MOLECULAR CHARACTERIZATION OF CASSAVA CHIPS LINE CMR-100 AND ITS PROGENIES USING SSR MARKERS

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By

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2016

DECLARATION

I hereby declare that this thesis entitled "**Molecular characterization of cassava chips line CMR-100 and its progenies using SSR markers"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellayani   Keerthana P. V.

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Produce tuber reduce hunger

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LIST OF ABBREVATIONS AND SYMBOLS

◦C	Degree Celsius
%	Percentage
µg	Microgram
µl	Microgram
A	Adenine
A_{260}	Absorbance at 260 nm wavelength
A_{280}	Absorbance at 280 nm wavelength
AFLP	Amplified Fragment Polymorphism
BC	Before Christ
bp	Base pair
CMD	Cassava mosaic disease
CTCRI	Central Tuber Crops Research Institute
cm	Centimetre
C	Cytosine
DNA	Deoxyribo nucleic acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylene diamine tetra acetic acid
EST	Expressed sequence tag
et al	And other co workers

Fig.	Figure
G	Guanine
Hrs	Hours
ISSR	Inter simple sequence repeat
KAU	Kerala Agricultural University
Kbp	Kilo base pair
kg	Kilogram
M	Molar
mg	Miligram
$MgCl_2$	Magnesium Chloride
min	Minute
ml	Millilitre
mM	Millimolar
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ng	Nanogram
OD	Optical Density
PCR	Polymerase Chain Reaction
PVP	Polyvinyl pyrrolidine
RAPD	Random Amplified Polymorphic DNA
RNase	Ribonuclease

rpm	Revolution per minute
s	Second
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
T	Thymine
Taq	*Thermus aquaticus*
TBE	Tris- EDTA Buffer
T_m	Melting Temperature
Tris HCl	Tris (Hydroxyl Methyl) aminomethane hydrochloride
UV	Ultraviolet
V	Volt
W	Watt

INTRODUCTION

INTRODUCTION

Cassava, (*Manihotesculenta*Crantz) is one of the most widely cultivated root cropthat feeds more than 800 million people in the developing world.Itis the second most important tuber crop used as staplefoodafter potato in Africa, Asia and Latin America.The root is rich in carbohydrates, calcium, vitamin B, vitamin C and essential minerals like phosphorus, potassium and iron.The leaves are used as vegetable as it contains 20-30% proteins along with vitamins A and B(Aregheore, 2012).Cassava is anadaptablecrop that grows in unfavorable environmental conditions like drought,soils with low fertilityand acidity(Edwards*et al*., 1970). It is propagated vegetatively by stem cuttings, and can be harvested in 8 to 10 months. The tuber is also used as an animal feed and also as a raw material for various industrial products.

Cassava breeding developedafter the establishment of the International Institute of Tropical Agriculture (IITA) in Nigeria and the International Center of Tropical Agriculture (CIAT) in Colombia (Ceballos *et al*., 2004).The breeding objectives of cassava includes yield, dry matter content, cooking quality, drought tolerant, disease resistance, β-carotene, early root bulking, cyanogenic glucosides and starch (Vincent *et al*.,2014;Jennings and Hershey, 1985).The cassava breedersproduce full-sib families by controlled pollination and half-sib familiesby open pollination.The open pollinated progenies of interspecific hybrids of cassava produce spontaneous tetraploids and triploids(Hahn *et al*., 1990).Genotypes can be improved using open pollinated progenies. The authentication of variety or hybrids is very significant for the reliability of breeding programme. Many techniques like GOT (Grow Out Test), biochemical markers and DNA based molecular markers are available for purity assessment.

Molecular markers are DNA sequences which reveal sites of variation in DNA. These are also known as genetic markers or DNA markers.The advances in DNA marker technology and their wide range of applications like germplasm characterization, diversity studies, marker assisted selection and phylogenetic analysispaved a new way forplant breeding and crop improvement. These markers

can be used foreasy and reliable identification of breeding lines, hybrids, cultivars, varieties and parental lines(Asif *et al*., 2009).Hashemi*et al*., 2009 emphasized that the fingerprinting of hybrids and identification of their genetic relationships are important for crop improvement, seed purity testing, DUS (Distinctness, uniformity and stability) testing, variety registration and protection of farmer's and breeder's rights. Many studies have been conducted to identify true hybrids in different crops using molecular markers like RAPD (Random Amplified Polymorphic DNA) (Shukla *et al*., 2015), ISSR (Inter Simple Sequence Repeats) (Bianco *et al*., 2011), AFLP (Amplified Fragment Length Polymorphism) (Schaber and Goldman, 2013) and SSR (Simple Sequence Repeats) (Bora *et al*., 2016). Among these DNA markers, SSR has wide application in characterization and clarification of parentage-offspring relationship as well as authentication of genotype identity (Gomez *et al*., 2008).

SSRs or microsatellites are short tandem repeats of DNA sequences present in the genomes of all eukaryotes. This co-dominant marker is simple, quick, discriminative, reliable, repeatable,efficient and amenable for high throughput analysisof hybrid identity and genetic purity(Selvakumar*et al*., 2010).SSR marker is used for the identification of true hybrids of cassava (Mohan *et al*., 2013;Vincent *et al*., 2014). To our knowledge there is no report of any work related to molecular makers being used to identify the parental lines of progenies with unknown parental or any related work to identify the unknown parent of half-sib lines.

The present study was formulated with the intension of understanding the capability of molecular markers for identifying the unknown parents of a progeny. In this work, SSR marker was used to identify the parents of CMR-100, which is an open pollinated progeny of cassava. This study was undertaken with the following objectives:

- To identify the parents of CMR-100 using SSR markers.
- To access the genetic diversity among open pollinated progenies of CMR-100 with cassava cultivars.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Cassava (*Manihot esculenta* Crantz, 2n = 36) also known as yucca, manioc, mandioca or tapioca belongs to the dicotyledon family Euphorbiaceae and is an important source of nutrition (Alves, 2002).

History

The center of origin of cassava is Brazil, where it shows maximum diversity. Cassava was domesticated in Peru earlier than 4000 BC (Ugent *et al*., 1986). European sailors carried out this crop to Africa in the 16th century. It was introduced into India from Brazil by Portuguese traders in Malabar region of Kerala during the 17th century (Edison *et al*., 2006). This crop was popularized in Kerala by the king of Travancore state, Sri Vishakham Thirunal, who introduced high yielding varieties from Malaya (Duraisamy *et al*., 2011).

Morphology

Cassava being a perennial woody shrub, possesses highly variable morphological characters due to interspecific hybridization. It is an erect growing shrub, branched or unbranched at the top.

Roots, the storage organ of cassava is not a tuberous root and cannot be used for propagation. The plants grown from true seeds produce tap root and secondary roots whereas plants grown from stem cuttings produce adventitious roots and later both tap and adventitious roots become storage roots. The bark, peel and parenchyma are three different tissues present in the storage roots of cassava and parenchyma is the edible part. The surface of bark can be wrinkled or smooth and is commonly seen in dark brown colour. The peel may be in white, cream or pink colours and is 1 to 2 mm thick. The edible flesh of cassava tuber is either bitter or sweet in taste (Alves, 2002).

The cassava stem is cylindrical with sympodial branching which produce lateral and reproductive branches. Leaves are simple, alternate with lamina and petiole. The lamina is deeply lobed with palmate veins. Petiole is variously

pigmented from light green to yellowish green, light purple and light pink or pink and the length normally ranges from 5 to 30 cm (Alves, 2002).

It is monoecious and protogynous, with 36 chromosomes and is highly heterozygous due to its out-crossing nature. The inflorescences are produced at the reproductive branches where male flowers develop near tip, while larger female flowers develop closer to the base of the inflorescence (Alves, 2002). Honeybee is the main pollinating agent. Some varieties flower frequently and regularly, while others flower rarely or not at all. Environmental factors, such as photoperiod and temperature influence flowering in cassava. The cassava fruit is six winged trilocular capsule with ovoid-ellipsoidal seeds of 1 cm in length (Palaniswami and Peter, 2008). It is vegetatively propagated mainly by means of stem cuttings and to some extent through seeds (Rajendran *et al*., 2005). It can be self-pollinated, but suffers from inbreeding depression.

Importance

This root crop grows well in poor soils with limited labor requirements. It provides food security during conflicts because the invader cannot easily destroy or remove the crop, since it conveniently grows underground. Cassava is usually intercropped with vegetables, plantation crops (such as coconut, oil palm, and coffee), yam, sweet potato, melon, maize, rice, groundnut or other legumes.

Apart from food, cassava is very versatile and its derivatives such as starch are applicable in many types of products such as foods, confectionery, sweeteners, snacks, glues, plywood, textiles, paper, biodegradable products, monosodium glutamate and drugs. Cassava chips and pellets are used in animal feeds and alcohol production.

The crop possesses some medicinal properties and is used for the treatment of various diseases. Bitter variety of *Manihot* is used for the treatment of diarrhoea and malaria (Idu and Onyibe, 2007) and the leaves are used to treat hypertension, headache and pain.

Production

The world cassava production is more than 291.3 million tonnes during 2014, of which 57% is contributed by sub-Saharan Africa. Nigeria is the largest producer, whereas Thailand is the largest exporter of cassava (FAO, 2014). In Asia, India ranks 4th in cassava production (FAO, 2014) and major contribution are from southern parts of the country especially Kerala and Tamil Nadu (Palaniswami and Peter, 2008).

Cassava production depends on a supply of quality stem cuttings. According to FAO (2014), the propagation of cassava with improved varieties leads to 2% increase in the production.

Disease and constraints

Cassava crop is affected by various diseases and pests. Cassava bacterial blight (CBB), cassava mosaic disease (CMD), cassava root rot (CRR), cassava mosaic virus (CMV), cassava brown streak disease (CBSD) and cassava frogskin disease (CFSD) are the important diseases that affect the cassava production. Cassava bacterial blight, induced by *Xanthomonas axonopodis pv. manihotis* is the major disease in South America. The fungal disease Super-elongation is caused by *Sphaceloma manihoticola* whereas cassava root rot is caused by *Phythophthora* species. Frogskin disease is associated with phytoplasma which directly affects root yields. Cassava brown streak disease is considered important in Tanzania, Uganda, Mozambique and coastal Kenia. The most important constraint to cassava production in tropical countries was cassava mosaic disease (CMD) and is caused by several cassava mosaic geminiviruses (Alvarez *et al*., 2006). In India, the CMD is caused by *Indian cassava mosaic begomovirus* (ICMV) and is a potential threat to cassava production causing yield loss ranging from 25-80% (Makeshkumar *et al*., 2006).

*Mononychellu*s mite complex, mealybugs (*P. herreni* and *P. manihoti*) the hornworm *Erinnyis ello*, whiteflies, lacebugs, stemborers, fruitflies, shootflies, scales, thrips and gallmidges are the important pests which are co-evolved with this crop. The generalist feeders that attack cassava include several *Tetranychus* mite

species, certain whitefly species (*Bemisia tabaci* and others), a complex of white grub species, termites, cutworms, grasshoppers, leaf-cutting ants, burrowing bugs, crickets, stemborers and others (Bellotti *et al*., 2012).

Breeding in Cassava

Crop improvements through plant breeding have great importance in the modern agriculture. Inorder to meet the requirement of growing population high yielding, disease resistant varieties were developed in many crops like rice, wheat etc. with the involvement of plant breeding techniques.

Cassava breeding started with the selection of good quality plants by farmers. In the early 20th century the scientific breeding of this tuber crop began and the priority increased after the formation of two international centres CIAT and IITA in late 1960s. In India, ICAR-CTCRI focused on the breeding and improvement of cassava along with other tuber crops (Jennings and Iglesias, 2002).

Cassava seeds produced through hybridization are used for most of the breeding programmes, in order to create more genetic variations. Sometimes open pollinated sib-lines are also used for this purpose. The utility of the crop determines the breeding objectives *ie*., productivity and high dry matter content is important for industrial use whereas cooking quality is focused if it is used for human consumption (Ceballos *et al*., 2004). Parental lines for the breeding were selected based on their performance and general combining ability (Hallauer and Miranda, 1988). Cassava mosaic disease is a serious threat to Indian and African cassava cultivation. So breeding activities were carried out to develop CMD resistant varieties and many varieties which are disease resistant and high yielding were released till now by ICAR-CTCRI and other organizations. Through breeding, carotene content of cassava has been improved (Iglesias *et al*., 1997). The difficulties faced by cassava breeders include seed dormancy and issues related to synchronization of flowering. In spite of this, the controlled pollination in the open field may be contaminated with illegitimate pollen right from the time of pollination, seed collection and nursery bed establishment to planting of the trials

(Vincent *et al*., 2014). So that verification of progenies obtained from the cross is essential for the success of breeding.

Importance of True Hybrids

Hybrids are produced by crossing two genetically different parental cultivars of a particular crop. Hybrid varieties have great importance in the modern agriculture. The development of improved cultivars through hybridization aided increased production and quality of different crop species (George *et al*., 2005). Rice production is increased by using hybrid varieties for cultivation (Viramani and Kumar, 2004). India is the second largest producer of cotton and is attained by using hybrid cotton varieties among 70% of the cultivated areas (Mehetre *et al*., 2007).

Significance of True Hybrid Identification

Crop production has to be increased to meet the demand of growing population. The supply of good quality hybrid seeds to farmer is essential for the increase of crop production. For the commercialization of any hybrids, it should possess high genetic purity. Genetic purity of hybrid/variety is referred as the absence of seeds of genotypes other than the specific one (Dongre *et al*., 2011). Hybrid seeds are often contaminated by crossing with foreign pollens or by selfing. Mao *et al*. (1998) stated that 1% impurity in the hybrid seed may decline the yield potential of hybrid by 100 kg/ha. So before the supply of hybrid, it is essential to assess the genetic purity of the hybrids in a short time. Varietal identification and purity testing has great impact on seed production, breeding as well as intellectual property rights point of view to ensure quality seeds (Rao *et al*., 2015). Besides these, the authentication helps to prevent the unauthorized commercial use of hybrids.

Methods for Hybrid Identification

1. Morphological markers

Conventionally hybrid identification is done using morphological markers. GOT (Grow Out Test) is method for authentication of hybrids based on these morphological markers. Heritable morphological characters of seeds, seedlings, leaves, fruits, stem, flowers etc. are used as markers for hybrid confirmation. An authentic standard sample must be available for comparison which is required to be treated and examined in the same way as the sample is tested. The differences in descriptors help to distinguish true hybrids from off-types (Ballester and deVincente, 1998). These morphological markers are not efficient because the expressions of specific morphological or physiological traits are highly influenced by environmental conditions (McDonald, 1995; Selvakumar *et al.,* 2010). Moreover, this method consumes money and time (Dongre *et al.,* 2011) for the identification. According to Selvakumar *et al*. (2010), morphological differences are not enough to distinguish true hybrids from off-types if the parents are genetically similar.

2. Biochemical markers

Biochemical markers are another alternative method for hybrid purity assessment. The biochemical markers include isozymes and other seed storage proteins which can be distinguished by electrophoresis techniques. Many studies use these biochemical markers as a tool for hybrid identification in crops like *Arachis sp*. (Lacks and Stalker, 1993) pumpkin (Yan, 2013), and sorghum (Kavimandon and Khan, 2011). Eventhough, the biochemical markers reduce the time for hybrid purity confirmation, these markers also have some drawbacks like low reproducibility, effect of developmental stage of plant etc. The lack of abundance and dependency on environmental factors make it less attractive for purity assessment of cultivars and hybrids (Selvakumar *et al.,* 2010). Sometimes the zymograms shows complex banding patterns due to polyploidy or gene duplication which may complicate the interpretation (Kumar *et al*., 2009). So there is a need of a technique which is independent of environmental factors for hybrid

This problem was solved with the introduction of DNA based molecular markers in plant breeding.

3. DNA based markers

DNA markers are molecular markers that detect variation at DNA level. These markers do not rely on the environmental conditions. This is an appropriate technique for genetic purity assessment more quickly and accurately at low cost. The efficiency of DNA markers to distinguish more closely related accessions make it more suitable for purity analysis. The ideal DNA markers are highly polymorphic, abundant, easily available, highly reproducible and detectable in plant tissues and do not relay on the growth, differentiation and defense status of crops. These properties make molecular markers indispensable for crop improvement.

These advanced markers have many applications other than purity assessment like genetic diversity analysis, gene identification, studies of hybridization and introgression *etc.* There are three types of DNA markers: hybridization based, PCR based and sequence based. The RAPD, AFLP, SSR, ISSR are PCR based whereas RFLP and SNP comes under hybrization and sequence based category respectively (Azhaguvel *et al*., 2006).

a. Random Amplified Polymorphic DNA

RAPD was the first PCR based marker developed by Welsh and McCelland (1991). RAPD primers are usually single 10bp synthetic primers of random sequence which are able to amplify DNA segments in the genome. Since it is a random primer, there is no need of sequence data for primer construction. In this technique, the primer anneals to the genomic DNA at two different sites on the complementary strands of template DNA. A discrete DNA product is formed in PCR amplification, if the priming sites are within an amplifiable range. The polymorphism in RAPD is because of variation in the primer annealing sites (Kumar *et al*., 2009) and these RAPD amplified products are visualized using agarose gel electrophoresis followed by gel documentation. The factors which influence the reproducibility of RAPD markers includes; quality and quantity of

template DNA, primer to template ratio, annealing temperature, PCR buffer, concentration of magnesium chloride, brand of Taq DNA polymerase and PCR machine (Wolff *et al*., 1994). Bartish *et al*. (2002) suggested that RAPD can be a sensitive method for detection of genetic variation according to the isolation-by-distance model. Fernandez *et al*. (2002) carried out phylogenetic studies in barley cultivars at different countries using this marker technique along with ISSR markers. Alzate-Marin *et al*. (1996) used RAPD marker for true hybrid identification from closely related progenitors of common bean. Tabbasam *et al*. (2006) carried out hybrid authentication of sorghum using 20 RAPD primers and suggested that this marker had great importance in genotype identification because simple agarose gel electrophoresis is used for detection.

