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# Studying the Disease Resistance and Morphological Variations in Cassava

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***Data Availability Statement:*** Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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## Abstract

Cassava is one of the most important tropical root crops with highest carbohydrate content feeding more than 800 million people all over the world. Cassava mosaic disease (CMD) is a geminiviral disease which causes serious yield penalties in cassava production in tropical and sub-tropical regions. The most reliable and long lasting mechanism to combat CMD is to utilize host plant resistance especially through R-gene mediated resistance. The present study aimed to identify polymorphism between cassava cultivars *viz.,* MNga-1 and CI-732 using resistant gene candidates (RGC) degenerate primers. This study utilized eight degenerate primers (*L6, I, RPS2, RPS5, RPP5, Xa-1, N, RGC2*) to detect polymorphism and were observed between cassava cultivars amplified with primer pairs (*I, RPS2, RPS5, RPP5*). A total of 60 single nucleotide polymorphisms (SNPs) were observed between two cassava cultivars which included 21 transitions and 39 transversions. The identified polymorphism was used to synthesize cleaved amplified polymorphic sequences (CAPS) marker computationally using CAPS designer tool of SOL GENOME NETWORK and identified three CAPS markers in susceptible cultivar amplified with *RPP5*. These cultivars were selected as male and female parent in CMD resistant breeding studies. True hybrid evaluations of the $F_1$ mapping populations were carried out using single allelic markers. Morphological characterizations of $F_1$ mapping population were carried out based on cassava descriptors mentioned by IITA, Nigeria and were grouped using cluster analysis. It was also found that more than half of the $F_1$ progenies (65) acquired morphological characters from female parent indicating the influence of female parent.

***Keywords:*** Cassava, CAPS markers, SNP, transition, transversion

## 1. Introduction

Cassava, also known as manioc, tapioca, and yucca, is one of the most important tropical root crops with highest carbohydrate content feeding more than 800 million people (Burns et al., 2010). The cultivated cassava (*Manihot esculenta* Crantz) comes under Euphorbiaceae family, domesticated over 5,000-7,000 years BC along the southern and or northern rim of the Amazon basin (Duputie et al., 2009). The broad agro-ecological adaptability, its ability to mitigate unfavourable environmental condition and its abilities to produce reasonable yield makes cassava a food security turned cash crop. The average global cassava production was estimated to be 270.2 MT in which 54.3% is accounted by Africa, 33.4% contributed by Asia and remaining 12.2% by Latin America (FAOSTAT, 2014). Indian production was accounted to be 4171000 ton

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from an area of 199000 ha (FAOSTAT, 2017). But the cassava production is always limited by Cassava mosaic disease (CMD), one of the most economically important Gemini viral disease causes a serious yield loss of 20 to 80%. A severe loss of more than 19 million tons of cassava valued above US $1.9 billion was reported in Africa in 2003 due CMD (Legg and Fauquet, 2004).

Plants have developed multiple mechanisms to defend various pathogens, of which viruses poses a serious threat to global agriculture as it cause a massive yield penalties in the concerned crop. To resist viral pathogen, plants relied on innate immune system contained multi-layered network of defense proteins. As an initial step of defense mechanism, plants developed pattern-triggered immunity (PTI) by which the immune system of the plant recognized a broad range of pathogens with conserved molecular patterns which were followed by effector-triggered immunity (ETI) acts inside the cell *via* various proteins encoded by a class of defense genes that can successfully intercept the invasion of pathogens were called R-genes (Hammond-Kosack and Jones, 1997; Dangl and Jones, 2001; Jones and Dangl, 2006; Lozano et al*.,* 2015). The immediate response in R-gene mediated resistance mechanism was the hypersensitivity reaction in the infected region that restricted the spread of pathogen. This local hypersensitive response immunizes the plant against future infection by the same or closely related pathogen via systemic acquired resistance (SAR) occurs in tissues that were different from the initial infection site (Jennifer et al*.,* 2005). R-genes were grouped into several classes based on the basis of different functional domains include the nucleotide binding leucine-rich repeats proteins (NLR), receptor-like proteins (RLP), receptor-like kinases (RLK), including LRR-kinases and lectin receptor kinases, and intracellular protein kinases (PK) (Bent, 1996; Altschul et al*.,* 1997; Hammond-Kosack and Jones, 1997). The most essential step in marker assisted breeding is the development of molecular markers to R-genes in the genome which is facilitated by the presence of conserved domain in the R-genes. Degenerate primers synthesized from the conserved consensus amino acid motifs of these functional domains were used to amplify Resistance gene analogues (RGAs) in several plant species (Azhar et al*.,* 2011; Tarr and Alexander, 2009; Chen et al*.,* 2007) and that had either provided structural CGs or useful marker for MAS. With this in mind, present study made an attempt to identify polymorphism between cassava cultivars using RGC primers and identified polymorphism were used to develop CAPS markers.

