INTROGRESSION OF MOSAIC RESISTANCE IN POPULAR SHORT DURATION CASSAVA VARIETIES OF KERALA THROUGH MARKER ASSISTED SELECTION

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"INTROGRESSION OF MOSAIC RESISTANCE IN POPULAR SHORT DURATION CASSAVA VARIETIES OF KERALA THROUGH MARKER ASSISTED SELECTION"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"INTROGRESSION OF MOSAIC RESISTANCE IN POPULAR SHORT DURATION CASSAVA VARIETIES OF KERALA THROUGH MARKER ASSISTED SELECTION"** is a record of research work done independently by Mr. Darshan.S,under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Out of strain of doing into peace of work done....

DARSHAN .S

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LISTOF ABBREVIATIONS

%	-	per cent
µ	-	Mean
µl	-	Micro litre
µM	-	Micro molar
^{0}C	-	Degree Celsius
ANOVA	-	Analysis of Variance
bp	-	base pairs
cm	-	centimeter
CTAB,	-	CetylTrimethylammonium Bromide
d.f	-	degrees of freedom
EDTA	-	Ethyl diamino tetra acetic acid
et al.	-	and co-workers/co-authors
F_1	-	First filial generation
Fig.	-	Figure
g		gram
ha	-	hectare
i.e.	-	that is
ISSR	-	Inter-simple sequence repeats
kg	-	kilogram
m	-	meter
M	-	Molar
mg	-	milligram
min	-	minutes
Nacl	-	Sodium chloride
ng	-	Nanogram
PCR	-	polymerase chain reaction
PVP	-	Polyvinyl pyrollidone
rpm	-	revolutions per minute

S.E(m)	-	Standard Error Mean
SE	-	Standard Error
spp.	-	Species
SSR	-	simple sequence repeat
t	-	tons
v/v	-	Volume/ volume
viz.	-	namely

INTRODUCTION

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is commonly known as a perennial woody shrub, belonging to the family Euphorbiaceae and found to be native of South America (Allen, 2002). This crop was recorded as a vital storage root crop worldwide (Ceballos *et al.*, 2004; El-Sharkawy, 2012). It is having crucial components of the diet of over 800 million people across many continents (El-Sharkawy, 2012). Cassava is known to produce 90% of starch from its total storage root dry mass (Jansson *et al.*, 2009), and about 70 million people obtain more than 500 kcal day^{-1} from consuming storage roots of cassava (Kawano, 2003; Burns *et al.*, 2011). This crop is presently the world's fourth most important staple crop and carbohydrate rich food crop (El-Sharkawy, 2012) along with a worldwide production of 257 million tonnes (MT), of which about 146 MT drawn from Africa (FAO, 2012). The crop average yield in the worldwide was only 12– 13 tons per hectare. However, under favorable conditions the potential yield attained was almost seven times larger units (80 tons/ha; FAO, 2013). As per FAO statistics (FAOSTAT, 2015), cassava world production rose to more than 263 million tons in 2013 and has been increased in production of 27% during the last 10 years.

Cassava crop has got several agronomic characters that gave comparative advantages under adverse environments where the farmers frequently lack the resources to enhance the income-generating ability of their land from purchased inputs. This crop is hardy in nature and tolerates to marginal soils, periodic, extended droughts and biotic stresses (Calle *et al.*, 2005; El-Sharkawy, 2007). Since the crop is having very much flexibility in time of harvest, it is highly suited for intercropping with many other types of crops. This crop has a huge variety of food, feed and industrial uses (Westby, 2002; Tonukari, 2004; Jansson *et al.*, 2009). These important features make crop as a significant in producing the food, generating the income, and in particular, helping the poor in the tropical regions of the world (Henry and

Hershey, 2002). Owing to its tolerance for poor soils and hard climatic conditions, this crop is commonly grown by smallholder farmers like a subsistence crop under a varied range of agricultural and food systems of cropping (Alves, 2002). As its storage roots may be harvested as and when needed, the crop can be classified as a classical food security crop (DeVries and Toenniessen, 2001).