Nair *et al*. (2006) had conducted characterization of 12 intergeneric hybrids of *Saccharum sp*. with *Erianthus sp*., in sorghum and maize using 20 RAPD markers. Without any uncertainity, these markers were able to identify genuine intergeneric hybrids of *Saccharum sp*. Out of 20 primers, 10 primers produced male specific bands in hybrids. On the other hand, RAPD produce 107 *Erianthus* specific bands, but all the four hybrids didn't show any of these bands. So a large number of RAPD primers were required for the screening of *Saccharum* X *Erianthus* hybrids.

Fifteen RAPD primers were used to authenticate four sorghum hybrids *viz*. CHS-14, CSH-9, CSH-19R and CSH-15R (Akhare *et al*., 2008). Based on the presence and absence of bands, markers are categorized into three. Category one includes markers which show bands in both hybrids and parents, and are suitable for identifying hybrid of their respective parents. Next category consists of markers which express bands in parents but not in hybrids which are useful for distinguish self and off-sibs from sib-lines. And the third category is non-parental bands appeared in the hybrids those helps to determine the specific hybrid and thereby protect the breeder's right.

Ali *et al*. (2008) successfully carried out the authentication of cotton hybrids with 16 RAPD primers. Among these primers GLG-17 shows maximum polymorphism (87.5%) whereas GLH-2 shows minimum (42.85%). RAPD banding pattern and dendrogram constructed with UPGMA (Unweighted Pair Group Method based on Arithmetic Mean) revealed the true hybrids. The hybrid (Paymaster x SLS1) shows 82.46% similarity to male parent SLS1 and 65% similarity to female parent Paymaster.

Ilbi (2003) determined the seed purity of five hybrid varieties of *Capsicum annuum* with RAPD analysis. George *et al*. (2005) confirmed the hybrids of black pepper. Since it is a dominant marker, male parent specific bands can only determine the true hybrids (Selvakumar *et al*., 2010). According to Shukla *et al*. (2015), RAPD is a reliable tool for seed certification, after evaluating true hybrids in eggplant. This marker technique requires high purity DNA to avoid unwanted amplification of DNA. Low reproducibility and poor locus specificity are other drawbacks of this method.

Kumar and Kalyani, (2016) successfully determined the genetic purity of tobacco hybrids using twenty RAPD primers. Among these 20 RAPDs employed, 3 primers (OPA-5, OPA-7 and OPA-9) produced specific bands for F_1 hybrids and for both the parents.

b. Amplified Fragment Length Polymorphism

Amplified Fragment Length Polymorphism (AFLP) is a combination of RFLP and PCR techniques. Selective Fragment Length Amplification (SFLA) and Selective Restriction Fragment Amplification (SRFA) are the synonyms of AFLP. It is a DNA fingerprinting technique which involves generation of a genomic library, restriction digestion of genomic DNA into fragments, amplification of the DNA fragments after adapter ligation and selective PCR amplification (Vos *et al*., 1995). The electrophoresis technique like polyacrylamide gel electrophoresis or capillary electrophoresis are used for detection of polymorphism and visualized with autoradiography or fluorescence methodology (Kumar *et al*., 2009).

This molecular marker is used in studies like genetic identity, parentage and identification of clones and cultivars, phylogenetic studies of closely related species, genetic diversity analysis and characterization of germplasm. This abundant marker is used for genetic diversity studies in crops like maize (Lubberstedt *et al*., 2000), peanut (Herselman, 2003) *etc.* and construction of genetic linkage maps in some crops (Komatsuda and Mano, 2002). Many diversity studies had been reported in cassava using AFLP (Fregene *et al*., 2000; Elias *et al*., 2000; Wong *et al*., 1999). Schaber and Goldman (2013) chose AFLP marker technique for hybrid identification in two vegetable crops, carrot and beetroot along with visual identification. The male parent specific fragments were used to score progenies and to determine the hybrid purity. On an average, about 64% of true hybrids were found in all the fertile crosses. Out of five carrot crosses, three crosses exhibited significant deviations from expected results in identification with respect to AFLP based progeny identification. The results of this study clearly demonstrate the inaccuracy of visual identification of hybrid progeny.

c. Inter Simple Sequence Repeat

Inter Simple Sequence Repeat (ISSR), discovered by Zietkiewicz *et al*. (1994) is a PCR based DNA marker which amplifies DNA segments present at an amplifiable distance in between two identical microsatellites. The amplification of DNA is done with PCR technique where microsatellite is used as primer and the polymorphism is detected with either agarose or polyacrylamide gel electrophoresis. This simple and quick technique requires only low quantity of DNA for assay.

ISSR markers along with cluster analysis can be used for hybrid purity determination and to predict the characteristics of F_1 hybrids in artichoke (Bianco *et al.,* 2011). Hybrids were identified with male specific bands produced by ISSR markers and three markers *viz*., 857c, 857g and 878 were sufficient to assess the hybrid purity. The morphological descriptors were also included in this study for confirmation.

Khajuparn *et al*. (2012) successfully verified true hybrids in legumes like mungbean with ISSR-PCR assay. Ten ISSR primers were used to authenticate hybrids from six cross combinations. Out of these 10 primers, 3 (UBC 807, UBC 857 and UBC 841) show maximum polymorphism. The demerits of ISSR include dominant inheritance, reproducibility problems and homology of co-migrating amplification products.

d. Simple Sequence Repeats

Simple Sequence Repeats (SSR) is short tandem repeats of DNA present in all eukaryotic genome. The introduction of SSR or microsatellites revolutionized the molecular marker technology. It is hypothesized that the variation or polymorphism of SSRs are a result of polymerized slippage during DNA replication or unequal crossing over (Levinson and Gutman, 1987). SSR markers developed from genomic libraries are analyzed by PCR-amplification of a short genomic region containing the repeated sequence. The size estimation of the repeat length is obtained from gel separation. SSR is a co-dominant marker which requires only very little DNA for analysis and is usually visualized on metaphor agarose or polyacrylamide gels.

SSR markers are potentially powerful for DNA fingerprinting and have wide spread application in plant genome analysis (Morgante and Oliveri, 1993). Microsatellite markers have been used for DNA fingerprinting by several researchers (Belaj *et al*., 2003). This single co-dominant marker is enough to differentiate true and false hybrids (Nandakumar *et al*., 2004) and is used for hybrid authentication in several crops like peanut (Gomez *et al*., 2008), sunflower (Iqbal *et al*., 2010), cotton (Selvakumar *et al*., 2010), maize (Hipi *et al*., 2013), rice (Gimhani *et al*., 2014), flax (Pali *et al*., 2014) *etc*.

Tamilkumar *et al*. (2009) tested the genetic purity of rice hybrids with eleven SSR markers. The study identified a unique SSR marker RM234 to verify the genetic purity of the hybrid CORH3. This marker produces bands of different sizes like 145bp and 170bp in TNAUCMS2A and CB87R respectively. The result

indicates that there is 2% contamination in the hybrid seeds. The result was also confirmed with GOT.

Maize hybrids are identified using ten SSR primers (Wu *et al*., 2010). In this study, four primers show polymorphism and those primers are used for hybrid purity analysis. The consistency of SSR markers were checked by screening 100 seeds of F_1 progenies. Isozyme assay also done along with this molecular maker technique. The isozyme esterase distinguish hybrid variety Nongda 108 but cannot distinguish hybrid variety Zhengdan 958 because latter is the progeny from closely related parents. This clearly indicates that SSR is more efficient than isozymes to identify hybrids from closely related parents.

Pallavi *et al*. (2011) was able to distinguish true hybrids from pollen shedders/off-sibs using microsatellites in sunflowers. The primer ORS 309 produced a polymorphic band of size 250bp, which is a female parent (CMS-17A) specific and male parent (RHA 95-C-1) specific band of 230bp. Another primer which shows this type of polymorphism was primer ORS170. Both ORS 309 and ORS 170 can identify the hybrid KBSH-44. The SSR ORS 811 amplifies specific bands of the parental lines of the hybrid KBSH-53 at different size and is suitable for hybrid verification.

Another hybrid identification study in maize was done by Sudharani *et al*. (2012). From this study it was evident that SSR marker Umc1600 can be used for the authentication of hybrid DHM-117.

The inter specific hybrids of the cross *Eucalyptus camaldulensis* x *E. tereticornis* were identified with 25 fluorescent labeled microsatellite markers and calculated the hybrid purity index which ranges from 85 to 100 per cent. Subashini *et al*. (2014) suggested that hybrid purity assessment can be used to develop genetic linkage maps, quantitative analysis of economically important traits and marker assisted selection (MAS).

Genetic purity of three commercially important maize hybrids (Pasha, Frida and PG1661) was confirmed by fifty SSR markers. The co-dominant SSR markers

were accurate for genetic purity assessment of maize hybrids. Twenty three primers exhibit polymorphism between the different types of hybrids with an average of 0.69 polymorphism information content (PIC) value and they showed more than 98 per cent homogeneity in the hybrid seeds (Elci and Hancer, 2015).

EST-SSR was employed for the verification of interspecific hybrid of napiergrass x pearlmillet (kinggrass) by Dowling *et al*. (2014). F_1 individuals expressed all parental specific EST-SSR and all individuals of kinggrass were true hybrids. Six co-dominant markers and three pearl millet specific markers were used in this study.

Zhang *et al*. (2014) tested the genetic purity of commercial soybean hybrids developed using a cytoplasmic male sterility (CMS) system with SSR markers. Among the 160 SSR used, eight markers showed polymorphism. The amplification with these markers showed both parental bands in true hybrids proved their heterozygosity and impurities showed only one parental allele. The study reported that the single polymorphic marker was enough for detection of contaminations and confirmation of hybrid purity.

Sixteen SSR markers were used for determination of true hybrids in cotton (Rao *et al*., 2015). The five SSRs *viz*., BNL-3449, BNL-3255, JESPR-148, BNL-1317 and BNL-3090 produced unique fingerprints for six hybrids. SSR markers which are efficient for genetic purity testing was also able to distinguish heterozygous and homozygous states.

Turchetto *et al*. (2015) had done hybrid identification in closely related wild species of *Petunia* genus using 14 EST-SSR markers with 126 wild and 13 putative morphological hybrids. The EST-SSR distributed in the *Petunia* genome is effective for verification of interspecific hybrids and genetic diversity studies.

Fifty one rice specific sequence tagged microsatellite primers were used for the assessment of genetic purity of eight rice hybrids. Out of 51 markers, 28 microsatellites showed 54.9 per cent polymorphism. A total of 98 alleles were obtained from 51 primer pairs with an average of 1.92 alleles per primer pairs. The

number of alleles amplified for each primer pair ranged from 1 to 4 (Bora *et al*., 2016).

The genetic purity of rice hybrids was determined with seventeen SSR markers. The fingerprinting of 15 hybrids with their parental lines was done and a total of 272 alleles were produced by 17 primers. Among these 17 markers, 7 markers together differentiated all the 15 hybrids and their parental lines. The marker RM 229 distinguished CMS lines and their corresponding restorer lines for most of the hybrids (Islam *et al*., 2016).

In cassava, there are two studies reported on the context of hybrid identification. Mohan *et al*. (2013) identified true hybrids from the cross between CO2 and MNga-1 with SSR markers. Fifty seven SSR markers showed polymorphic bands and among these 6 primers produce single allelic band that differentiated both parents. Using these six SSR primers, 153 progenies were screened and 12 out of 153 were found to be selfed progenies of the female parent CO2, because only female specific bands were amplified in those progenies. The study suggested that SSR markers were highly efficient for early identification of true hybrids and the disposal of unwanted types.

With the help of microsatellites and capillary gel electrophoresis, Vincent *et al*. (2014) had done the identification of F_1 cassava progeny. Three hundred and sixty four F_1 progenies from four controlled crosses were tested with 12 SSR primers for authentication of hybrids. Among 364 progenies, 44 were self-pollinated progenies and 3 were outcross progenies. So the percentage of true hybrids was 87.1 per cent and rest of them are off-types. Vincent *et al*. (2014) recommended that the errors in pollination can be minimized if the true-to-type identity is verified before genetic analysis and it should become a common practice.

e. Single Nucleotide Polymorphism

Single Nucleotide Polymorphism (SNP) is a sequence based DNA marker which shows polymorphism in single base and can be present in both coding and non-coding regions. SNPs are often associated with morphological changes (Lindblad-Toh *et al.,* 2000). Yuan *et al*. (2010) used SNP along with RAPD for the confirmation of Clematis hybrids. Since SNP require sequencing, this technique is very expensive and made it less suitable for hybrid verification by commercial seed companies.

Table1. Hybrid Identification in Crop Plants using DNA markers

Sl. No.	Crop	Reference
Random Amplified Polymorphic DNA		
1	Common bean: Soy bean: 2 primers	Alzate-Marin *et al*. (1996)
2	Chilly pepper: 12 primers	Ilbi (2003)
3	Black pepper: 35 primers	George *et al*. (2005)
4	Saccharum: 20 primers	Nair *et al*. (2006)
5	Sorghum: 20 primers	Tabbasam *et al*. (2006)
6	Sorghum: 15 primers	Akhare *et al*. (2008)
7	Cotton: 16 primers	Ali *et al*. (2008)
8	Eggplant: 29 primers	Shukla *et al*. (2015)
9	Tobacco: 20 primers	Kumar and Kalyani (2016)
Amplified Fragment Length Polymorphism		
10	Beetroot: 2 primers Carrot: 2 primers	Schaber and Goldman (2013)
Inter Simple Sequence Repeats		
11	Artichoke: 20 primers	Bianco *et al*. (2011)
12	Mungbean: 10 primers	Khajudparn *et al*. (2012)
Simple Sequence Repeats		
13	Rice: 9 markers	Nandakumar *et al*. (2004)
14	Peanut: 24 primers	Gomez *et al*. (2008)
15	Melon: 15 primers	Ju-Fen *et al*. (2008)
16	Rice: 48 markers	Sundaram *et al*. (2008)
17	Rice: 11 primers	Tamilkumar *et al*. (2009)
18	Sunflower: 20 primers	Iqbal *et al*. (2010)
19	Cotton: 6 primers	Selvakumar *et al*. (2010)
20	Maize: 10 primers	Wu *et al*. (2010)
21	Sunflower: 58 primers	Pallavi *et al*. (2011)
22	Maize: 13 marker	Sudharani *et al*. (2012)
23	Maize: 5 marker	Hipi *et al*. (2013)
24	Cassava: 6 primers	Mohan *et al*. (2013)
25	Castor: 283 EST-SSRs	Shankar *et al*. (2013)
26	Kinggrass: EST-SSR	Dowling *et al*. (2014)
27	Rice: 6 markers	Gimhani *et al*. (2014)
28	Brinjal: 30 markers	Kumar *et al*. (2014)
29	Indian flax: 28 primers	Pali *et al*. (2014)
30	Eucalypt: 25 markers	Subashini *et al*. (2014)
31	Cassava: 12 markers	Vincent *et al*. (2014)
32	Soybean: 160 markers	Zhang *et al*. (2014)
33	Maize: 50 primers	Elci and Hancer (2015)
34	Petunia sp.: 14 EST-SSR primers	Turchetto *et al*. (2015)
35	Rice: 51 primers	Bora *et al*. (2016)
36	Rice: 17 markers	Islam *et al*. (2016)

_ **Table1. Hybrid Identification in Crop Plants using DNA markers (cont.)**

Sequence Related Amplified Polymorphism (SRAP)		
37	Coffee: 31 markers	Mishra *et al*. (2011)
Internal Transcribed Spacer (ITS)		
38	Sugarcane: 6 primers	Zhang *et al*. (2009)
Resistance Gene Analog Polymorphism		
39	Waxflower: 2 markers	Shan *et al*. (2010)
Combination of two markers		
40	Chilly pepper: 53 RAPD primers and 10 SPAR markers	Ballester and Vicente (1998)
41	Cotton: 3 SSR primers and 3 RAPD primers	Asif *et al*. (2009)
42	Rice: 14 SSR markers and 15 RAPD primers	Hashmei *et al*. (2009)
43	Lentil: 35 SSR primers and 67 RAPD primers	Solanki *et al*. (2010)
44	Clematis: 60 RAPD and 3 SNP primers	Yuan *et al*. (2010)
45	Pepper: 11 SSR and 80 RAPD	Jagtap *et al*. (2016)
Multiple markers		
46	Cotton: 25 SSR, 20 RAPD and 19 ISSR	Dongre and Parkhi (2005)
47	Cabbage: 44 SSR, 157 RAPD, 54 ISSR and 84 SRAP	Liu *et al*. (2007)
48	Cotton: 33 SSR, 20 RAPD and 19 ISSR	Dongre *et al*. (2011)

Genetic diversity studies in cassava using SSR markers

Siqueria *et al*. (2009) successfully studied the genetic diversity of 42 cassava land races from five regions in Brazil. Nine SSR primers were used for the diversity analysis and got high percentage of polymorphism ranges from 88.8 to 100 per cent. The land races from Mato Grosso exhibited highest differentiation. Forty six alleles were observed in 42 land races with an average of 3.3 alleles per locus. All the 42 cassava land races showed high total species diversity value of 0.635. The accessions from different regions shows some resemblance and human migration may be the probable reason for this similarity.