In agricultural crops, true hybrid evaluation is a routine exercise carried out using DNA markers especially co-dominant markers were often used for the evaluation (Wu et al., 2006; Selvakumar et al., 2010; Mbanjo et al., 2012; Semagn et al., 2012; Otti et al., 2011). The most desirable co-dominant markers were RFLP and SSR (Gomez et al*.,* 2008) that were capable of showing homozygous polymorphic alleles in parents. Among the co-dominant markers, SSR markers were widely used because the heterozygosity of the hybrids could be easily determined by the presence of alleles from both the parents i.e., markers with one allele size per parent or markers with two allele sizes for one parent and a single allele size for the other parent. The characterization of plant materials using morphological traits has being used to assess the genetic diversity of cassava (Sambatti et al*.,* 2001; Raghu et al*.,* 2007; Benesi et al*.,* 2010; Asare et al., 2011), to determine the divergence among genotypes (Rimoldi et al*.,* 2010), and to verify the correlation among agronomic traits. Thus the present study focused to identify CMD resistant SNP variations in cassava and also study the true hybrids and morphological variations in $F_1$ mapping populations.

## 2. Materials and Methods

### *2.1. Studying the disease resistance in cassava using RGC degenerate primers*

#### *2.1.1. Sample collection*

In this study, two cassava cultivars were selected based on CMD resistance *viz.,* CI-732, CMD susceptible local cultivar and MNga-1 (TMS30001), CMD resistant cultivar developed by the International Institute for Tropical Agriculture (IITA), Nigeria. Samples collected and all the field trials were conducted in ICAR-Central Tuber Crop Research Institute (ICAR-CTCRI) from 2011-2015. The resistance in these cultivars was indexed as 5-score and 1-score respectively based on symptom severity index (SSI) explained by Hahn et al. (1980; Table 1).

#### *2.1.2. DNA isolation and PCR amplification*

The genomic DNA was isolated from these cassava genotypes using Cetyl trimethyl ammonium bromide (CTAB) method

Table 1: CMD symptom severity score to determine the level of resistance

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

(Doyle and Doyle, 1990). Weighed 100 mg of leaf samples and powdered properly using liquid nitrogen with sterile mortar and pestle. Added pre-warmed CTAB extraction buffer (1M Tris-HCl (pH 8.0); 0.5M Ethylene diamine tetraacetic acid (EDTA) pH 8.0; 5M NaCl; 2 g CTAB powder) containing 2% polyvinylpyrrolidine (PVP) and 0.2% β-mercaptoethanol to the powdered leaf samples and grinded properly. The samples were incubated in water bath (Memmert) at 65 °C for about 30 min and afterwards centrifuged the samples at 12,000 rpm for 10 min at room temperature. Transferred the supernatant to fresh tube and added equal volume of chlorform and isoamyl alcohol in 24:1 proportion into the supernatant. After thorough vortex, the samples were centrifuged at 12000 rpm for 10 min. The aqueous phase was transferred and added 5 µl of RNase A (10 mg $ml^{-1}$) into the samples. After quick vortex, the samples were incubated at 37 °C for 1 hr. Again added equal volume of chloroform:isoamyl alcohol (24:1) to the samples and centrifuged the samples at 12,000 rpm for 10 min. Transferred the aqueous phases in to new sterile tubes and added 500 µl of ice-cold isopropanol, slightly inverted the tubes. The samples were then spun down at 12000 rpm for 5 min. Discarded the supernatant and retained the pellets. Washed the pellets with 500 µl of 70% ethanol and centrifuged at 5000 rpm for 5 min. The pellets were recovered after discarding the supernatant. The pellets were then dried and were finally suspended in 100µl of sterile double distilled water. The quality and purity of the DNAs were checked using 0.8% agarose gel and stained with ethidium bromide solution. The DNA bands were visualized using UV illumination system and gel images were taken using Gel documentation system (Alpha Imager). The quantification of DNA was determined on the basis of optical density readings (ODs) read at wavelengths of 260 nm and 280 nm. The concentration of DNA samples were then adjusted to 50 ng $µl^{-1}$.

