dendrogram generated using UPGMA cluster analysis separated the 6 accessions of cassava in to 3 major groups with 2 accessions each. The accessions C1 (Narrow leaved - MNS-13-1) and C5 (MNS-AICRP) formed a ball cluster with 86% genetic similarity were found to be highly related. Likewise, C3 (Pullad Kappa - MNS-13-5) and C4 (Karutha Malabar) also formed a ball cluster showing 86% genetic similarity. C3 (Pullad Kappa - MNS-13-5) and C4 (Karutha Malabar) recorded 80% genetic relatedness with C1 (Narrow leaved - MNS-13-1) and C5 (MNS-AICRP). C2 (Devan Kappa - SRA-13-1) and C6 (Pullad Kappa) also showed 80% genetic similarity with each other. C2 (Devan Kappa - SRA-13-1) and C6 (Pullad Kappa) recorded only 67% genetic relatedness with C3 (Pullad Kappa - MNS-13-5), C4 (Karutha Malabar), C1 (Narrow leaved - MNS-13-1) and C5 (MNS-AICRP). However, the two accessions C3 and C6 of the landrace Pullad Kappa got separated with only 67% genetic similarity. The dendrogram generated grouped the accessions and species on the basis of Nei genetic distance using unweighted pair group method with arithmetic average (UPGMA) cluster analysis. The result indicated that the primers selected for the present study will be useful for future molecular profiling of cassava germplasm.

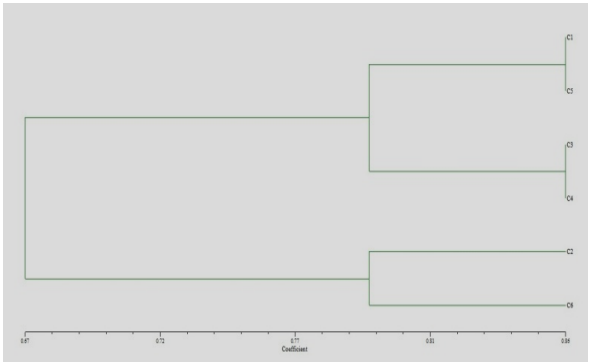

Fig. 5. Dendrogram showing the clustering pattern in 6 cassava genotypes using 4 ISSR markers

4. Conclusion

Cassava is the most important tropical root crop for its starchy roots that forms a major source of dietary energy for

more than 500 million people. According to the United Nations Food and Agriculture Organisation (FAO), cassava ranks fourth as a food crop in the developing countries, after rice, maize and wheat. The leaves are relatively rich in protein and can be consumed. Cassava can be stored in the ground for several seasons, thereby serving as a reserve food when other crops fail. Cassava is an efficient producer of carbohydrate under optimal growth conditions like uncertain rainfall, infertile soil and limited input encountered in tropical areas. This makes cassava an attractive source of food, feed and renewable industrial raw materials. In India cassava has been used both for direct consumption and for the production of starch and sago mainly in the southern states of Andhra Pradesh, Kerala and Tamil Nadu.

In the present study, the genetic variability among 6 accessions of cassava germplasm maintained in the field genebank of ICAR-CTCRI and morphologically characterized were subjected for molecular analysis using 5 selected ISSR primers. UBC 808 produced highest number of bands (12) and UBC 817 produced the lowest number of bands (3) while UBC 845 is not amplified. The dendrogram generated using UPGMA cluster analysis separated the 6 accessions of cassava in to 3 major groups with 2 accessions each. By this analysis the accessions C1 (MNS-13-1) and C5 (MNS-AICRP) formed a ball cluster with 86% genetic similarity were found to be highly related. Likewise, C3 (Pullad Kappa - MNS-13-5) and C4 (Karutha Malabar) also formed a ball cluster showing 86% genetic similarity. While C2 (Devan Kappa - SRA-13-1) and C6 (Pullad Kappa) recorded only 67% genetic relatedness with C3 (Pullad Kappa - MNS-13-5), C4 (Karutha Malabar), C1 (Narrow leaved - MNS-13-1) and C5 (MNS-AICRP). However, the two accessions C3 and C6 of the landrace Pullad Kappa got separated with only 67% genetic similarity. The result indicated that the ISSR primers selected for the present study will be useful for future molecular profiling of cassava germplasm.

Genetic diversity serves as a way likely that some individuals in a population will possess variations of alleles that are suited for the environment. The genetic diversity study in cassava is very much needed for the accomplishment of breeding programs, since genetic variance produces high heterotic effect. The results of the study is in line with the process of selecting core collection of cassava as refined by the use of molecular markers. The studies on genetic diversity of an *in vitro* germplasm collection of African cassava clones evaluated using RFLP (Beeching *et al.*, 1993), multilocus finger printing (Bertram, 1993) and RAPD markers (Manney *et al*., 1994; Yu and Nguyen, 1994) supports the use of molecular markers in the germplasm characterization and diversity studies in cassava. In all these cases, the genetic diversity and cultivar relationships were assessed with germplasm collected as bulked samples. Moreover, genetic variation at microsatellite loci can be used for the investigation in evolutionary and geographical origins of cassava and population structures of cassava wild relatives (Olsen and Shaal, 2001).

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