The genetic structure and diversity in 83 Brazilian cassava accessions, including several land races, in the Cerrado biome in Mato Grosso do Sul, Brazil were investigated using nine SSR markers. All these markers exhibit polymorphism, with an average of six alleles per locus. The observed heterozygosity value was 0.32 and total genetic variability was 0.668. Cluster and structure analyses grouped accessions into two major groups and a significant genetic versus geographic correlation were found (Siqueira *et al*., 2010).

Twenty SSR primers along with fourteen morphological descriptors were employed for the diversity study of 43 Ghanaian preferred cassava accessions. SSR markers were able to differentiate accessions which showed similarity in morphological descriptors. This study proved that SSR markers are more efficient than morphological descriptors for diversity analysis and can be used for germplasm management. The allele frequencies of all the primers were below 0.95 and gene diversity varying from 0.07 to 0.78. The primer SSRY 181 showed least heterozygosity whereas eight primers showed the highest (Asare *et al*., 2011).

Ribeiro *et al*. (2011) conducted a study to estimate the genetic similarity between 93 accessions of cassava with fourteen SSR primers. The genetic similarity was calculated using dice coefficient and similarity ranges between 0.16 and 0.96. A total of 26 alleles were produced by polymorphic primer with 2.2 alleles per locus on an average. The dendrogram obtained with dice coefficient and UPGMA method

had twenty similarity clusters. Among the 93 accessions, the clones UFLA-60 and UFLA-76 express maximum diversity. The observed heterozygosity value ranges from 0.00 to 0.56 which was lower than expected heterozygosity value (0.34 to 0.69), which signifies that most of the loci were in homozygous condition. The primer GA-131 produced more number of alleles than any other primers used in this study.

Genetic diversity of 51 farmer preferred cassava landraces and 15 elite accessions in Uganda were evaluated by Turyagyenda *et al*. (2012) with twenty six microsatellite markers. All these markers exhibit polymorphism and amplified 154 alleles in which, 37 alleles were unique to landraces and four were unique to elite accessions. SSRY 100 produced maximum number of alleles. When compared with landraces, the elite accessions expressed less genetic diversity and observed heterozygosity. High gene diversity values of 0.834 were obtained for landraces and 0.824 for elite accessions. The PIC values ranged from 0.370 for SSRY 155 to 0.813 in SSRY 64 with an average of 0.604. The accessions collected from Eastern Uganda expressed more gene diversity whereas accessions from Northern Uganda expressed the least. The less diversity in elite accessions suggested that of narrow genetic base. However, the use of landraces in cassava breeding may aid to widen the genetic base of this crop. Likewise, the negative inbreeding coefficient across landraces and elite accessions indicated that there is no inbreeding within landraces and elite accessions. Turyagyenda *et al*. (2012) reported that cassava landraces in Uganda may be very helpful for identification of novel and unique alleles.

Twenty SSRs were used to evaluate genetic variation in 21 Tanzanian farmer preferred cassava landraces collected and maintained at Mikocheni Agricultural Research Institute laboratory in Tanzania. Two West African cassava genotypes and one Kenyan cassava were included in the clustering analysis. The cluster analysis with Neighbours joining had grouped the accessions into three groups. The first three axes of principle component analysis (PCA) with positive Eigen values accounted for 22.76, 15.93 and 8.48% of the total variations, which supported the results of cluster analysis. Average gene diversity among the Tanzanian cassava

was high (0.71) with an average heterozygosity of 0.46 and a mean PIC value of 0.67 (Elibariki *et al*., 2013).

Fu *et al*. (2014) used 35 SSR markers to compare genetic diversity of 266 cassava clones that were collected from 80 farms in eight provinces with 16 cassava landraces and varieties released since the 1970s through genotyping. The study had found a large regional heterogeneity in cassava diversity, with a strong genetic differentiation of the assayed clones among the 80 farms (19.8%) and across the eight provinces (11.8%). The study showed no significant genetic differentiation (0.9%) between the 266 farm clones and 16 reference varieties.

Ndung'u *et al*. 2014 identified genetic constitution of 69 cassava accessions from different regions of Kenya using seven pairs of SSR molecular markers which detect 21 polymorphic alleles. Genotyping with high informative polymorphic SSR markers grouped the genotypes into five clusters using the software POPGENE version 1.31.

The molecular level characterization of genetic diversity and population structure of 60 sweet cassava accessions was studied by (Ferreira *et al*., 2015). A total of 88 alleles were produced by 19 SSR markers, with an average of 4.63 alleles per marker and allelic frequencies were used in order to assess the genetic diversity indexes for each marker. The mean of PIC and observed heterozygosity (Ho) were 0.525 and 0.862 respectively. This indicated that the markers were highly polymorphic and informative. The genetic diversity mean was of 0.601 and revealed significant genetic diversity among the genotypes.

Table2. Genetic diversity studies in cassava with different DNA markers

Sl. No.	Crop	Reference
RAPD		
1.	31 Brazilian cassava clones	Colombo *et al*. (1998)
2.	Brazil and CIAT collections	Carvalho and Schaal (2001)
3.	50 accessions- Ghana	Asante and Offei (2003)
AFLP		
4.	8 accessions	Wong *et al*. (1999)
5.	69 accessions wild species	Elias *et al*. (2000)
6.	20 landraces 9 accessions	Fregene *et al*. (2000)
7.	African cassava landraces and elite cultivars	Raji *et al*. (2009)
8.	93 accessions from Malawi	Benesi *et al*. (2010)
ISSR		
9.	Cassava 4 cultivar	Zayed *et al*. (2013)
SSR		
10.	220 accessions and 33 wild	Olsen and Schaal (2001)
11.	283 accessions of cassava landraces	Fregene *et al*. (2003)
12.	38 accessions	Elias *et al*. (2004)
13.	5 improved lines, 62 CMD resistant and 10 CMD susceptible landraces	Lokko *et al*. (2006)
14.	245 accessions – Uganda	Kizito *et al*. (2007)
15.	36 genotypes	Moyib *et al.* (2007)
16.	160 accessions	Peroni *et al*. (2007)
17.	58 accessions	Raghu *et al*. (2007)
18.	Cassava accessions in Costa Rica	Rocha *et al.* (2008)

Table 2. Genetic diversity studies in cassava with different DNA markers (cont.)

19.	42 landraces from Brazil	Siqueira *et al*. (2009)
20.	83 Brazilian cassava accessions, including several landraces	Siqueira *et al*. (2010)
21.	43 accessions in Ghana	Asare *et al*. (2011)
22.	12 released varieties and 24 Central Kerala collections	Lekha *et al*. (2011)
23.	Cassava from 162 locations across Puerto Rico	Montero-Rojas *et al*. (2011)
24.	93 accessions	Ribeiro et al. (2011)
25.	Cassava genotypes in African gene pool	Kabeya et al. (2012)
26.	51 farmer-preferred landraces and 15 elite accessions	Turyagyenda et al. (2012)
27.	21 farmer preferred cassava landraces from Tanzanian	Elibariki et al. (2013)
28.	69 accessions from Kenyan germplasm	Ndungu et al. (2014)
29.	266 elite varieties from Thai	Fu et al. (2014)
30.	61 sweet cassava accessions	Ferreira et al. (2015)

Cassava clone – CMR-100

CMR-100 (Cassava Mosaic Resistance-100) is a chips line identified through cassava improvement programme in ICAR-CTCRI. This clone has some characteristics like middle branching, light yellow flesh (carotene content), non-bitter and high dry matter (43%) and uniform tuber shape (cylindrical) which make it ideal for fried chips making. The peeling of the tuber is easy, raw tuber has sweet taste and with very good cooking quality. Since this chips line is a product of open pollination the parentage of this clone is not identified. The authentication of parents is essential for the release of this promising clone.

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Plant material

The present study was carried out at ICAR-Central Tuber Crop Research Institute (ICAR-CTCRI), Thiruvananthapuram. For the purpose of parental identification, 13 cassava varieties (Table 3) which showed morphological resemblance with CMR-100 (Fig. 1) were selected and used in this study. For studying genetic variability, 27 open pollinated progenies of CMR-100 and ten cassava lines were selected. The stem cuttings of CMR-100 and 27 OP progenies were planted in the field at ICAR-CTCRI (Fig. 2 and 3). The details of the planting materials are listed in Table 3.

3.2 METHODS

3.2.1Scoring of CMD

The cassava accessions used in the study were scored visually for the symptoms of CMD after three months. The level of resistance was determined based on scores of 1-5 which is established as per the severity of the symptoms.

Symptom description	Score
Unaffected shoots, no symptoms	1
Mild chlorosis, mild distortions at bases of most leaves, while the remaining parts of the leaves and leaflets appear green and healthy	2
Pronounced mosaic pattern on most leaves, narrowing and distortion of the lower one-third of the leaflets	3
Severe mosaic distortion of two thirds of most leaves and general reduction of leaf size and stunting of shoots	4
Very severe mosaic symptoms on all leaves, distortion, twisting, misshapen and severe leaf reductions of most leaves accompanied by severe stunting of plants	5

Table 3. List of cassava accessions used for the study

Sl. No.	Name	Code	Sl. No.	Name	Code
1.	Narayanakappa	C1	23.	CMR-100/16	C23
2.	Ambakadan	C2	24.	CMR-100/17	C24
3.	MNga-1	C3	25.	CMR-100/19	C25
4.	CI-732	C4	26.	CMR-100/22	C26
5.	CI-731	C5	27.	CMR-100/24	C27
6.	C-114	C6	28	CMR-100/25	C28
7.	C-54	C7	29.	CMR-100/27	C29
8.	C-76	C8	30.	CMR-100/31	C30
9.	C-68	C9	31.	CMR-100/37	C31
10.	C-129	C10	32.	CMR-100/43	C32
11.	C-53	C11	33.	CMR-100/45	C33
12.	C-15	C12	34.	CMR-100/47	C34
13.	S-8-2	C13	35.	CMR-100/50	C35
14.	CMR-100	C14	36.	CMR-100/52	C36
15.	CMR-100/2	C15	37.	CMR-100/53	C37
16.	CMR-100/3	C16	38.	CMR-100/56	C38
17.	CMR-100/5	C17	39.	CMR-100/57	C39
18.	CMR-100/6	C18	40.	CMR-100/59	C40
19.	CMR-100/8	C19	41.	CMR-100/60	C41
20.	CMR-100/12	C20	42.	Harsha	C42
21.	CMR-100/13	C21	43.	BR-2	C43
22.	CMR-100/15	C22	44.	BR-5	C44

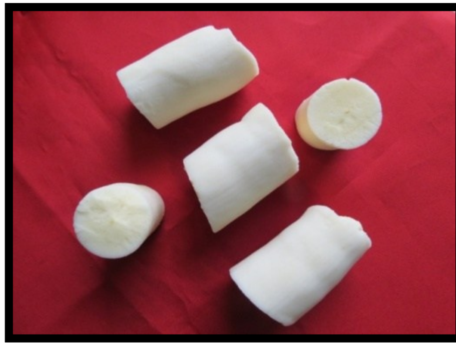


Fig: 1. CMR-100 cassava line

Fig: 2. Field view of CMR-100 cassava line

Fig: 3. Field view of OP progenies of CMR-100

3.2.2Extraction of DNA

DNA was extracted from selected cassava lines by Sodium dodecyl sulphate (SDS) method proposed by Dellaporta *et al*.(1983) with some modifications (Appendix). The fresh, young leaves were collected from each individual lines. For each sample, 10-15 leaves were taken, mid-ribs were removed and placed in autoclaved pestle and mortar. The samples were homogenized in liquid nitrogen and transferred to sterilized centrifuge tubes containing 15 ml of extraction buffer, 100 mg polyvinylpyrolidone (PVP) and 20 µl β-mercaptoethanol. The homogenized mixtures were kept in ice for few minutes and 1 ml of 20 percent SDS was added into it. After proper mixing, the extracts were incubated for 30-60 minutes at 65°C in the water bath (Memmert) with occasional swirling. Five ml of 5 M potassium acetate was added and mixed, then incubated in the ice for half an hour. After incubation, the contents were centrifuged (SIGMA Laborzentrifge) at 12,000 rpm for 12 minutes. The supernatant was collected into a fresh centrifuge tube. After 30 min incubation nucleic acid was precipitated using 10 ml chilled isopropanol and the DNA was pelleted at 12,000 rpm for 20 minutes. The supernatant was discarded and the pellet was resuspended in sterile distilled water. The suspension was transferred to 2 ml eppendorf tube and was added with 10 µl of RNase (10 mg/µl) and incubated at 37°C for 1 hour. After RNase treatment, 750 µl of chloroform: isoamyl alcohol (24:1) was added, mixed and centrifuged for 20 minutes at 10,000 rpm. The supernatant was transferred to a new eppendorf. The chloroform: isoamyl alcohol step was repeated once to remove all the protein contamination. Along with supernatant 1/10th volume of 3 M sodium acetate and 2 volumes of chilled ethanol were added, mixed gently and incubated at 4°C for 2 hours. After a spin at 10,000 rpm for 5 minutes, the supernatant was discarded, pellet is washed twice with 70 per cent ethanol, air dried and resuspended in sterile distilled water and stored at -20°C as stock.

3.2.3 Determining the quality and quantity of DNA

a. Quality analysis of DNA samples

The quality of the isolated DNA of each sample was determined using agarose gel electrophoresis. The DNA samples were electrophoresed on 0.8 per cent agarose gel. 0.8 g agarose was weighed and transferred to a conical flask containing 100 ml of 1X Tris Borate EDTA Buffer (TBE) (Appendix). Agarose was completely melted by heating in a microwave oven (Neutron) for 2 minutes. When the solution is cooled to 40-50°C and immediately, 1 µl of ethidium bromide (0.5 µg/ml) was added and mixed uniformly by gently swirling the conical flask. The gel was poured into casting tray pre-fitted with combs. The gel was poured without forming any bubbles and was kept for solidification. After solidification, the comb was removed and the casting tray containing gel was placed in the buffer tank filled with 1X TBE. The 3 µl of DNA samples were mixed with 2 µl of loading dye (Appendix) and were loaded into the wells. Electrophoresis was set at 80 V in a horizontal electrophoresis unit for one hour. The bands were documented with the help of gel documentation system (Alpha Imager, Alpha Innotech, USA). The gel photos were used to assess the quality of the DNA.

b. Quantification of DNA samples

DNA concentration was determined by taking the readings directly from the Nanodrop Bio-spectrophotometer (DeNovix, DS11 Spec). For standardization, sterile distilled water was used as blank. The DNA samples were diluted to working concentration of 50 ng/µl by adding suitable quantity of sterile distilled water.

3.2.4 PCR amplification

A total of 21 SSR primers were used for parental identification study. Ten primers were used for genetic diversity analysis of OP progenies of CMR-100 and other cassava varieties. The details of the primers used in this study are listed in Table 4.