Polymorphism in these selected genotypes were carried out using RGC primers developed by Vasquez and Lopez (2012) (Table 2). A 20 µl PCR reactions were performed containing 20 ng DNA, 1X buffer (10mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 Mm dNTPs, 0.5 µM each of forward and reverse primers and 0.2U Taq polymerase (GeNei). Reaction cycle includes initial denaturation of 95 °C for 3 min; 45 cycles of 94 °C for 45s, respective annealing temperature for each primer set at 45s and 1 min of extension at 72 °C; and final extension at 72 °C for 5 min. The amplified products were resolved in 1.2% agarose gel stained with ethidium bromide. Then the PCR bands of corresponding size were eluted and purified using the QIA quick® gel extraction kit (Qiagen). These products were directly sequenced using ABI 3500 automated sequencer. The entire process includes DNA isolation, PCR amplification and elution and sequencing maintained two replications.

*2.1.3. Data analysis*

BLASTN was performed with the identified sequence and cassava genome in NCBI with default parameters. Functional annotations of the resulted sequences were and NCBI databases (Altschul et al*.,* 1990) and Phytozome v12 (Goodstein et al*.,* 2012). Expected value (E-value) gives an indication of the statistical significance of a given pair-wise alignment to infer sequence homology. Lower the E-value greater the sequence homology. The resultant sequences were submitted in NCBI and DDBJ.

*2.1.4. Detection of polymorphism and CAPS marker design*

The sequences of the both genotypes were checked by pair-wise alignment using Clustal X version 2.0 (Larkin et al., 2007) and the variations were used to identify CAPS sites using CAPS designer tool in SOL GENOMICS NETWORK (Bombarely

Table 2: List of RGC primers used to study polymorphism in cassava

| Primer Name | Sequence in 5'-3' | Base pair size | Annealing temperature |
|---|---|---|---|
| L6 | R: TTTCAGAGGTGGAGATACCCGCAA<br>L: AAGCTCGTCTAGGCACCATCTTGA | 211 | 52 °C |
| I | R: CTTTGCACAAGGCATGAGCAGGAT<br>L: TGACCATGCCAAGGCGACATGTAT | 635 | 52 °C |
| RPS2 | R: AGACAGGCTTCCAACTCCAACTCA<br>L: TAAGCTCATTGGACATTGCCGTGC | 480 | 54 °C |
| RPS5 | R: TGAAGCAGAGAAACACTGGTGGGA<br>L: AGGGTAGTGTAATGGGAGGAAATGGG | 304 | 58 °C |
| RPP5 | R: AAGGCCTAGAAAGGCACTAAGCGA<br>L: TGTCGACACGGTTAAGGTATGGCA | 493 | 50 °C |
| Xa-1 | R: TGGGCCAAGATTTCTCACATCCCT<br>L: GCTCGTATATGCAGTGCTCCACTT | 666 | 54 °C |
| N | R: GAGCTAAGCACTTCGGAGCTTTCA<br>L: TCTGACGAGCTTGTTCGATATTGT | 428 | 52 °C |
| RGC2 | R: TGATGTGTTGATGTGCTTCGTCCC<br>L: GGTATTTCTATGGACTAGCCGTGC | 253 | 50 °C |

et al., 2011).