All the parts of cassava plant can be much utilised, but its starchy storage roots are comparatively most common used plant part (Ceballos *et al.*, 2004; Ojulong *et al.*, 2007). The storage roots of crop are consisting of abundant source of carbohydrates (Westby, 2002; Baguma, 2004; Jansson *et al.*, 2009; El-Sharkawy, 2012) in terms of starch (31% of fresh mass), with lesser amounts of free sugars (less than 1% of fresh mass). The storage roots of Cassava also possess a maximum content of riboflavin, thiamine, dietary fibre, sodium, magnesium, citrate and nicotinic acid (Bradbury and Holloway, 1988). They are having low level of 0.53% protein and high level of 1.5% ash (Westby, 2002). In Africa, the leafy parts of crop are frequently used as a vegetable and are the cheap source of vitamins A, B and C, proteins, and other mineral nutrients (Fregene *et al.*, 2000; IITA, 2001 Benesi, 2005).

Among all the disease concerned, cassava mosaic disease (CMD) caused by the Indian cassava mosaic virus (ICMV) or the Sri Lankan cassava mosaic virus (SLCMV) is the major problem affecting crop tuber yield in regions of Kerala. The cassava mosaic virus causes withering of the leaves and limiting the root growth of cassava plant. A major famine was occurred in areas of Africa during 1920's due to an outbreak of this virus. The virus is spread by the whitefly and also through the use of infected planting material. Continuous vegetative propagation resulted in very high virus load and led to clonal deterioration of many varieties. Cassava virus was found to be infected for a number of indigenous cultivars namely Kalikalan, Ariyan and Burmah. The discovery of the host plant resistance, polygenic / monogenic dominant,

is helpful in the development of resistant varieties using a combination of conventional and marker assisted breeding methods.

Early storage root yield (SRY) is an important character that farmers are presently concentrating due to its importance in giving quick food, income and also in escaping late season drought effects, attack of pests and diseases (Suja *et al.,* 2009; Tumuhimbise *et al.*, 2012). The most important characters of cultivars of cassava that the farmers are preferring for high storage root yield, dry mass content, earliness and resistance for pests and diseases (Tumuhimbise *et al.,* 2012). Efforts to develop early-bulking cassava varieties are in a weak position due to absence of the distinct trait indicators in the Cassava.

Based upon these considerations, the proposed investigation was planned to achieve the objective; Introgression of cassava mosaic disease resistance to short duration varieties of cassava through marker assisted selection (MAS) and to study the inheritance of early bulking nature.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. GENERAL INTRODUCTION

Cassava is an important and staple food crop for over 800 million people in areas of sub-Saharan Africa, South America and Asia. More than half of the world's cassava is produced in areas of Africa and it comprises a major source of calories for about 40% of the whole population. The crop is highly efficient in producing the carbohydrates and adapting to an extensive range of environments.

2.2 ORIGIN AND BOTANY OF CASSAVA

2.2.1 The origin and distribution of cassava

The genetical, geographical and agricultural basis of Cassava has been originated from Latin America. Allen (2002) recorded that its domestication began 5000 - 7000 years BC in Amazon and Brazil regions and was distributed from Europeans to the rest of the world (Henry & Hershey, 2002). The crop was taken from Brazil to the West coast of Africa by Portuguese navigators in the sixteenth century (Jones, 1959 and Nweke, 1994). In the eighteenth century, Cassava was brought to East Africa by the Portuguese from Cape Verde and into Mozambique from Zanzibar Island (Leitao, 1970). In the late eighteenth and early ninetieth centuries, it was introduced in to the majority of Asia and the Pacific regions. Cassava production was started in India, Indonesia (Java) and Philippines by European explorers who had procured it from South America (Onwueme, 2002).