PCR reaction was run on Bio-Rad C1000^{TM} Thermal Cycler and the amplification was performed in 20 µl reaction volumes containing 50 ng/µl cassava DNA, 10X

Table 4. Sequence and product size of SSR primers used for the study

Sl. No.	Primer	Sequence		Product size (bp)
		Forward 5'-3'	Reverse 3'-5'	
1.	SSRY 6	TTTGTTGCGTTTAGAAAGGTGA	AACAAATCATTACGATCCATTTGA	298
2.	SSRY 7	TGCCTAAGGAAAATTCATTCAT	TGCTAAGCTGGTCATGCACT	250
3.	SSRY 8	AGTGGTTTGAGAAGACTGGTGA	TTTCCAAAATGGAACTTCAAA	271
4.	SSRY 21	CCTGCCACAATATTGAAATGG	CAACAATTGGCTAAGCAGCA	192
5.	SSRY 28	TTGACATGAGTGATATTTTCTTGAG	GCTGCGTGCAAAACTAAAAT	180
6.	SSRY 31	CTTCATCACGTGTTAATACCAATC	ATTGTTGTGGTTGCAGGACA	188
7	SSRY 32	CAAATTTGCAACAATAGAGAACA	TCCACAAAGTCGTCCATTACA	298
8.	SSRY 34	TTCCAGACCTGTTCCACCAT	ATTGCAGGGATTATTGCTCG	279
9.	SSRY 35	GCAGTAAAACCATTCCTCCAA	CTGATCAGCAGGATGCATGT	282
10.	SSRY 36	CAACTGTTTCAACCAACAGACA	ATTCTCGTGAACTGCTTGGC	134
11.	SSRY 39	TCAATGCATAGGATTTTGAAAGTA	AATGAAATGTCAGCTCATGCT	293
12.	SSRY 40	TGCATCATGGTCCACTCACT	CATTCTTTTCGGCATTCCAT	231
13.	SSRY 43	TCAGACGTTGATACCTCACTTCA	CCAGAGCATGGTCTTTCTGA	255
14	SSRY 49	TGAAAATCTCACTGGCATTATTT	TGCAACCATAGTGCCAAGC	300
15.	SSRY 50	CCGCTTAACTCCTTGCTGTC	CAAGTGGATGAGCTACGCAA	271
16.	SSRY 103	TGAGAAGGAAACTGCTTGCAC	CAGCAAGACCATCACCAGTTT	272
17.	SSRY 106	GGAAACTGCTTGCACAAAGA	CAGCAAGACCATCACCAGTTT	270
18.	SSRY 235	CAGCTTTGCCATCCAATTTT	CAGCAAAATGACATGAGTGTATCTC	216
19.	SSRY 324	CGCTTACAACACCACCTTCA	GCTTGATCTCAGCCATGTCA	206
20	NS 158	GTGCGAAATGGAAATCAATG	TGAAATAGTGATACATGCAAAGGA	166
21.	NS 198	TGCAGCATATCAGGCATTTC	TGGAAGCATGCATCAAATGT	170-210

Taq buffer A, 10 mM dNTPs (Deoxynucleotide triphosphates) (dATP, dGTP, dCTP and dTTP), 2 mM SSR primer and 3 units of Taq polymerase. The 20 µl PCR cocktail was prepared (Table 5) and run based on the PCR profile (Table 6). SSR products were electrophoresed on 2 per cent agarose gels and were visualized under UV (Ultra Violet) light and documented.

3.2.5 Polyacrylamide gel electrophoresis

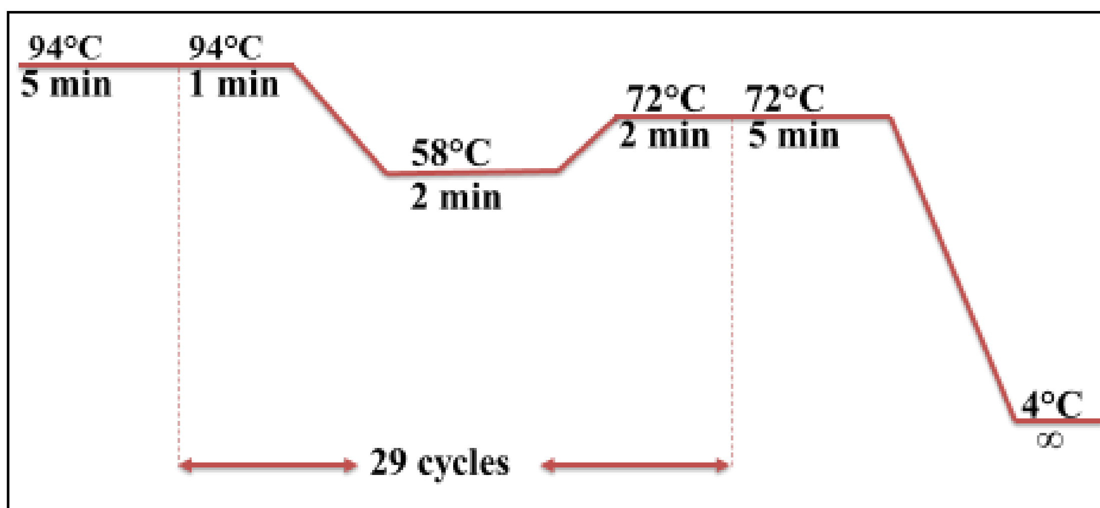
The amplified SSR products were run on vertical PAGE gel. At first the large plate and small plate were washed and wiped with distilled water followed by wiping with absolute ethanol. Then laboline (repellent) was uniformly spread in large plate whereas bind silane was used in small plate. The spacer and comb was wiped with ethanol. After the spacer was placed on the large plate, the small plate was kept on it (coated side towards spacer). Assembled the unit with side clamp, bottom is assembled and the unit is locked. The prepared 6 per cent acrylamide gel (Appendix) added with 10 per cent ammonium per sulphate and TEMED (1:10 ratio) is injected using a syringe, uniformly in between the plates without air bubbles. After solidification, the pre-running was done for 15 minutes. The PCR products with loading dye were denatured (94°C for 5 minutes) using thermal cycler and 4 µl of the denatured PCR products were loaded in the gel. The gel was run at 100 W constantly for 20 to 45 minutes. Duration depends upon the size of PCR products. After running, the small plate was separated from the assembly and silver staining was carried out.

Table 5.PCR cocktail

Items	Volume (µl)
DNA (50 ng/µl)	2.0
Primer (2 mM)	2.0
Taq buffer A (10 X)	2.0
dNTPs (10 mM)	0.1
Taq polymerase (1 unit)	0.1
Sterile distilled H_2O	13.8
Total volume	20.0

Table 6. PCR Profile

Step	Specification		Temperature (°C)	Time (min)
1	Initial denaturation		94	5
2	Denaturation	29 cycles	94	1
3	Annealing		58	2
4	Extension		72	2
5	Final extension		72	5
6	Hold		4	∞



3.2.6 Silver Staining

The staining procedure has different steps. It starts with the fixing of the gel in 10 per cent acetic acid for 30 minutes followed by washing in distilled water for 30 minutes and staining with 0.1% $AgNO_3$ and 3 ml formaldehyde for 30 minutes. This is again, washed with distilled water for 10 seconds, developing (ice cold 3% Na_2CO_3, 3 ml formaldehyde and 400 µl sodium thiosulphate) until the ladder developed properly, followed by 1 min fixing and washing for 30 minutes. The scoring was done after the gel plate get dried. The gel plate also scanned to store the image for future use.

3.2.7 Data Analysis

a. Scoring of bands in the gels

The bands were scored visually as 1 and 0 based on the presence and absence of bands respectively. The data scoring was done for all 21 primer products across 13 cassava lines and CMR-100. The binary matrix scoring done for OP progenies with CMR-100, its expected parents and other varieties amplified using 10 SSR primers for genetic diversity studies. Both scored data was analyzed in NTSYSpc programme.

b. Cluster Analysis

The scored data was analyzed using NTSYSpc (version 2.21f) software. The similarity was calculated for qualitative data with SM (Simple matching) coefficient and used for cluster analysis. The dendrogram was constructed by the Unweighted Pair-Group Method based on Arithmetic Mean (UPGMA) with cluster analysis data (Sneath and Sokal, 1973).

c. Principal Component Analysis (PCA)

The similarity matrix was constructed with SimQual programme and used for PCA. The 2-D and 3-D images were generated using Eigen vector in NTSYSpc software (Rohlf, 2009).

d. Polymorphism Information Content (PIC)

The PIC value of SSR primers used in this study were calculated using the formula *PIC=1∑pi2*where *pi* is frequency for the ith allele (Nei, 1973). The PIC value was calculated to characterize the efficiency of each primer to detect the polymorphic loci among the cassava varieties.

3.2.8 Estimation of Drymatter Content and Starch Content

To estimate the Drymatter Content (DMC), 50 g of freshly collected cassava tuber was chopped into thin slices after removing the cassava rind. The materials were dried in hot air oven (BESTON) at 60°C for 72 hours and the dry weight was measured. DMC was calculated using the formula,

$$\text{DMC (\%)} = \frac{\text{Dry weight of the tuber (g)}}{\text{Fresh weight of the tuber (g)}} \text{ X } 100$$

For estimating total starch content using DMC, the following formula reported by Wang *et al*. (1989) was used.

$$\mathbf{y} = 0.86945\mathbf{x} - 6.34587$$

Where, 0.86945 and 6.34587 areconstants.

y = starch content

x = percentage DMC

RESULTS

RESULTS

The present study on “Molecular characterization of chips line CMR-100 and its progenies using SSR markers” have done to identify the parents of CMR-100 and to assess the genetic variation in OP progenies of CMR-100 and selected cassava cultivars. The results obtained in the experiments are described as below.

4.1 Selection of plant material

A total of 13 cassava lines which show morphological resemblances with CMR-100 were selected for the study (Fig. 4). For the genetic variation analysis, twenty seven OP progenies of CMR-100 with 10 cassava lines were selected (Fig. 5).

4.2 Scoring of CMD

All the cassava lines used for the study were analysed for cassava mosaic disease incidence by visual screening were detailed in Table 7. Out 13 lines selected for parental identification, 5 lines were CMD susceptible. The ten OP progenies of CMR-100 were also susceptible for the disease. Among the susceptible progenies, 5 progenies were severely affected by the disease. Eventhough, CMR-100 is a resistant variety, mild symptoms are observed very rarely.

4.3 Molecular Marker analysis

In the present study, 13 cassava accessions were studied for parental identification of chips line CMR-100 using 21 SSR markers. The genetic variation of OP progenies, CMR-100, expected parents and seven cultivars have done with ten SSR markers.

a. Extraction of genomic DNA

The SDS method (Dellaporta *et al.*, 1989) was good for the isolation of DNA from all the leaf samples (44 samples) used for the study.

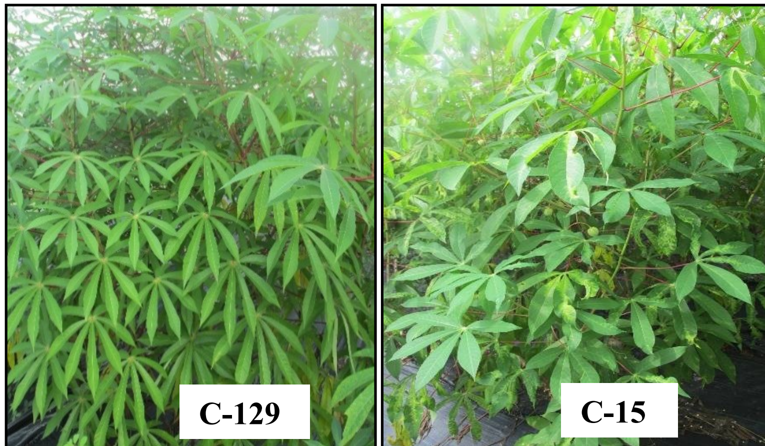


Fig: 4. Selected cassava lines used for parental identification

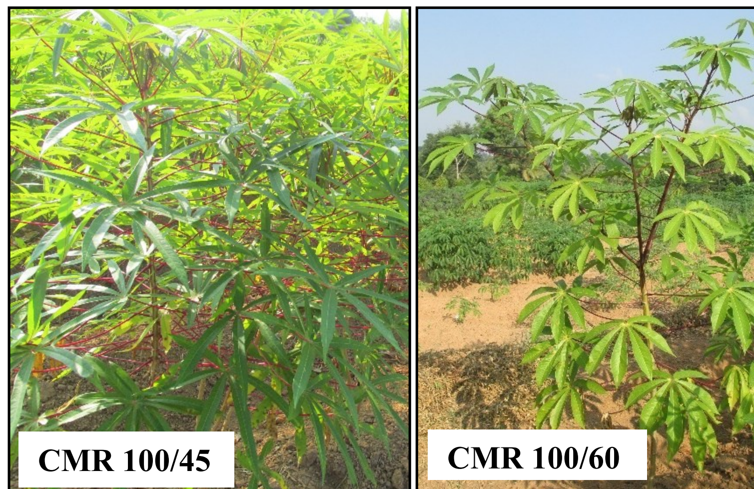


Fig: 5. Selected OP progenies of CMR-100

Table 7. CMD incidence in the cassava accessions used

Sl. No.	Name	CMD Incidence	Score
1.	Narayanakappa	S	3
2.	Ambakadan	S	3
3.	MNga-1	R	1
4.	CI-732	S	2
5.	CI-731	S	1
6.	C-114	R	1
7.	C-54	R	1
8.	C-76	R	1
9.	C-68	R	1
10.	C-129	R	1
11.	C-53	R	1
12.	C-15	S	2
13.	S-8-2	R	1
14.	CMR-100	R	1
15.	CMR-100/2	S	4
16.	CMR-100/3	S	5
17.	CMR-100/5	R	1
18.	CMR-100/6	R	1
19.	CMR-100/8	R	1
20.	CMR-100/12	S	3
21.	CMR-100/13	R	1
22.	CMR-100/15	R	1

Sl. No.	Name	CMD Incidence	Score
23.	CMR-100/16	R	1
24.	CMR-100/17	R	1
25.	CMR-100/19	R	1
26.	CMR-100/22	S	5
27.	CMR-100/24	S	4
28	CMR-100/25	S	4
29.	CMR-100/27	R	1
30.	CMR-100/31	R	1
31.	CMR-100/37	R	1
32.	CMR-100/43	R	1
33.	CMR-100/45	R	1
34.	CMR-100/47	S	5
35.	CMR-100/50	R	1
36.	CMR-100/52	S	2
37.	CMR-100/53	R	1
38.	CMR-100/56	R	1
39.	CMR-100/57	S	4
40.	CMR-100/59	S	5
41.	CMR-100/60	R	1
42.	Sree Harsha	R	1
43.	BR-2	R	1
44.	BR-5	R	1

b. Determination of DNA quality

The quality determination of DNA was accomplished by analysing the DNA on 0.8 per cent agarose gel (Plate 1) and very sharp good quality DNA bands without protein and RNA contamination were obtained.

c. Quantification of DNA

The concentration of the DNA samples was determined by measuring the absorbance at 260, 280 and 230 nm. The concentration of DNA ranges from 390 to 11403 ng/µL. The 260/230 values obtained were in the range of 2.0-2.2 which indicated that the DNA was not contaminated with EDTA, carbohydrates and phenol. The DNAs were diluted to 50 ng/µL and confirmed the concentration.

I. SSR Analysis for Parental Identification

a. Detection of PCR amplification on agarose gel

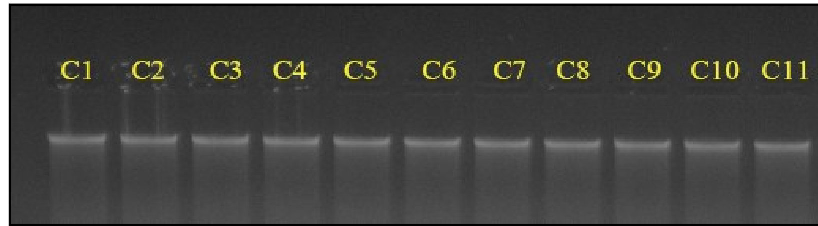
All the 14 samples were amplified with 21 SSR primers. The PCR amplified products were run on 2 per cent agarose gel (Plate 2) to detect polymorphic bands among CMR-100 and selected cassava lines.

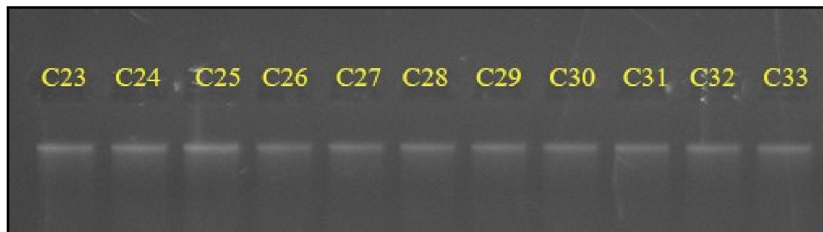
b. Detection of PCR amplified products on Polyacrylamide gel

The amplified products of 21 SSR primers were electrophoresed on vertical denaturing PAGE unit for resolved band separation. The separated bands on 6 per cent acrylamide gels were visualized after silver staining and polymorphism among the 14 cassava lines were detected (Plate 3).

c.Polymorphism Detection

A total of 21 primers were used to identify the parents of CMR-100. The analyses using all the SSR primers were produced clear, scorable, unambiguous and polymorphicbands. The total number of amplicon detected was 75. The number of bands produced with each primer is ranged from 2 to 6 (Table 8). The maximum numbers of alleles (6 alleles) were produced by two primers, SSRY 40 and NS 158. The percentage of polymorphic bands was 100% for all other primers.