*2.2. Studying the morphological variations in $F_1$ mapping population*

*2.2.1. Development of $F_1$ mapping populations*

The sexual hybridization was employed in the development of hybrid seeds. A total of 200 hybrid seeds were collected from hybridization between CI-732 and MNga-1, which were dried in sunlight and stored. Before sowing, the seeds were soaked in water for 12 hours in night to ensure early germination. The non-viable seeds get floated on the surface and were eliminated. The submerged seeds were viable and were separated from water. A total of 158 viable seeds thus obtained were sowed in seed bed prepared in open field and were watered regularly. The sprouts started appearing on eight day onwards from the date of seed sowing. The rate of germination was evaluated for about one month and maximum germination was recorded on 16$^{th}$ day (9 /10 seeds). A total of 130 seedlings were germinated and the remaining seeds did not germinate. The seedlings were maintained as such for one more month which developed single tap root. Later the seedlings were transplanted into individual mounts after removing the tap root.

*2.2.2. True hybrid evaluation of $F_1$ mapping population*

True hybrid evaluation of $F_1$ mapping population were carried out using 7 markers (SSRY83, SSRY339, NS890, SSRY95, SSRY33, SSRY47 and MeSSRY10) which showed single allelic size polymorphism among parental lines.

*2.2.3. Studying the morphological variations in $F_1$ mapping population*

Morphological characterizations of $F_1$ mapping population were carried out based on cassava descriptors mentioned by IITA, Nigeria (Fukuda et al., 2010). Each character was studied within the $F_1$ progenies and was then compared with both parents to study the segregation pattern and categorized the entire population accordingly (Table 3).

## 3. Results and Discussion

An attempt was undertaken to study the polymorphism in cassava genotypes using RGC primer pairs. Plant disease resistance genes are those genes which have the ability to detect the pathogen attack and facilitate counter attack against pathogen. The two commonly occurring R-genes were NBS-LRR-Toll/interleukin-1-receptors (TIR or TNL) and NBS-LRR-coiled coil (CC) or CNL. Both R-genes contained several LRR at the C-terminus of their proteins which played a role in protein–protein interactions specifically while sensing the Avr molecules (Marone et al*.,* 2013). Also TNL genes were found only in eudicot plants whereas CNL genes were found in both eudicots and monocots. The degenerate primers synthesized from R-genes helped to target the highly conserved domain of NBS domain. In this study, eight RGC primer pairs were used to detect the polymorphism in cassava genotypes includes *L6, I, RPS2, RPS5, RPP5, Xa-1, N, RGC2*. Among these primers, *L6, RPP5* and *N* were grouped in TNL R-genes and *I, RPS2, RPS5, Xa-1* and *RGC2* were belonged to CNL R-genes. Out of eight primer pairs used, four primers produced considerable variations between the selected

Table 3: Morphological traits used to evaluate $F_1$ mapping populations

| SL. No. | Morphological descriptor | Phenotypic classes | Time (MAP) |
|---|---|---|---|
| 1. | Colour of apical meristem | Light green, Dark green, Purple green and purple | 3 |
| 2. | Leaf retention | Scale 1- very poor retention, Scale 2 - less than average, Scale 3 – average, Scale 4 - better than average, Scale 5 - outstanding | 6 |
| 3. | Shape of central leaflet | Lanceolate and Ellipitical-lanceolate | 6 |
| 4. | Leaf colour | Light geen and dark green | 6 |
| 5. | Number of leaf lobes | 3 lobes, 5 lobes and 7 lobes | 6 |
| 6. | Petiole colour | Green, Greenish-red, Reddish-green and Pink | 6 |
| 7. | Flowering | Non flowering, Green, Green-pink, Cream/white, Green-purple and Pink | 6 |
| 8. | Colour of stem exterior | Silver, Orange, Light brown and greenish yellow | 9 |
| 9. | Colour of stem cortex | Light green and dark green | 9 |
| 10. | Shape of the plant | Compact and open | At harvest |
| 11. | Tuber skin colour | Dark brown, Light brown and Cream/white col-our | At harvest |
| 12. | Tuber rind colour | Pink, White, Cream and Yellow | At harvest |
| 13. | Tuber flesh colour | White, Cream and Yellow | At harvest |
| 14. | Primary branching | | At harvest |
| 15. | Secondary branching | Depends on the number of branches | At harvest |
| 16. | Tertiary branching | | At harvest |