Cassava was introduced into India from Brazil during the 17th century by the Portuguese while they landed in the regions of Malabar, currently part of Kerala state. The popularization of the crop in Kerala was credited to the renowned king of Travancore State, Sri. Visakham Thirunal by introducing popular varieties from Malaya and other places. India is one among the major Asian countries growing

cassava and it obtained importance in the global cassava scenario owing to world's higher productivity. Cassava cultivating area, production and productivity of India is 2, 28, 300 ha, 81, 39,400 MT and 35.7 t/ha respectively (NHB, database 2013-14).

2.2.2 Cytology and Taxonomy of Cassava

Cassava is known as "manioc" in French, "yuca" in Spanish and in Portuguese, "mandioca". The crop has been classified under the class Dicotyledoneae, family Euphorbiaceae, tribe Manihoteae, genera Manihot Tournefort and species *Manihot esculenta* Crantz (Alves, 2002). The genus *Manihot,* consists of about 100 species, among which cassava, *Manihot esculenta* Crantz, is widely distributed in the tropics and subtropics and the only species commercially cultivated as a food crop (Fauquet and Fargette, 1990 and Alves, 2002). A small number of other *Manihot* species contained minor uses, especially as another sources of latex for production of rubber (*M. glaziovii* and *M. caerulescens*) (Franche *et al.,* 1991). Cultivated cassava belongs to the *Fructicosae* and is regarded as a cultigen unknown in the wild (Rogers and Appan, 1973). The chromosomes number of all species of *Manihot* have 2n = 36 and can be considered as allopolyploid (n = 18), although the species studied have usual bivalent pairing and perform as diploids species (Jennings, 1976).

Jennings and Iglesias (2002) reported that the crop was placed in the *Fruticosae* section of the genus *Manihot*, which was a member of the Euphorbiaceae family and they also stated that the *Fruticosae* section contained low-growing shrubs which were adapted to savannah, grassland or desert and was considered as less primitive than *Arboreae* section of Euphorbiaceae, which contained tree species.

The Cassava plants can also be classified based on their morphological characters and cyanogenic glycosides content. Based on cyanogenic glycoside, cassava cultivars are classified into bitter and sweet. Onwueme (1982) opined that

care should be taken while using the glycosides levels as a distinguishing trait for cassava cultivars because the exact level of glycosides in a particular cultivar would vary as per the environmental conditions under which the plant is cultivated. The glycoside content of a cultivar might be high or low depending on the conditions. Sweet cultivars were known to have a short growing season with their storage roots maturing early (Nassar and Ortiz, 2006; Amenorpe *et al.*, 2007).

2.2.3 Plant morphology and growth of Cassava

The crop is a dicotyledonous shrub, with intermediate growth habit, and possessing a wide spectrum of variability (Mandal, 1993). It is a perennial crop, but farmers commonly harvest it during the first or second year. Onwueme (1978) reported that abandoned stands of cassava continued to grow for many years and most frequently associated with other crops (Hershey, 1993).

It is propagated mainly from stem cuttings. However, under natural conditions, as well as in plant breeding, propagation by seed is quite common. Plant establishment is considerably slower when propagated by seed. Osiru *et al.*, (1996) considered that the seedlings were genetically segregate into different types, a few weeks after emergence or sprouting, the shoot lengthened and the root extended downwards and they also reported that the plant shoot system consistsed of the leaves, stem, inflorescence and the root system consisted of feeder roots and storage roots.

2.2.4 Crop Ecology

It was considered as a tropical crop and distributed between the latitudes of 30^0 North and 30^0 South (Alves, 2002 and Costa and Silva, 1992). The congenial crop growth temperature ranged from 24 to 30^0C (IITA, 1990). Alves (2002) recorded that the crop could be grown in the semi-arid tropics with an annual rainfall less than 600 mm, but with the ideal rainfall of 1000 to 1500 mm per year, sandy to light soils

where storage roots could develop easily and also documented that crop can also tolerated with low soil p^H.