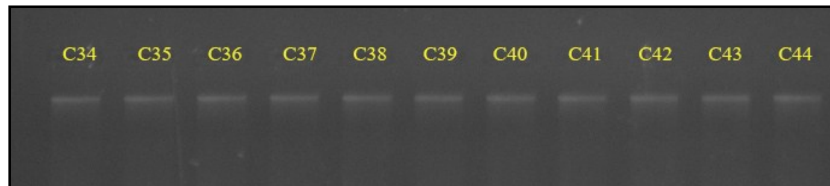


Plate: 1. Quality analysis of 44 genomic DNA

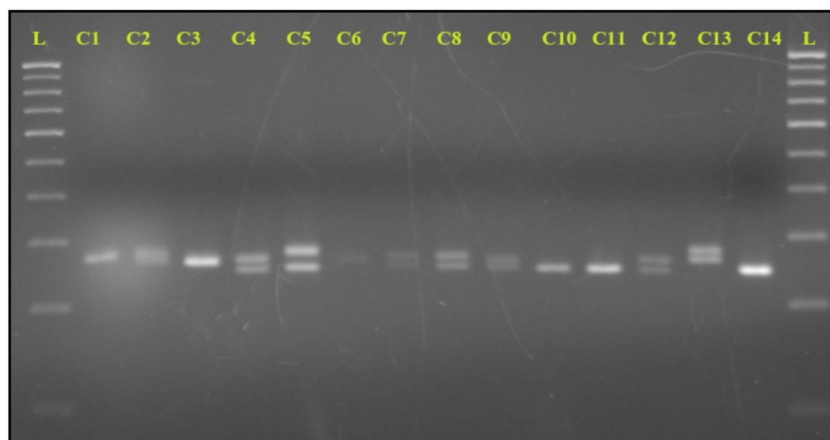
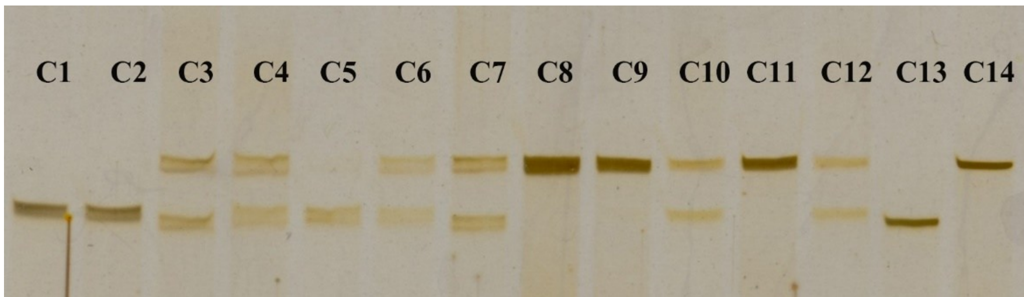
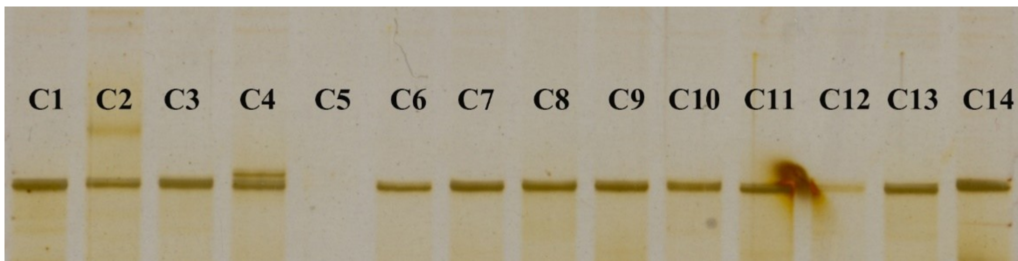


Plate: 2. PCR amplified products of SSRY 106

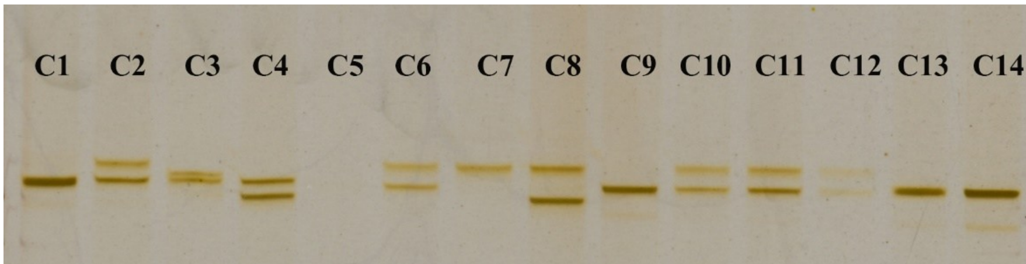
SSRY 235



SSRY 7



SSRY 40



SSRY 106

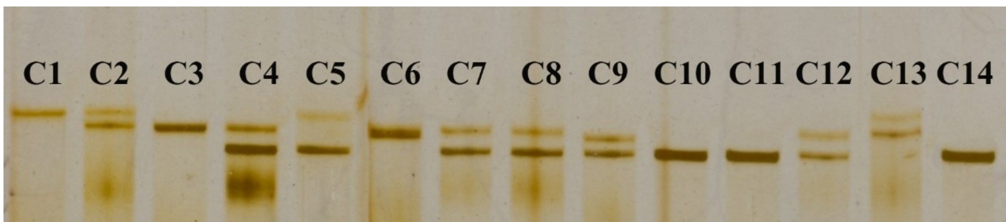


Plate: 3. SSR banding profile on PAGE gel

Table 8. Polymorphic bands produced by 21 SSR primers

Sl. No.	SSR primers	Total no. of bands	No. of polymorphic bands	Percentage of polymorphism
1	SSRY 6	3	3	100
2	SSRY 7	3	2	66.67
3	SSRY 8	4	4	100
4	SSRY 21	3	3	100
5	SSRY28	5	5	100
6	SSRY 31	4	4	100
7	SSRY 32	2	1	50
8	SSRY 34	2	2	100
9	SSRY 35	3	2	66.67
10	SSRY 36	4	4	100
11	SSRY 39	3	3	100
12	SSRY 40	6	6	100
13	SSRY 43	5	5	100
14	SSRY 49	3	3	100
15	SSRY 50	2	2	100
16	SSRY 103	4	4	100
17	SSRY 106	4	4	100
18	SSRY 235	3	3	100
19	SSRY 324	3	3	100
20	NS 158	6	6	100
21	NS 198	3	3	100

except SSRY 7, SSRY 32 and SSRY 35. Similarly, the lowest polymorphism was seen in the primer SSRY 32 (50%).

d. Cluster Analysis

i. Similarity index

The similarity index values obtained for each pair wise comparison among 13 lines along with CMR-100 were listed in Table 9. The similarity index value based on Simple Matching coefficient among cassava lines ranged from 0.38 to 0.97. CMR-100 shows 0.93 similarity with C-129, which is the highest value obtained whereas C-15 showed 0.84. The lowest value obtained was 0.4 showed between many lines (Ambakadan and C-53, MNga-1 and CI-731, C-114 and CI-731, C-15 and CI-731). Among 13 accessions used, Ambakadan showed lowest value of 0.44 with CMR-100. Similarly, the cassava lines C-129 and C-15 also obtained high value of similarity (0.9). Based on the similarity index data, a frequency graph was drawn and shown in Fig. 6. From the graph high frequency obtained between 0.5-0.59.

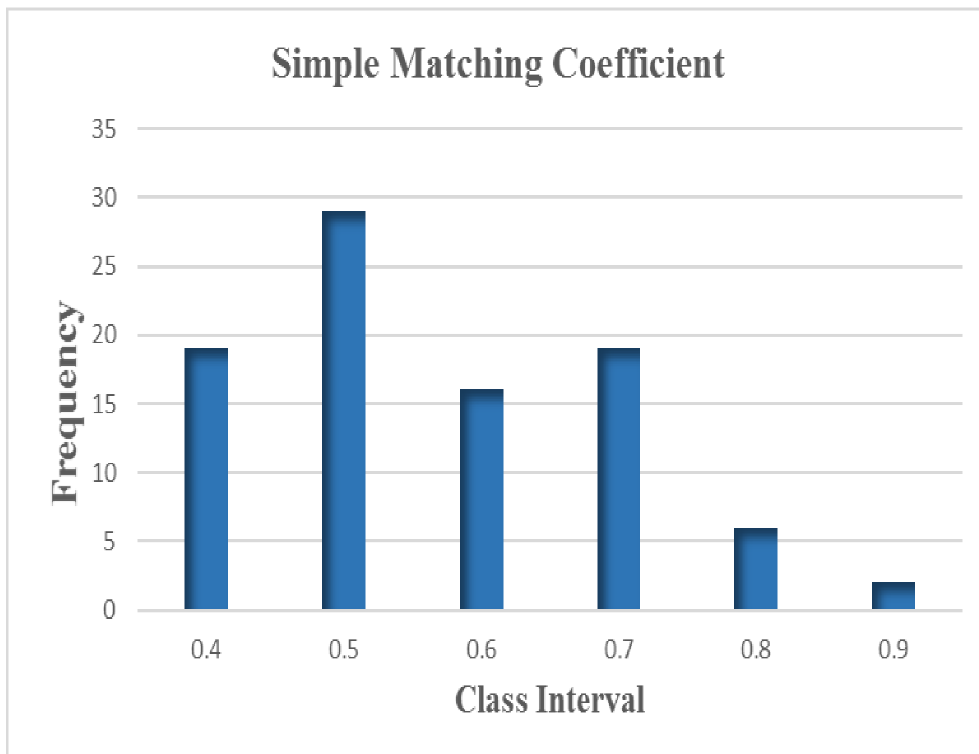
ii. Cluster based Dendrogram

The similarity coefficient matrix was calculated and dendrogram was constructed based on Simple matching (SM) coefficient using UPGMA cluster analysis. The similarity dendrogram displayed CMR-100 and two cassava accessions C-129 and C-15 in a single cluster (Fig. 7). Among the 13 cassava lines studied, two lines express more than 90 per cent similarity with CMR-100. The cassava line C-129 express 96 per cent similarity to CMR-100 whereas C-15 express 91 per cent similarity. The cassava line CI-731 was an outlier which expresses only 69 per cent similarity with other lines. The carotene lines included in the study shows 86 per cent similarity and except C-76. Similarly, MNga-1 showed 86 per cent similarity with S-8-2.

Table 9. Similarity coefficient matrix of 14 cultivars

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14
C1	1													
C2	0.74	1												
C3	0.55	0.54	1											
C4	0.55	0.73	0.54	1										
C5	0.65	0.51	0.4	0.54	1									
C6	0.53	0.54	0.62	0.49	0.4	1								
C7	0.50	0.46	0.76	0.57	0.43	0.76	1							
C8	0.59	0.47	0.62	0.61	0.49	0.57	0.73	1						
C9	0.55	0.46	0.59	0.57	0.49	0.76	0.73	0.73	1					
C10	0.55	0.46	0.57	0.52	0.46	0.73	0.70	0.68	0.76	1				
C11	0.58	0.40	0.62	0.52	0.44	0.65	0.76	0.81	0.81	0.81	1			
C12	0.49	0.50	0.61	0.58	0.4	0.74	0.74	0.72	0.72	0.90	0.74	1		
C13	0.55	0.51	0.65	0.49	0.42	0.54	0.65	0.65	0.62	0.59	0.62	0.64	1	
C14	0.59	0.44	0.58	0.5	0.47	0.72	0.72	0.69	0.8	0.93	0.83	0.84	0.64	1

Fig: 6. Frequency distribution of SSR marker based on similarity matrix



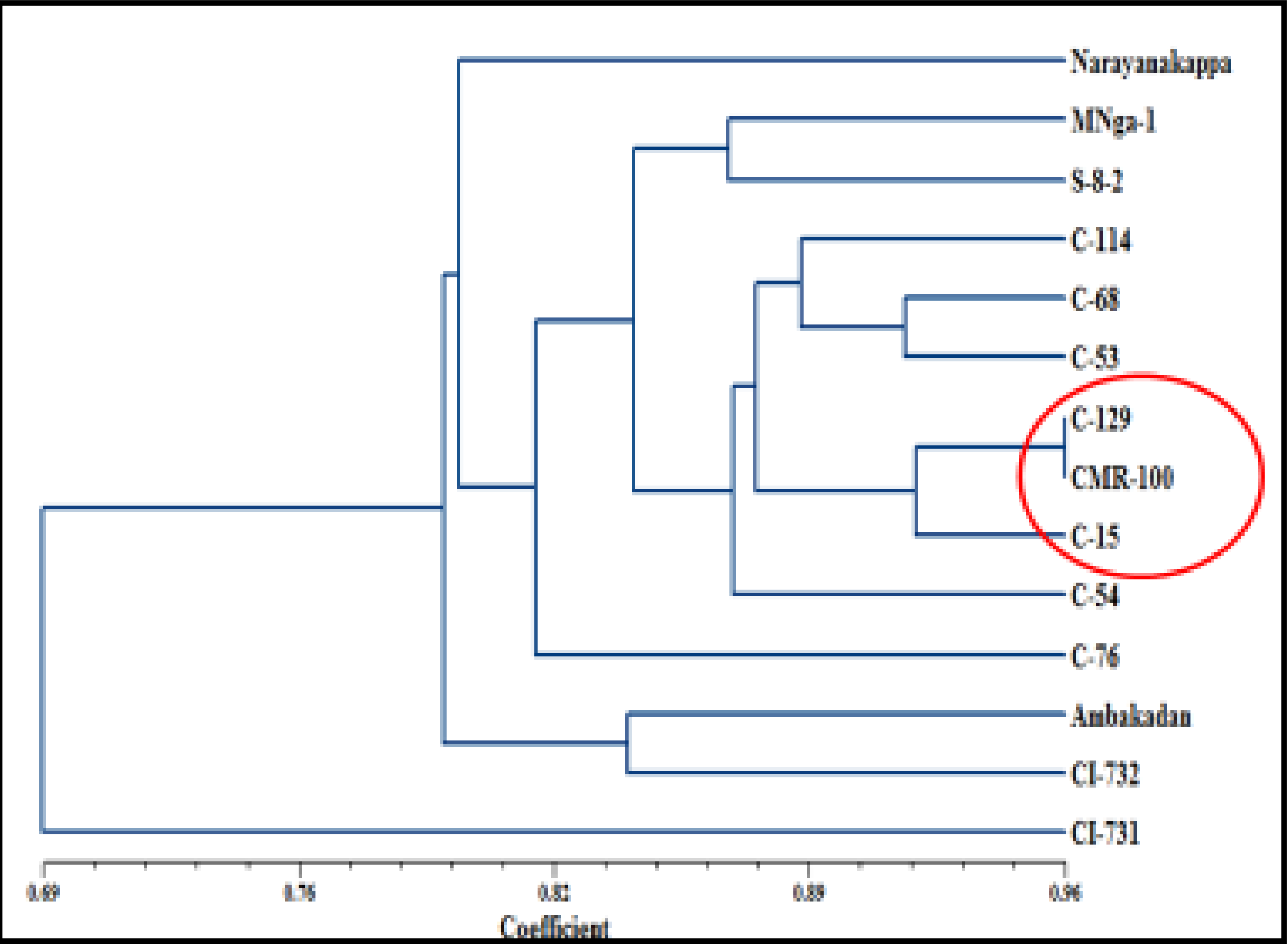


Fig: 7. Cluster based dendrogram of 14 cassava cultivars

iii. Principle Component Analysis

The PCA was performed using Eigen vector and 2-D and 3-D plot were obtained (Fig. 8 and 9). PCA analysis revealed that CMR-100 is genetically closer to the carotene lines C-129 and C-15. The accession CI-731 was showed high level of variation from other cultivars followed by Ambakadan. The cassava line MNga-1 is genetically closer to S-8-2 and Narayanakapa with CI-732.

e.PIC and Heterozygosity (He) of Primers

The PIC and heterozygosityvalue of each primer used for parental identification were calculated and details were given in Table 10. The PIC value ranges from 0.31 to 0.75 and heterozygosity from 0.36 to 0.78. Out of 21 primers, 14 primers *viz.,* SSRY 8, SSRY 21, SSRY 28, SSRY 31, SSRY 36, SSRY 39, SSRY 40, SSRY 43, SSRY 49, SSRY 103, SSRY 106, SSRY 235, SSRY 324 and NS 158 show PIC value greater than 0.5 which indicated that these primers are highly polymorphic and informative.

f. Confirmation of Parentage

Inorder to confirm the parentage of CMR-100, only the cassava lines showed high similarity with CMR-100 were amplified with 19 different SSR primers and bands were separated in 6 per cent PAGE gel electrophoresis (Plate 4). The segregation patterns of all the alleles of CMR-100 were compared with alleles of other two lines C-129 and C-15. The result indicates that, at least one of the alleles in both C-129 and C-15 were present in CMR-100.

II.SSR Analysis for Genetic variation study

a. Detection of PCR amplification on agarose gel

All the 37 samples which consist of OP progenies of CMR-100, CMR-100, its expected parentsand seven cassava cultivars were amplified with ten SSR primers. The PCR amplified products were run on 2 per cent agarose gel (Plate 5) to detect bands.