cassava cultivars (*I, RPP5, RPS2* and *RPS5*) and the remaining four primer pairs did not show any sequence variations (*L6, Xa-1, RGC2* and *N*) (Figure 1 and Figure 2). The primer *I* were reported to resist *Fusarium oxysporum* sp *licopersicum, RPS2* and *RPS5* resists *Pseudomonas syringae* bacteria and *RPP5* against *Hyaloperonospora arabidopsidis*. A total of 60 SNPs were observed between two cassava cultivars *viz.,* MNga-1 and CI-732. Among these SNPs, 21 were showing transitions and 39 were transversions. The transistion-transversion ratio in each primer pair were calculated and found to be 9:18 in *RPP5*, 5:10 in *RPS5*, 5:10 in *I* and 2:1 in *RPS2*. In addition to SNP variations, InDels were also found between the genotypes amplified with primer *I* and primer *RPP5*. A total of four InDels

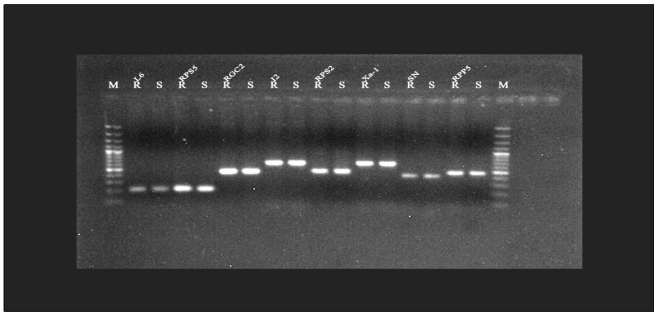



Figure 1: Amplifed products of RGC primer pairs in agarose gel

were identified two with primer I and two with primer *RPP5.* Similar study was carried out by Vásquez and Lopez (2012) and observed a total of 37 SNPs were identified by comparing the sequence of CM2177-2 and TMS 30572 constituted 48.6 % transitions and 45.9% transversions.

The amplified fragments of each genotype with primer pairs *viz., L6, I, RPS2, RPS5, RGC2 RPP5* and *Xa-1* were analyzed using BLASTN using pair-wise alignment against cassava genome in NCBI and Phytozome v12 and the resultant sequences from both cutivars were submitted in NCBI (MF984391, MF984392, MF984393) and DDBJ (LC322308, LC322309, LC322310, LC322311). All the eight sequences were identified as disease resistance gene RFL-related. Each sequence was individually analyzed in NCBI and found that most of the sequences belong to either *RPS2* or *RPP13* disease resistant gene (Table 4).

The presence of SNP variations or InDel variation in resitriction edonuclease site could be used as CAPS markers. The present study also made an attempt to identify CAPS markers using CAPS designer tools in SOL GENOME NETWORK and identified three CAPS markers computationally in cassava genotypes amplified with those primers which showed polymorphism between cassava genotypes (Table 5). CAPS markers for disease resistance were based on the principle that CAPS site should present in resistant genotype and absent in susceptible genotype. In this study, it was found three CAPS sites were observed in susceptible genotype and no CAPS in resistant genotype amplified with *RPP5* amplified sequences with remaining primers (*RPS2, RPS5* and *I*) produced CAPS sites in both resistant and susceptible genotypes.

Cassava being an out crossing crop, the possibility of occurrence of contaminants in controlled hybridization was

**PRIMER - I**

TACAAATATTTTCACCGCTGTTACATGTACCGA—GATGATGAGTTGACTC
CAACTATACTA(A/T)GGAAC(A/T)C(C/T)TAGT(C/T)CTTAACTAG
AGATTT(G/C)ACCAGGAAGCTT(-/A)C(A/C)TTTG(T/A)GGAGGTA
CAGAACTGCAAATCTCC(A/-)GTATGTGACTAAGGAATC(C/T)TAACT
GTACGTGAAATTCTACTCTCTGCTCTTTACATGTCTACAGACTAAAATAT
TTGTATATTGGCCATTGAATTTA(A/T)GAGAAAGA(A/G)CAAAGAACC
(G/A)TTCTCTC(T/A)ATGTTTAAAATTTGAAGTACATGCCTTCCTGCA
(A/T)T(C/G)TTGTA(T/A)AATGCTTCCCCTTTGAGCTGCTTGAGTTG
GAGTTGGAAGCCTGTTATTAAA

**PRIMER - RPS2**

TAGATTAAGG-GTGCAGTAAACTTCTTATTTTTACCATTTTTG—CTTCTG
-GT ATA(C/G)ACACAAGTACATTCCAG(A/G)AA(G/A)CT---AGCA
AAAACATG-GAGTCACCTCTGTTACAGAAACCATTATGAAGAGTTGAATC
CAACTTACAGTTA-AGATTCCTCAGTCACATACAGGTTAATGCAGTTCTG
TACCTCCTCAAATGTGAGCTTCCTGG—TGAAATCTCTAGTTAATGACTAA
GTGTTCCTTAGTATACTTGAAGTCATAACATCTGCTCTTTACATGTCTAT
GCACTAAAATATTTGTAAATTGGCCATTGAATTTCAGAGAAAGAACAAAG
AACCATTCTCTCTTATTTTTAGACTTGTATTACATGCATTCATGCGATGT
TGTATAATTCTTCCCCTTTGAGCTGCTTGAGTTGGAGTTGGAAG-CCTGTC

**Primer – RPS5**

GCTTTTCAGTTCTGTTTCCAAGATCTTGGGAGACGGTCTTGATTTTCATT
TGTGCAGTAGTTTCTGTTCTACAAATTTTTGAATTTGTCCGTTGTGTTCC
ATTTATTAAACCATAAAGCTTCTCATACTGTTTTGAAGGCAATTGTATTA
GTAATAA(G/T)GAG(C/G)ATGAC(C/G)ATTGTATTACTATTTTGACA
TTATGTT(A/G)TAC(A/T)G(A/G)A(A/G)TTATTA(A/T)CAAGAT
(A/G)TTCCC(A/C)TTTC(C/T)TCCC(A/C)TTACACTACCCAGGGAC
CCCTTA(T/G)GTC(T/G)GT(T/G)GTTCGATACTTAATGTTTCC
TTTTTCATGGGTCAGGGCCCCTTTTCCTCCCATT

**Primer – RPP5**

CGGCCCTTTCCAGGG-TGAGGCACCTCTCTTTGAGGCGCTTGCCTTAGA-
GA
AAA(-/A)GGCGCTAGGCGAGAGGTAGGCGAGGC(T/G)TCACTTGG(T/C)
GCA(T/A)TTGCTTTT(-/A)TTTTATTATTAACTTTTTTAACTTTCA(A/C)
GGG(T/A)TTTTAACTTAGAACCCCAAAGTTATAACGACAGCTAGGTATGT
CTCTCACTCTCCCCTTTCTCTCTTTCTTTTTCTTCTATGCCCGCTCC(C/T)
GTAGGCATTATACAATACTTTATTTTTTTTCTT(T/C)TCCCTTTTT(C/T)
CCCCTC(T/C)TCCCCCC(T/C)CCC(T/C)CCCAACACCAAACA(A/C)CC
TTTTTTTTATCT(T/A)CA(A/T)TTTTTTTTTA(T/A)TACTTTATGTA
(T/C)CC(T/G)TGTTGTTTT(A/T)T(T/G)GC(T/A)AATTTT(T/A)G
AAAAATTTTA(T/A)AAATTATA(G/T)AT(T/C)ACTTGC(T/A)AAT
(T/A)TATTTTA(A/T)ATAGGTTCTGATACTTTGACTCTACTTTTTCCCA
TACCACAACCGTGTCAACAA

Figure 2: Identification of SNP variations in cassava genotypes using RGC primer pairs

more. So the evaluation of the obtained seeds as true hybrid was an essential criterion for the establishment of true segregating population. The true hybrid analysis of $F_1$ mapping population was carried out using size specific polymorphic markers especially markers produced single alleles in parental lines. Seven single allelic size specific polymorphic markers (SSRY95, SSRY33, SSRY83, SSRY339, SSRY47, MeSSR10 and NS890) obtained from parental analysis were used in true