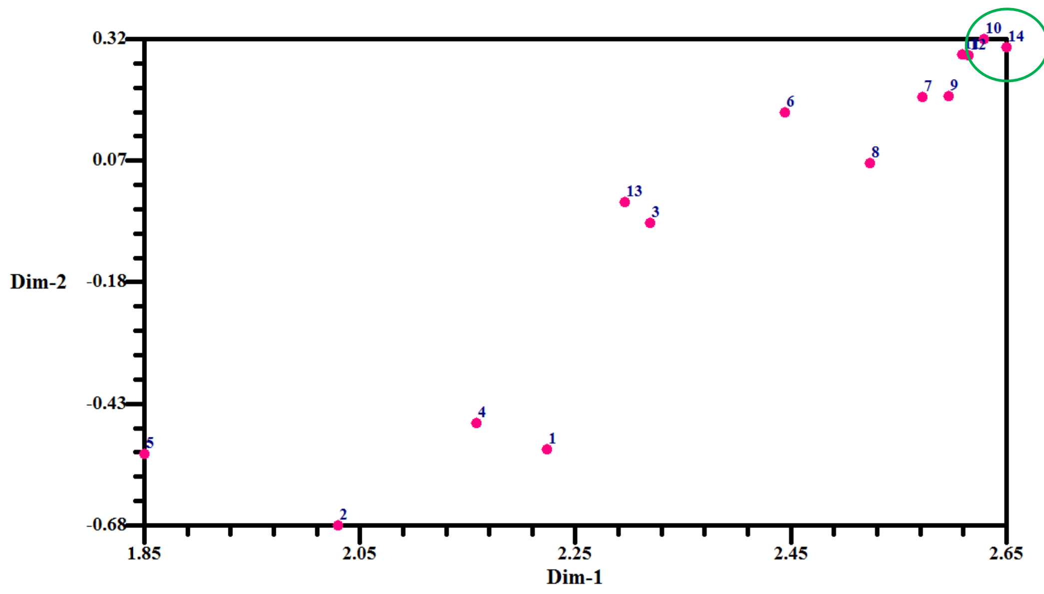


Fig: 8. 2D plot of PCA in parental identification

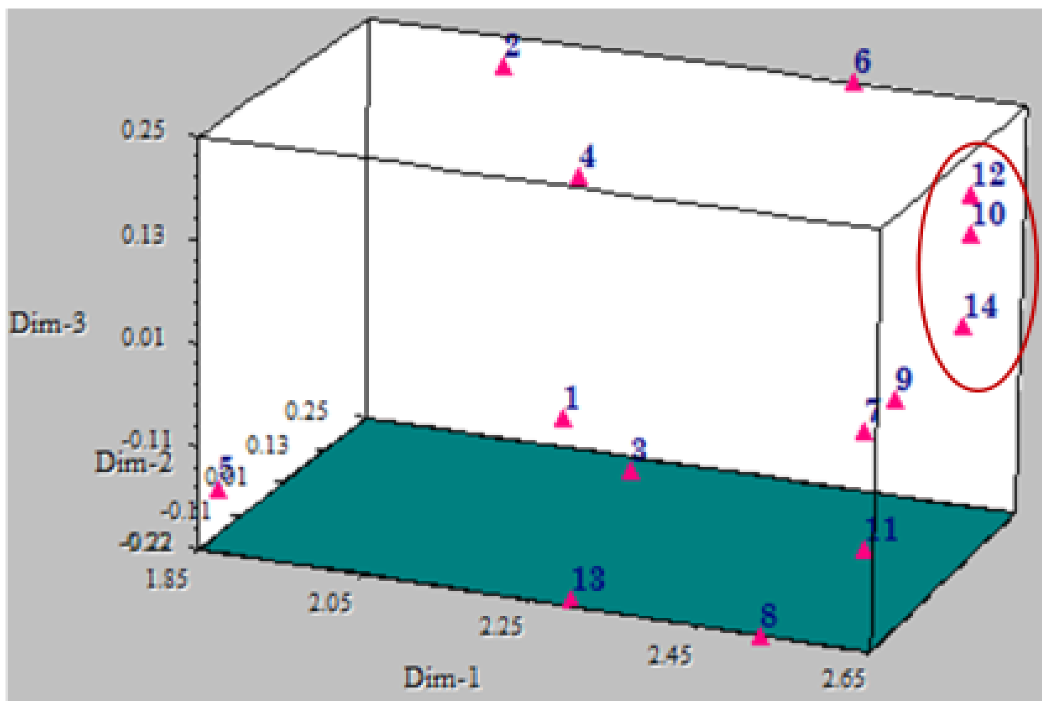


Fig: 9. 3D plot of PCA in parental identification

Table 10. PIC and Heterozygosity of Different Primers

Sl. No.	Primer	PIC	He
1.	SSRY6	0.46	0.54
2.	SSRY7	0.42	0.50
3.	SSRY8	0.57	0.64
4.	SSRY21	0.51	0.60
5.	SSRY28	0.57	0.62
6.	SSRY31	0.63	0.69
7.	SSRY32	0.28	0.34
8.	SSRY34	0.30	0.36
9.	SSRY35	0.34	0.37
10.	SSRY36	0.61	0.66
11.	SSRY39	0.52	0.60
12.	SSRY40	0.74	0.77
13.	SSRY43	0.72	0.76
14.	SSRY49	0.50	0.57
15.	SSRY50	0.36	0.46
16.	SSRY103	0.63	0.69
17.	SSRY106	0.63	0.69
18.	SSRY235	0.53	0.60
19.	SSRY324	0.53	0.60
20.	NSY158	0.75	0.78
21	NSY198	0.49	0.55

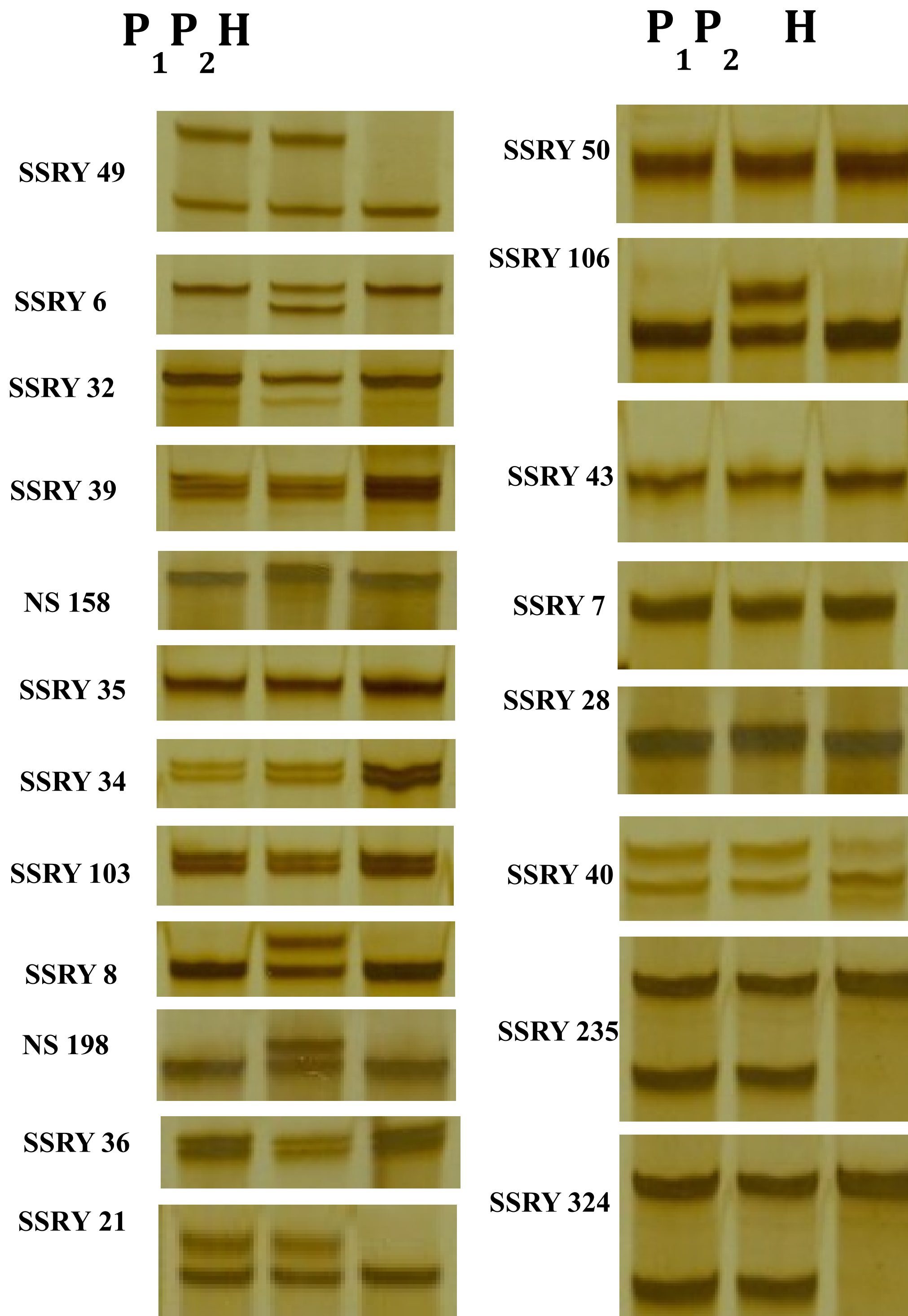
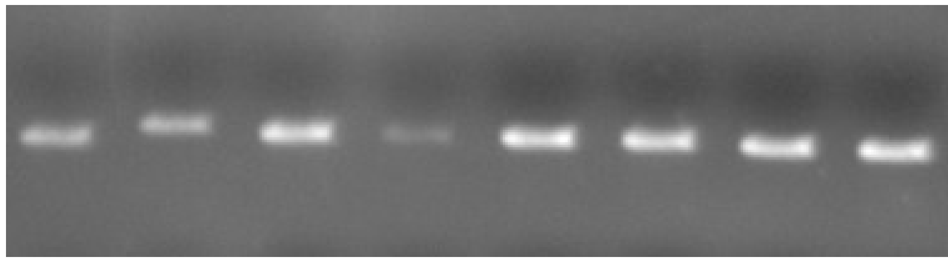


Plate: 4. Validation of parental identity using SSR primers



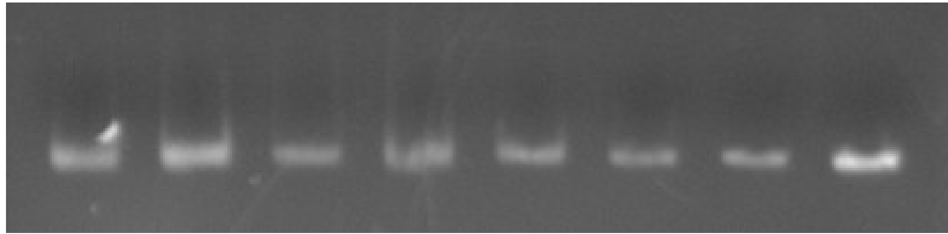


Plate: 5. PCR amplified products

b. Detection of PCR amplified products on Polyacrylamide gel

The amplified products of ten SSR primers were electrophoresed on vertical denaturing PAGE unit. The separated bands on 6 per cent acrylamide gels were visualized after silver staining and polymorphism among the cassava lines were detected (Plate 6).

c. Data Analysis

A total of 34 amplicons were produced with ten SSR loci analysed in 37 accessions. Out of these 34 alleles, two alleles were monomorphic and rest of them were polymorphic. The number alleles produced by each of the primers ranged from 2 (SSRY 7, SSRY 32, SSRY 235 and NS 158) to 7 (SSRY 103) per locus (Table 11). The percentage of polymorphic bands was 100 per cent for all other primers except SSR 7 and SSR 235 which produced 1 monomorphic band each.

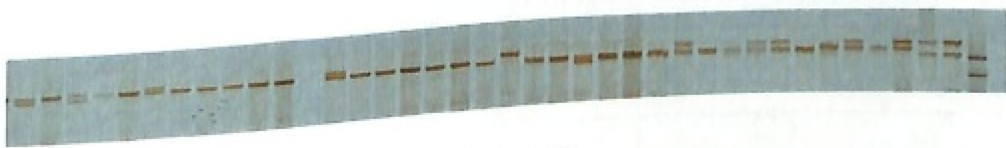
i. Similarity index

The similarity index values obtained for each pair wise comparison among 37 accessions listed in Table 12. The coefficient of similarity between 37 cassava lines ranged from 0.38 to 0.97. The highest genetic similarity (0.97) was observed among accessions CMR 100/8 and CMR 100/17;CMR 100/8 and CMR 100/31; CMR 100/25 and CMR 100/31; CMR 100/53 and CMR 100/56; CMR 100/60 and CMR 100, while the lowest genetic similarity was observed between Narayanakapa and BR-2 with 0.38 similarity Based on the similarity index data, a frequency graph was drawn and shown in Fig. 10. From the graph high frequency obtained between 0.7-0.0.79.

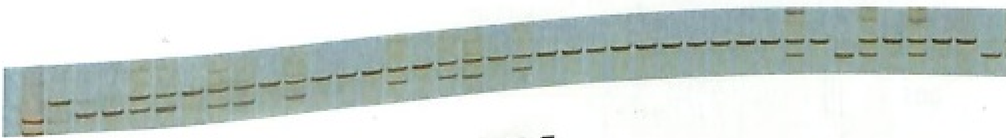
ii. Cluster based Dendrogram

The dendrogram (Fig. 11) in the cluster analysis indicates genetic variability among analyzed accessions and it was observed that the amplitude in the similarity coefficient of SM ranged from 0.57 to 0.97. After estimating the genetic similarity and considering the similarity of 61% among lines, three main clusters were formed. The clustering of cassava lines showed that CMR-100, its expectedparents

SSR 40



SSR 235



SSR 7

SSR 32

Plate: 6. SSR banding profile on PAGE gel

Table 11. Details of polymorphic bands produced by 10 SSR primers

Sl. No.	SSR primers	Total no. of bands	No. of polymorphic bands	Percentage of polymorphism
1	SSRY 7	2	1	50
2	SSRY31	4	4	100
3	SSRY 32	2	2	100
4	SSRY 36	3	3	100
5	SSRY 40	5	5	100
6	SSRY 50	3	3	100
7	SSRY 103	7	7	100
8	SSRY 106	4	4	100
9	SSRY 235	2	1	50
10	NS 158	2	2	100

Table 12. Similarity coefficient matrix of 37 cassava cultivars

	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31	C32	C33	C34	C35	C36	C37	C38	C39	C40	C41	C14	C10	C12	C9	C3	C2	C1	C42	C43	C44
C15	1																																				
C16	0.74	1																																			
C17	0.56	0.588	1																																		
C18	0.62	0.647	0.941	1																																	
C19	0.53	0.617	0.735	0.794	1																																
C20	0.59	0.676	0.735	0.735	0.764	1																															
C21	0.68	0.705	0.823	0.823	0.794	0.852	1																														
C22	0.705	0.676	0.735	0.794	0.823	0.823	0.911	1																													
C23	0.764	0.735	0.617	0.676	0.764	0.647	0.735	0.764	1																												
C24	0.5	0.588	0.705	0.764	0.97	0.735	0.764	0.794	0.735	1																											
C25	0.71	0.62	0.68	0.74	0.76	0.76	0.85	0.94	0.76	0.74	1																										
C26	0.76	0.79	0.56	0.62	0.65	0.65	0.735	0.823	0.764	0.617	0.823	1																									
C27	0.5	0.647	0.823	0.823	0.794	0.911	0.764	0.735	0.617	0.764	0.676	0.558	1																								
C28	0.53	0.558	0.676	0.735	0.941	0.764	0.794	0.823	0.705	0.911	0.764	0.647	0.735	1																							
C29	0.617	0.647	0.647	0.705	0.794	0.794	0.823	0.911	0.676	0.764	0.852	0.794	0.705	0.794	1																						
C30	0.558	0.588	0.705	0.764	0.97	0.794	0.823	0.852	0.735	0.941	0.794	0.676	0.764	0.97	0.823	1																					
C31	0.647	0.617	0.676	0.735	0.823	0.764	0.852	0.941	0.764	0.794	0.882	0.764	0.676	0.823	0.911	0.852	1																				
C32	0.735	0.764	0.705	0.764	0.794	0.852	0.882	0.852	0.794	0.764	0.794	0.794	0.764	0.794	0.823	0.823	0.794	1																			
C33	0.558	0.647	0.647	0.705	0.735	0.794	0.764	0.794	0.617	0.705	0.794	0.617	0.705	0.735	0.764	0.764	0.735	0.764	1																		
C34	0.588	0.676	0.676	0.676	0.764	0.823	0.852	0.882	0.647	0.735	0.823	0.764	0.735	0.764	0.911	0.794	0.823	0.794	0.794	1																	
C35	0.558	0.588	0.647	0.647	0.852	0.676	0.764	0.735	0.676	0.823	0.676	0.617	0.647	0.852	0.705	0.882	0.735	0.705	0.647	0.735	1																
C36	0.647	0.617	0.676	0.735	0.764	0.705	0.794	0.882	0.647	0.735	0.882	0.764	0.676	0.764	0.794	0.794	0.823	0.735	0.676	0.764	0.794	1															
C37	0.529	0.558	0.617	0.676	0.882	0.705	0.735	0.764	0.647	0.852	0.705	0.588	0.676	0.941	0.735	0.911	0.764	0.735	0.676	0.705	0.911	0.823	1														
C38	0.558	0.588	0.647	0.705	0.911	0.735	0.764	0.794	0.676	0.882	0.735	0.617	0.705	0.911	0.764	0.941	0.794	0.764	0.705	0.735	0.941	0.852	0.97	1													
C39	0.617	0.705	0.529	0.588	0.735	0.735	0.705	0.735	0.617	0.705	0.676	0.676	0.647	0.735	0.705	0.764	0.676	0.705	0.705	0.735	0.823	0.735	0.794	0.823	1												
C40	0.588	0.5	0.676	0.676	0.647	0.764	0.676	0.705	0.529	0.617	0.705	0.529	0.676	0.705	0.676	0.676	0.647	0.676	0.735	0.647	0.676	0.705	0.764	0.735	0.676	1											
C41	0.5	0.529	0.647	0.647	0.852	0.794	0.705	0.735	0.617	0.823	0.676	0.558	0.764	0.852	0.705	0.882	0.735	0.705	0.647	0.676	0.882	0.794	0.911	0.941	0.764	0.794	1										
C14	0.529	0.558	0.676	0.676	0.823	0.823	0.735	0.705	0.647	0.794	0.647	0.529	0.794	0.823	0.676	0.852	0.705	0.735	0.617	0.647	0.852	0.764	0.882	0.911	0.735	0.764	0.97	1									
C10	0.529	0.558	0.676	0.676	0.764	0.764	0.676	0.705	0.588	0.735	0.647	0.588	0.735	0.764	0.676	0.794	0.647	0.676	0.676	0.647	0.852	0.764	0.823	0.852	0.794	0.823	0.911	0.882	1								
C12	0.588	0.558	0.558	0.558	0.705	0.705	0.617	0.647	0.588	0.676	0.588	0.588	0.617	0.705	0.617	0.735	0.647	0.617	0.617	0.588	0.794	0.647	0.764	0.794	0.794	0.764	0.852	0.823	0.882	1							
C9	0.617	0.529	0.647	0.647	0.676	0.735	0.647	0.617	0.617	0.647	0.558	0.558	0.705	0.676	0.588	0.705	0.617	0.647	0.529	0.558	0.705	0.558	0.676	0.705	0.705	0.676	0.764	0.794	0.735	0.852	1						
C3	0.705	0.558	0.735	0.735	0.647	0.823	0.735	0.705	0.529	0.617	0.647	0.588	0.794	0.647	0.676	0.676	0.647	0.735	0.617	0.647	0.558	0.588	0.588	0.617	0.617	0.705	0.676	0.705	0.647	0.705	0.852	1					
C2	0.735	0.705	0.705	0.705	0.5	0.676	0.647	0.617	0.676	0.47	0.617	0.735	0.647	0.5	0.588	0.529	0.558	0.705	0.588	0.558	0.47	0.5	0.441	0.47	0.529	0.676	0.529	0.558	0.617	0.617	0.705	0.735	1				
C1	0.647	0.735	0.676	0.676	0.588	0.705	0.676	0.647	0.588	0.558	0.588	0.647	0.735	0.529	0.617	0.558	0.588	0.676	0.5	0.588	0.5	0.588	0.529	0.558	0.617	0.588	0.617	0.647	0.588	0.647	0.676	0.764	0.735	1			
C42	0.588	0.558	0.558	0.558	0.47	0.588	0.558	0.529	0.588	0.5	0.529	0.529	0.558	0.529	0.441	0.5	0.47	0.558	0.558	0.411	0.441	0.411	0.47	0.441	0.558	0.588	0.5	0.529	0.588	0.647	0.676	0.647	0.735	0.705	1		
C43	0.617	0.47	0.529	0.588	0.617	0.558	0.588	0.617	0.676	0.647	0.617	0.558	0.529	0.676	0.588	0.647	0.558	0.705	0.647	0.558	0.529	0.558	0.617	0.588	0.47	0.558	0.529	0.558	0.558	0.441	0.411	0.5	0.529	0.382	0.558	1	
C44	0.588	0.441	0.617	0.617	0.588	0.588	0.558	0.588	0.647	0.558	0.588	0.529	0.617	0.647	0.558	0.617	0.529	0.676	0.617	0.529	0.5	0.529	0.588	0.558	0.441	0.588	0.558	0.588	0.588	0.47	0.5	0.588	0.617	0.47	0.588	0.911	1