Table 4: Homology search of isolated sequences from cassava genotypes in NCBI and Phytozome V12

| Sl. No. | Primers used | Transition | Trans-version | Length | Search Homology | E- value | Accession numbers |
|---|---|---|---|---|---|---|---|
| 1. | I | 5 | 10 | 353 | Manihot esculenta disease resistance protein RPS2 | 8e-88 | MF984391, LC322308 |
| 2. | RPS2 | 2 | 1 | 425 | Manihot esculenta disease resistance protein RPS2 | 3.1e-39 | MF984392, LC322309 |
| 3. | RPS5 | 5 | 10 | 319 | Manihot esculenta probable disease resistance protein | 4e-120 | MF984393, LC322310 |
| 4. | RPP5 | 9 | 18 | 465 | NB-ARC domain (NB-ARC) // Leucine Rich Re-peat (LRR_3) // TIR domain | 1.8e-170 | LC322311 |
| | Total | 21 | 39 | | | | |

Table 5: CAPS markers identified in cassava genotypes amplified with RPP5 primer

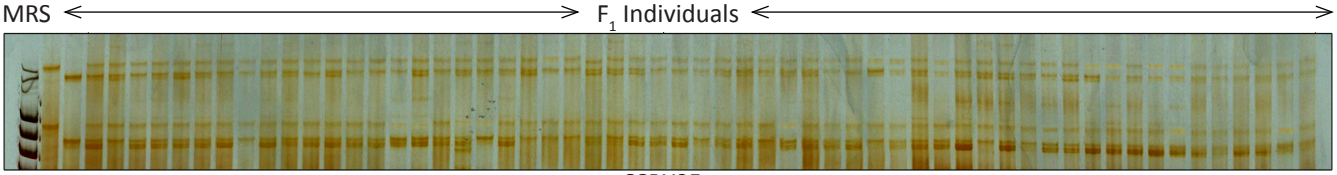
| Enzyme | Recognition sequences | R current site | S current site | CAPS site |
|---|---|---|---|---|
| BsaHI | G[A\|G]CG[C\|T]C | None (467) | 79 (79, 388) | R: gtaggcgaGGCTTCacttgg<br>S: gtaggcgaGGCGTCacttgg |
| MaeIII | GT.AC | None (467) | 80 (80, 387) | R: taggcgaggcTTCACttggt<br>S: taggcgaggcGTCACttggc |
| RsaI | GTAC | None (467) | 343 (343, 124) | R: tactttatGTATccttgttg<br>S: tactttatGTACccgtgttg |

hybrid evaluation. For heterozygous population, possession of alleles from both parents was considered as true hybrid. It was found that out of 130 seedlings obtained, only 114 $F_1$ progenies were identified as true hybrid and were used in further CMD mapping study (Figure 3 and Figure 4). The utility of these markers in true hybrid evaluation was evident from previous studies done in cassava by Otti et al. (2011) and Mohan et al. (2013). True hybrid validation at the seedling stage is one of the essential criteria for inter-specific hybrid production and also for clonal propagation. Such hybrids were essential to map genes controlling various economically important traits. Molecular markers based hybrid purity tests have been used in many crop species such as rice (Yashitola et al., 2002; Sundaram et al., 2008; Tamilkumar et al., 2009, Bora et al., 2016), maize (Asif et al., 2006; Salgado et al., 2006), cotton (Selvakumar et al., 2010) and safflower (Naresh et al., 2009), Eucalyptus (Subashini et al., 2014).

| Sl. No. | Parents | | $F_1$ Progeny | | | | SSR markers |
|---|---|---|---|---|---|---|---|
| 18 | R | S | $P_1$ ac | $P_2$ ad | $P_3$ bc | $P_4$ bd | |
| | | c — | | | c — | | SSRY83, SSRY339, NS890, SSRY95, SSRY33, SSRY47, MeSSRY10 |
| | b — | | | | b — | | |

Figure 3: Segregation pattern of single allelic markers in $F_1$ mapping population

The characterization of plant materials using morphological traits has being used to assess the genetic diversity of cassava (Sambatti et al., 2001; Raghu et al., 2007; Benesi et al., 2010; Asare et al., 2011), to determine the divergence among genotypes (Rimoldi et al., 2010), and to verify the correlation among agronomic traits. Based on the morphological characters, the entire $F_1$ genotypes and parents (MNga-1 and CI-732) were grouped into two main clusters. First cluster contained male parent, MNga-1 and 49 $F_1$ genotypes, of which KM-33, KM-1, KM-62, KM-84 and KM-133 were closer to MNga-1. Second cluster contained female parent, CI-732




SSRY95

Figure 4: Segregation pattern of SSRY95 in PAGE

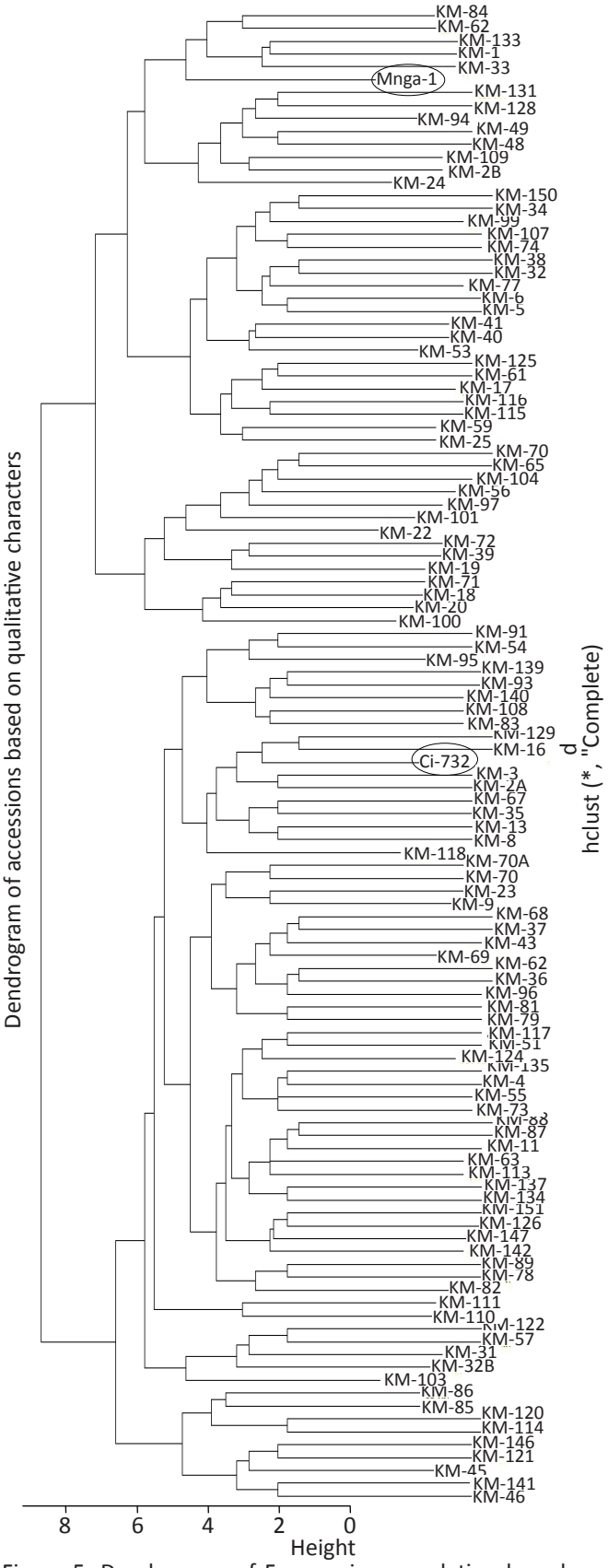



Figure 5: Dendrogram of $F_1$ mapping population based on qualitative characters

and 65 $F_1$ genotypes, of which KM-2A, KM-3, KM-87, KM-16 and KM-129 were closer to CI-732. From cluster analysis, it was also found that more than half of the $F_1$ progenies (65) acquired morphological characters from female parent indicating the influence of female parent (Figure 5).

## 4. Conclusion

The present study identify CAPS markers and that CAPS markers could be used to map $F_1$ mapping population developed from MNga-1 and CI-732 and thereby able to locate the regions associated with resistance genes in future. In this study true hybrid evaluation of $F_1$ mapping population was carried out to check the hybrid purity which is an essential criteria in inter-hybrid evaluation. Also the molecular characterization of cassava helped to understand the parental contribution in each characters of $F_1$ mapping population.

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