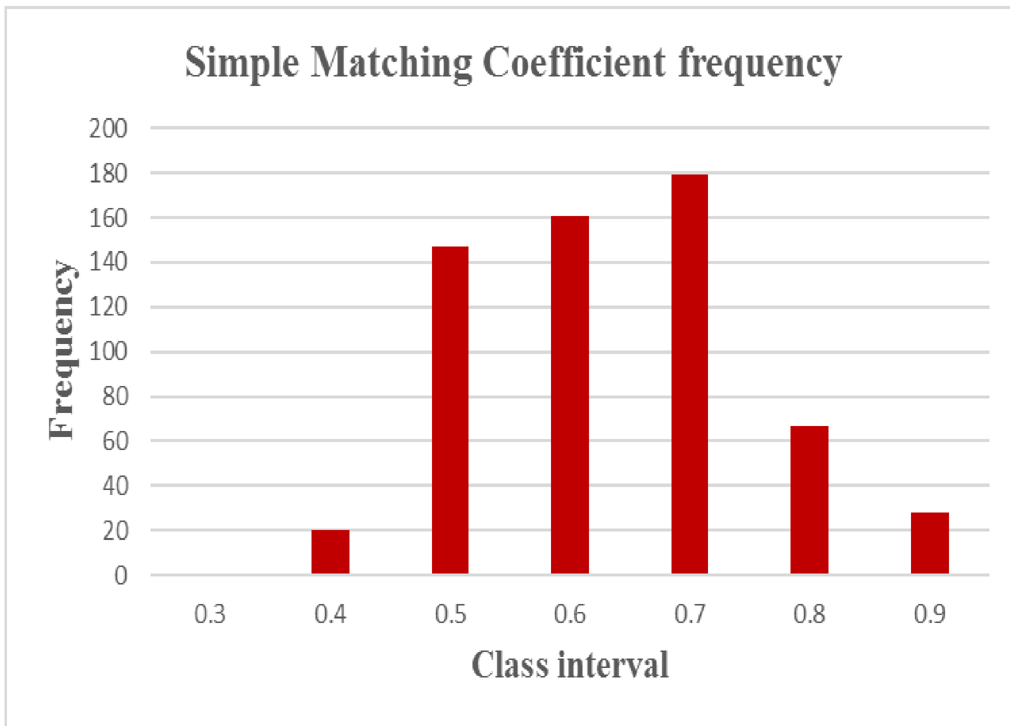


Fig: 10. Frequency distribution of SSR markers based on similaritymatrix

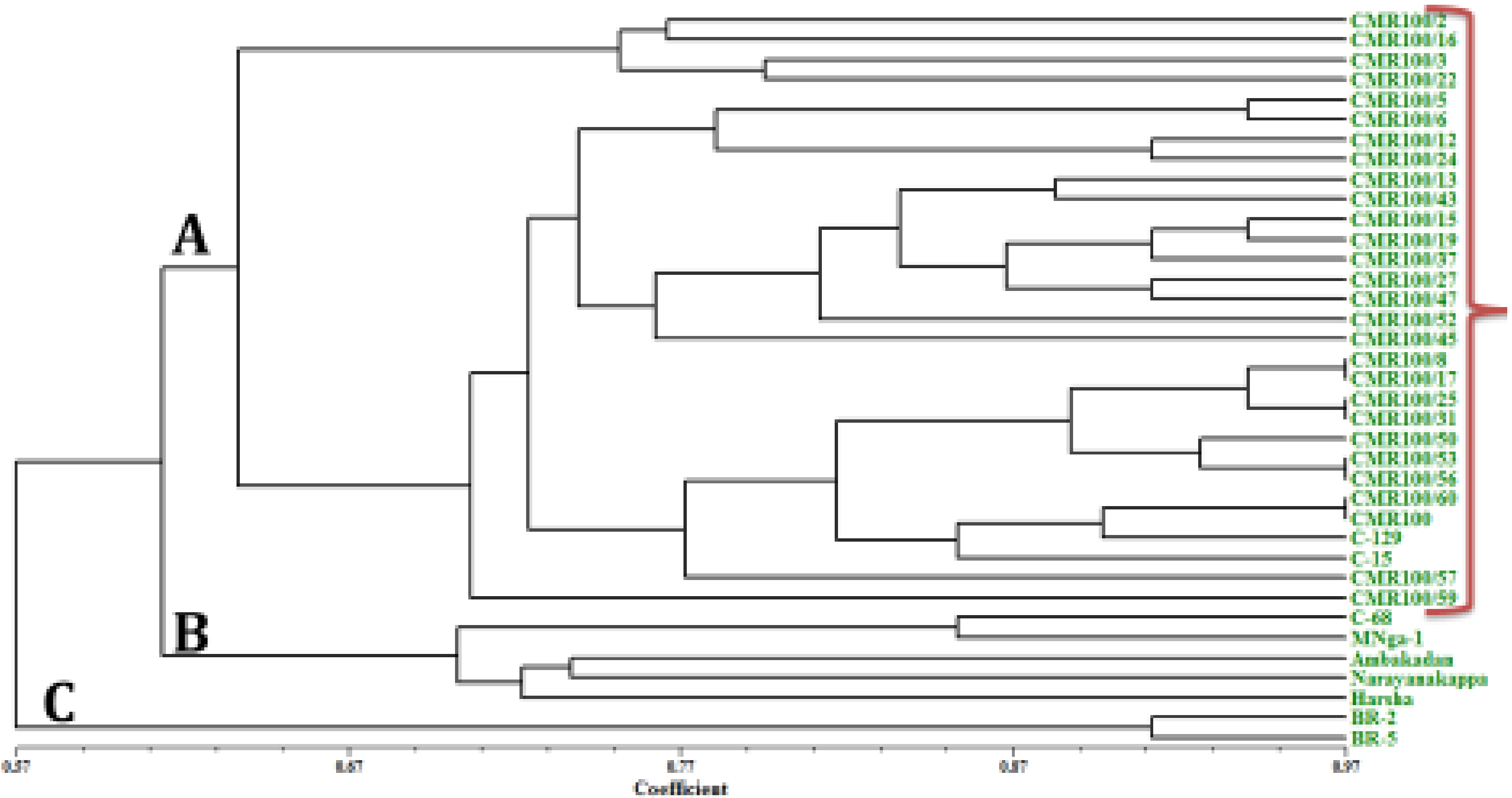


Fig: 11. Cluster based Dendrogram of 37 cassava cultivars

and OP progenies were grouped into cluster-A which is the largest cluster. The cluster-A was further divided into two clusters, cluster-A1 and cluster-A2 at a coefficient value 0.63. The cluster-A1 includes 4 OP progenies of CMR-100 and rest of the progenies were included in the cluster-A2 cluster along with CMR-100 and its expected parents. The progeny CMR-100/60 shows 97 per cent similarity with CMR-100, indicating that this could be the selfed progeny of CMR-100. Ambakadan, Narayanakkapa, MNga-1, C-68 and Sree Harsha were grouped in cluster-B. The BR lines BR-2 and BR-5 were grouped in cluster-C.

iii.Principle Component Analysis

The PCA was performed and 2-D and 3-D plots were obtained using NTSYSpc software (Fig. 12 and 13). In the 2D plot obtained reveals that, BR 2 and BR 5 were genetically closer. The cassava line Sree Harsha was showed high level of variation from other cultivars in both 2D and 3D. Two progenies of CMR-100, *viz.*, CMR 100/2 and CMR 100/2 were genetically closer to Ambakadan and Narayanakapa. The 3D plot also revealed similar result as in the dendrogram that the progeny CMR 100/60 was genetically closer to CMR-100.

d.PIC and Heterozygosity of Primers

The PIC and heterozygosity value of each primer used for genetic variability assessment were calculated and details were given in Table 13. The PIC value ranges from 0.21to 0.78 and heterozygosity from 0.24 to 0.81. The lowest heterozygositywas observed in the primer SSRY7 whereas highest was observed in SSRY103. Out of 10 primers, 3 primers *viz*., SSRY31, SSRY103 and SSRY106 show PIC value greater than 0.5 which indicated that these primers are highly polymorphic and informative.

4.4 Estimation of Dry Matter Content (DMC) and Starch Content

The DMC of CMR-100 and its27 OP progenies of were calculated (Table 14). The DMC obtained for CMR-100 is 43 per cent. Among the 27 progenies, 13 progenies show DMC greater than 40% and highest DMC is for CMR 100/6 (50%). The progenies, CMR 100/59 and CMR 100/60 have lowest DMC of 15%.

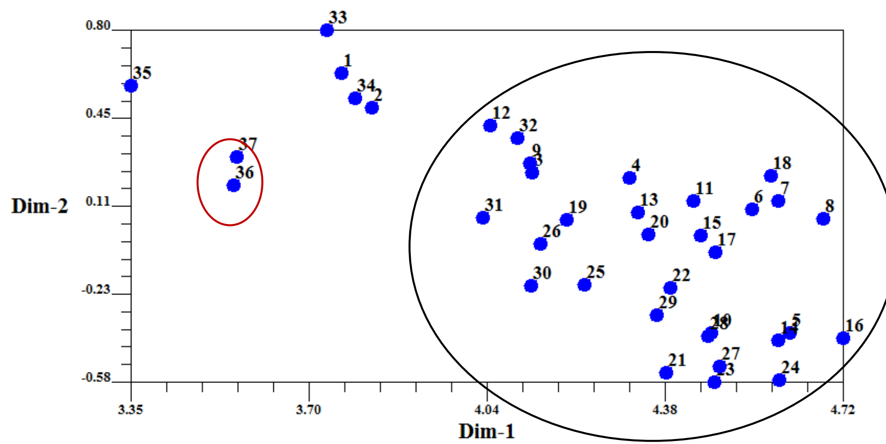


Fig: 12. 2D plot of PCA in genetic variability study

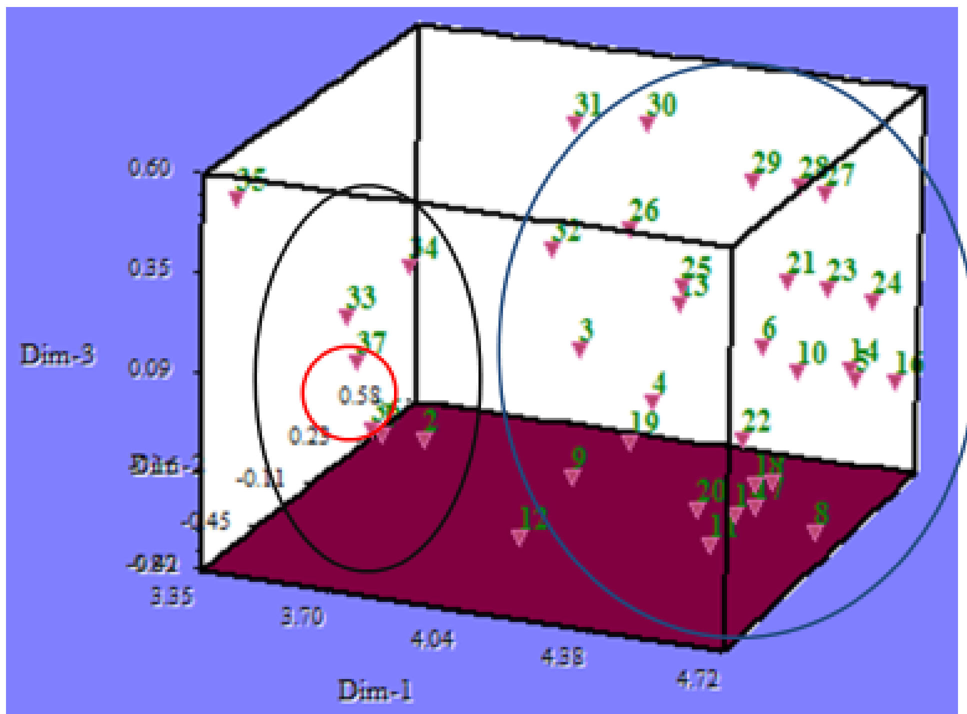


Fig: 13. 3D plot of PCA in genetic variability study

Table 13. PIC and Heterozygosity of Different Primers

Sl. No.	Primer	PIC	He
1.	SSRY7	0.21	0.24
2.	SSRY31	0.60	0.66
3.	SSRY32	0.36	0.48
4.	SSRY36	0.45	0.51
5.	SSRY40	0.47	0.51
6.	SSRY50	0.44	0.53
7.	SSRY103	0.78	0.81
8.	SSRY106	0.68	0.73
9.	SSRY235	0.26	0.31
10.	NS158	0.37	0.49

Table 14. Data analysis of DMC and starch content

		Dry Matter Content		Starch Content
Sl. No.	Name	Average	Per cent	Per cent
1	CMR 100/2	24.63	49.26	36.48
2	CMR 100/3	21.59	43.18	31.19
3	CMR 100/5	24.06	48.12	35.49
4	CMR 100/6	25.06	50.12	37.23
5	CMR 100/8	20.66	41.32	29.58
6	CMR 100/12	15.66	31.31	20.87
7	CMR 100/13	15.31	30.62	20.27
8	CMR 100/15	22.68	45.36	33.09
9	CMR 100/16	21.74	43.47	31.45
10	CMR 100/17	20.30	40.59	28.94
11	CMR 100/19	23.62	47.24	34.72
12	CMR 100/22	19.74	39.47	27.97
13	CMR 100/24	13.69	27.38	17.46
14	CMR 100/25	10.49	20.98	11.89
15	CMR 100/27	21.05	42.10	30.26
16	CMR 100/31	20.03	40.05	28.47
17	CMR 100/37	19.87	39.74	28.20
18	CMR 100/43	17.87	35.73	24.72
19	CMR 100/45	12.86	25.72	16.01
20	CMR 100/47	16.25	32.49	21.90
21	CMR 100/50	14.23	28.45	18.39
22	CMR 100/52	21.70	43.40	31.38
23	CMR 100/53	15.26	30.51	20.18
24	CMR 100/56	22.33	44.66	32.48
25	CMR 100/57	10.82	21.64	12.47
26	CMR 100/59	7.72	15.44	7.06
27	CMR 100/60	7.71	15.41	7.05
28	CMR 100	21.63	43.26	31.05

The percentage starch content of these progenies was determined using DMC to starch conversion formula. The starch content of CMR-100 is 31%. The nine OP progenies have high starch content greater than 30%. The progeny CMR 100/6 has highest starch content of 37%. The lowest starch content obtained was 7% for CMR 100/60. In genetic variability studies, among the 27 OP, CMR 100/27 is the best progeny having DMC of 42 DMC of 42% and starch content of 30% with high yield (6 kg/plant) and CMD resistance.

DISCUSSION

DISCUSSION

Cassava (*Manihot esculenta* Crantz) is a major food security crop which produce starchy tuberous root under low input conditions in tropics. Itis the second most important tuber crop used as staplefoodafter potato in tropical countries. Cassava is a very versatile crop which is used as raw material for many starch based industries and many value added products like fried chips, pastha etc. are produced.

Cassava breeding improved after the establishment of the IITA and the CIAT (Ceballos *et al*., 2004).The breeding objectives of cassava includes yield, DMC, cooking quality, drought tolerant, disease resistance, β-carotene, early root bulking, cyanogenic glucosides and starch (Vincent *et al*.,2014;Jennings and Hershey, 1985).The cassava breedersproduce improved varieties through hybridisation and clonal selection.Genotypes can be improved using open pollinated progenies. Many molecular techniques are used for improving the cassava cultivars.

Molecular markers are important for plant breeding and maker assisted selection. These markers were extensively used for crop genetic diversity studies and phylogenetic analysis. DNA based molecular markers are excellent tool for analysing the genetic relationship between the species. Many studies have reported that the application of molecular markers like SSR, ISSR, RAPD, SNP, AFLP etc. in the hybrid authentication analysis (Khajudparn *el al*., 2012; Schaber and Goldman, 2013; Dowling *et al*., 2014; Shukla *et al.,* 2015). SSR markers have many advantages over other markers because, it is a codominant, highly abundant, reliable and reproducible markers which is highly efficient for breeding related studies in plants.

The present study was under taken to identify the parents of cassava chips line CMR-100 and to assess the genetic variability of OP progenies of CMR-100 using codominant SSR markers.

Parental Identification

To our knowledge, this is the first use of DNA markers for the parental identification in crops. Thirteen cassava lines and CMR-100 were evaluated using molecular markers inorder to identify the parents of CMR-100. In this study, good

quality DNA was obtained using Dellaporta method. Gomez *et al*. (2008) conducted true hybrid identification in peanut and reported that Dellaporta method was satisfactory for DNA isolation from crosses involving cultivated genotypes but not for wild species of peanut.

In the current study, vertical PAGE which has good resolving capacity was used to separate bands instead of agarose gels. In contrast to this, Gomez *et al*. (2008) used horizontal PAGE with good resolving potential for band separation and suggested that the method is cheaper than vertical gels.

In the present study, 21 SSR markers amplifies a total of 75 alleles, which include 2 alleles in 3 SSR markers, 3 alleles in 9 SSR markers, 4 alleles in 5 SSR markers, 5 alleles in 2SSR markers and 6 alleles in 2 SSR markers. Similar results were obtained in rice hybrid identification study conducted by Sundaram *et al*. (2008), in which the number of alleles ranges from 2 to 7. In the study, the lowest polymorphism of was seen in the SSRY 32 (50%), whereas RAPD primer GLH2 showed lowest polymorphism of 48% in the research on hybrid authentication in cotton (Ali *et al*., 2008).

The lowest similarity coefficient value of 0.4 was showed between many lines (Ambakadan and C-53, Mnga-1 and CI-731, C-114 and CI-731, C-15 and CI-731) in the present study. Likewise, in the hybrid authentication study using RAPD analysis reveals that genotype Paymaster and the hybrid SLS1 x CIM-511 observed low value of similarity coefficient (0.53) (Ali *et al*., 2008). The above results point out that these lines are highly diverse from each other.

The present research work revealed that the genetic similarity observed for 14 cassava cultivars including CMR-100 ranged from 0.4 to 0.93 using Simple Matching coefficient in cluster analysis. The similar results were obtained in the study of genetic purity assessment in hybrid rice done by Nandakumar *et al.* (2004). In that study the cluster analysis based on Jaccard similarity coefficient showed the value in the range of 0.33 to 0.92.

Bianco *et al.* (2011) conducted a study to identify F_1 hybrids of artichoke using ISSR markers in which the dendrogram based on Nei genetic distance coefficient clustered hybrids of the three crosses and their respective male and

female parents together which substantiated the result obtained in the study. In present study CMR-100 clustered with C-129 and C-15 in a single group based on Simple Matching coefficient showing more than 90% similarity.

The PCA analysis done in this study showed that CMR-100 is genetically closer to C-129 and C-15. Similarly, male parent SP2 was genetically closer to the female parent MS6 in 2D plot of PCA analysis in the study identification of F_1 hybrids of artichoke using ISSR markers (Bianco *et al.,* 2011).

The present investigation revealed that 14 SSR primer which has PIC and more heterozygosity value more than 0.5 which are highly polymorphic and informative. The primer SSR 21 showed PIC value of 0.5, similar finding was reported in the cassava genetic diversity study conducted by Turgyenda *et al.* 2012 indicates that this primer is highly informative.

The banding patterns of 19 SSR markers shows that at least one allele from the parent is present in the offspring, CMR-100. Mendelian inheritance states that, for a diploid organism at least one allele per locus for codominant marker will be shared between a parent and a progeny.

The CMR-100 often expresses mild symptoms of CMD which may be contributed by the parent C-15, which is CMD susceptible.

The study suggests that codominant molecular markers like SSR primers have the potential to identify the parents of open pollinated progenies of cassava.

Genetic Variability Assessment

A total of 37 cassava accessions were used for the genetic variabilityassessment study.The number of bands obtained from ten different SSR primers ranged from 2 to 7. Similarly, Asare *et al*. (2011) obtained a range of 2 to 9 alleles among 43 cassava accessions using 20 SSR primers. Ribeiro *et al.*(2011) studied cassava accessions with 14 microsatellites produced 2 to 4 alleles per locus. Fu *et al*. (2014) also obtained 2 to 21 alleles per locus using 35 SSR markers in 282 cassava clones.

Out of ten SSR primers used in the study, eight primers showed 100% polymorphism and the ranges varied from 50 to 100 per cent. The high percentage

of polymorphism varying from 88.8 to 100 per cent were reported in a study conducted on cassava genetic diversity assessment by Siqueira *et al*. (2009).

In the current study, the PIC value of the SSR primers ranged from 0.21 to 0.78. Among the SSR primers used, SSRY 103 is the most informative and polymorphic primer with a PIC value of 0.78. Asare *et al.*(2011) reported PIC ranging from 0.07 to 0.75, where SSR 103 shows slightly lower PIC value of 0.68. Moyib *et al*. (2007) studied genetic diversity of cassava accessions by SSR markers; obtained PIC value ranging from 0.19 to 0.66. The high PIC value obtained in SSR primers suggested that these codominant markers are highly polymorphic for diversity studies.

In genetic diversity studies, the primer which is showing more heterozygosity are highly valuable. In the present study high heterozygosity value of 0.81 was showed by the primer SSRY 103. Likewise, Asare *et al*. 2011 obtained observed heterozygosity value of 0.77 in genetic variability assessment in cassava using SSR markers.

The results from this study indicate that SSR markers techniques along with cluster based dendrogram are highly efficient to estimate the relatedness between the cassava lines. In contradictory, Ribeiro *et al.* 2011 reported the genetic relatedness were deficient in the genetic variability assessment study conducted in 93 cassava accessions with 14 microsatellites.

The current study, it was found that the OP progenies showed more similarity with the female parent CMR-100. A different result was reported in the study of hybrid authentication in cotton using RAPD molecular markers conducted by Ali *et al.* (2008). The progeny expressed more similarity with male parent as compared to female parent.

The present investigation was not able to correlate CMD resistance with the SSR markers used in the study.

The findings from the study revealed that SSR markers are able to differentiate OP progenies of a variety efficiently. The expected parents of CMR-100 and its OP progenies are grouped in a single cluster and other varieties are

clustered in different groups. Therefore, this codominant marker can be used to discriminate closely related progenies of a variety.

SUMMARY

SUMMARY

The study entitled “Molecular characterization of cassava chips line CMR-100 and its progenies using SSR markers” was carried out in Division of Crop Improvement, ICAR-CTCRI, Thiruvananthapuram during 2015-2016 with an aim to identify the parents of CMR-100 and to assess the genetic variations among the OP progenies of CMR-100 with selected cassava cultivars. The significant findings of the study are summarized below.

I. Parental Identification

To identify the parents of CMR-100, 13 cassava lines which show morphological resemblance were selected. The genomic DNA was extracted using the method described by Dellaporta *et al*., 1983. Quality and quantity of DNA was determined using agarose gel electrophoresis and Nanodrop Bio-spectrophotometer respectively. The good quality DNA obtained was used for the PCR amplification with 21 SSR primers. The denatured PCR products were separated on 6 percent acrylamide gel and clear bands were scored in the binary (1, 0) form. A total of 75 alleles were produced by 21 primers across 14 accessions. The primer NS 158 produced six alleles and showed maximum PIC value (0.75) and He (0.78). Among the 21 primers, 14 primers were highly polymorphic and informative. The UPGMA based dendrogram using Simple Matching coefficient clustered CMR-100, C-129 and C-15 in a single cluster with more than 90 per cent similarity. The similarity index value obtained between CMR-100 and C-129 is 0.93 whereas C-15 shows 0.84 with CMR-100. The similar results were obtained from the 2D and 3D plots of PCA analysis. The banding pattern of the alleles in CMR-100, C-129 and C-15 produced by 19 primers were compared for analysing the segregation of alleles. The result showed that atleast one of alleles of both C-129 and C-15 were present in CMR-100 in combined form.

II. Genetic Variability Assessment

For the genetic variability analysis, 27 OP progenies of CMR-100, expected parents of CMR-100 and seven cassava cultivars were used for the study. The isolated DNAs were amplified with 10 SSR primersand bands were separated in 6 per cent acrylamide gel. The bands obtained were scored in the binary (1, 0) form. A total of 34 alleles were produced by 10 SSR primers. The number of bands produced ranged from 2 (SSRY 7, SSRY 32, SSRY 235 and NS 158) to 7 (SSRY 103). The binary matrix was used for data analysis with NTSYSpc software. The coefficient of similarity between 37 cassava lines ranged from 0.38 to 0.97. The lowest genetic similarity was observed between Narayanakapa and BR 2 with 0.38 similarities. The dendrogram based on Simple Matching coefficient was obtained and the 37 accessions were clustered into 3 major groups at a coefficient value of 0.61. OP progenies of CMR-100, CMR-100 and its expected parents were clustered in a single group (Cluster A). Two BR lines were clustered separately in cluster C and the rest of accessions included in cluster B. The progeny CMR-100/60 shows 97 per cent similarity with CMR-100, indicating that this could be the selfed progeny of CMR-100. The 2D and 3D plots were obtained after PCA analysis with Eigen vector. The 2D plot reveals that, BR 2 and BR 5 were genetically closer. The cassava line Sree Harsha was showed high level of variation from other cultivars. Two progenies of CMR-100, *viz*., CMR 100/2 and CMR 100/3 were genetically closer to Ambakadan and Narayanakapa. The 3D plot also revealed similar result as in the dendrogram that the progeny CMR 100/60 was genetically closer to CMR-100. The PIC value ranges from 0.21to 0.78 and heterozygosity from 0.24 to 0.81. Out of 10 primers, three primers *viz*., SSRY 31, SSRY 103 and SSRY 106 show PIC value greater than 0.5 which indicated that these primers are highly polymorphic and informative.

DMC and starch content of CMR-100 was 43% and 31% respectively. Some OP progenies of CMR-100 shows high DMC and starch content than the female parent CMR-100. Among the 27 OP progenies, CMR 100/27 is the best progeny having DMC of 42% and starch content of 30% with high yield (6 kg/plant) and CMD resistance which can be used for further breeding purpose.

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APPENDIX

APPENDIX

Reagents for DNA extraction

1. **Extraction buffer**

Tris – HCl (pH 8.0) - 100mM
EDTA (pH 8.0) - 0.5 M
NaCl - 5 M
β-mercaptoethanol - 0.2% (v/v)
PVP - 1% (w/v)

(β-mercaptoethanol and PVP: Added freshly prior to grinding)

2. **SDS – 20%**
3. **Potassium acetate – 5M**
4. **Sodium acetate – 3M**
5. **Chloroform isoamyl alcohol mixture (24:1)**
6. **Ethanol – 70%**
7. **RNase**

10mg/ml (RNase A was dissolved in TE buffer and boiled for 15 minutes at 100°C to destroy DNase and stored at -20°C)

Reagents for Agarose gel electrophoresis

1. **TBE buffer (10X)**

Tris base - 107 g
Boric acid - 55 g
EDTA - 82 g

Final volume made up to 1000 ml with distilled water and autoclaved before use.

2. **Ethidium bromide**

Weigh 5 mg of ethidium bromide powder and dissolve in 1 ml of distilled water.

3. **Loading dye**

Formamide - 50 ml
Xyline cyanol - 50 mg
Bromophenol blue - 50 mg
EDTA (0.5M) - 1 ml

Reagents and solutions for Poly acrylamide gel electrophoresis

1. **Bind silane**

Absolute ethanol (99.5%) - 497.5ml

Acetic acid (0.5%) - 2.5ml

Bind silane - 1µl

2. **Acrylamide (40%)**

Acryl amide - 38 g

Bis-acrylamide - 2 g

Final volume made upto 100 ml using distilled water

3. **Ammonium per sulphate**

100mg APS was weighed in aluminium foil covered eppendorf tube and add 1ml of distilled water. Vortex the contents well.

4. **6% polyacrylamide gel containing 7M urea**

42 g urea was dissolved in a beaker containing 10 ml TBE buffer (10X) and 15 ml distilled water by heating in a microwave oven for 40 s. 15 ml acrylamide solution (19:1) was filtered and added to a measuring cylinder followed by melted urea solution. The final volume was made upto 100 ml using distilled water and stored in dark till use. 60 µl TEMED and 600 µl APS (100 mg/ml) (1:10) was added and mixed before casting the gel.

5. **Empty well dye**

Loading dye - 50 µl

Sterile distilled water - 50 µl

6. **100bp marker**

100bp marker - 5 µl

Loading dye - 40 µl

Sterile distilled water - 55 µl

Final volume made upto 100 µl.

7. **Fixer**

Acetic acid - 200 ml

Distilled water - 1800 ml

8. Silver stain

2g silver nitrate dissolved in distilled water and 3ml formaldehyde and final volume made upto 2000ml.

9. Developer

60g sodium carbonate was dissolved in distilled water and the final volume made upto 2000ml and stored at -20°C. 3ml of formaldehyde and 400µl sodium thiosulphate (10mg/ml) was added and mixed thoroughly before use.

ABSTRACT

MOLECULAR CHARACTERIZATION OF CASSAVA CHIPS LINE CMR-100 AND ITS PROGENIES USING SSR MARKERS

By

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ABSTRACT

The study entitled "Molecular characterization of cassava chips line CMR-100 and its progenies using SSR markers" was carried out in Division of Crop Improvement, ICAR-CTCRI, Thiruvananthapuram during 2015-2016 with an aim to identify the parents of CMR-100 and to assess the genetic variations among the OP progenies of CMR-100 with selected cassava cultivars.

Cassava chips line CMR-100 was identified in ICAR-CTCRI and is ideal for chips making, because it has uniform cylindrical tuber shape. The parents of this cassava line are unknown since it is a product of open pollination. The marker analysis for the parental identification of this chips line reveals 97 percent similarity with C-129 and 90 percent with C-15 in cluster based dendrogram. This result suggesting that these two lines may be the parents of CMR-100. Similar results were obtained in both 2D and 3D plots. CMR-100 shows 0.93 similarity index value with C-129 which is the highest coefficient value obtained whereas C-15 shows 0.84. The banding patterns of these three lines were compared using 19 SSR products, which reveals that atleast one parental allele is present in the hybrid. The PIC value ranges from 0.30 to 0.75 and heterozygosity from 0.36 to 0.78 and 14 SSR markers were found to be highly polymorphic and informative.

The genetic variability of OP progenies using molecular markers reveals 67 percent similarity of progenies suggesting that female characters are expressed more in the progenies than male characters. All the OP progenies of CMR-100 along with CMR-100 and its expected parents were grouped in a single cluster at similarity coefficient value 0.63. The progeny CMR-100/60 shows 97 per cent similarity with CMR-100 suggesting that it is a selfed progeny of CMR-100. The PIC value ranges from 0.21 to 0.78 and heterozygosity from 0.24 to 0.81 and three SSR markers were highly polymorphic and informative.