2.3 ECONOMIC IMPORTANCE OF CASSAVA

Nweke *et al.*, (2002) reported that the crop played an important role in development of Africa as it act as a famine-reserve crop, rural staple food, cash crop for both rural and urban households and to a minor extent, raw material for feed and chemical industries. The crop was a major part of the diet for nearly a billion people in approximately 105 countries mostly in sub-Saharan Africa, Asia, the pacific and South America (FAO, 2008) and the crop was the 3rd most important source of calories in the tropics after rice and maize (FAO, 2010).

2.3.2 Uses and nutritional composition of cassava

Industrial uses of cassava starch include the production of adhesive and glue for use in paper and in the production of ethyl alcohol (Asiedu *et al.*, 1989). Cassava exports account for less than 0.1% of the total production, in form of meal or more generally, pellets account for 10-15% of world production. The bulk of world trade in dried cassava is conducted between Thailand (with annual exports of 13.6 million tonnes) and Europe, which imports about 12.2 million tons annually (Silvestre, 1989).

2.3.3 Chemical and nutritional composition of cassava roots

The roots and leaves of the crop were used for human consumption and animal feed (Dahniya, 1994 and Buitrago, 1990). The general chemical composition of roots and leaves is shown in Table 1.

Table. 1 Chemical composition of roots and leaves of cassava (Buitrago, 1990)

Nutrient	Storage root		Leaves	
	Fresh weight basis (%)	Dry weight basis (%)	Fresh weight basis (%)	Dry weight basis (%)
Dry matter	35.00	100.00	28.00.	100.00
Starch	30.21	85.10	16.23	39.00
Crude protein	1.10	3.10	6.80	24.00
Fat	0.47	1.30	1.50	6.50
Crude fiber	1.10	3.10	5.80	20.60
Ash	0.70	1.90	1.70	6.20
Calcium	0.10	0.33	0.43	1.50
Phosphorus	0.15	0.44	0.08	0.27

2.4 MORPHOLOGICAL CHARACTERIZATION

Rogers and Appan (1973) reported that classical breeding approaches utilized the recorded morphological traits of plants which were grown in the field as bases for identification and they have been effectively used as a powerful tool in the classification of cultivars and the study of their taxonomic position.

Stegemann (1984) stated that most of the plant descriptors were ambiguous and have limited usage. Kochert (1994) opined that breeders and geneticists used many morphological characteristics in leaves and flowers to follow segregation of genes and hybrids. However, most agronomic characters were not associated with easily observable phenotypic markers and such traits were controlled by multiple genes and were subjected to the varying degrees of environmental modifications and interactions.

Reports from several studies indicated the use of markers that were not environmentally influenced and phenotypic markers still played an important role in classical plant breeding and complement the use of molecular tools. However, It was used in the identification of specific markers linked to traits of interest and QTL (Kizito *et al.*, 2007; Akano *et al.*, 2002; Mkumbira *et al.*, 2002; Okogbenin *et al.*, 2002 and Fregene *et al.*, 2000).

The crop grown to a height of 1 to 4 m and its morphological characteristics were highly varied, indicated to a high degree of interspecific hybridisation (Alves, 2002; Chavarriaga and Halsey, 2005). The crop was normally established with the use of stem cuttings. However, in breeding programmes, its propagation in the first cycle is by sexual seeds (Fukuda, *et al.*, 2002; Alves, 2002). Cuttings, which were used as propagation materials, roots grown first and the buds grown later into stems (Alves, 2002; Fukuda, *et al.*, 2002; El-Sharkawy, 2003).

Izumi *et al.*, (1999) named as root bulking for the formation and growth of storage roots which showed secondary thickening. Root bulking resulted from increased number of cells due to cell division, proliferation and their accumulation of starch content. Storage root weight increased by the accumulation of photosynthates and root size increased by increase in cell number and cell size (Alves, 2002; El-Sharkawy, 2003; Ravi *et al.*, 2009). So, storage root bulking depended on the sink strength, the potential of leaves to export photosynthates and on the photosynthetic efficiency of leaves (Keutgen *et al.*, 2001).

Storage root bulking process involved secondary growth by the origin of a circular primary vascular cambium in addition to several irregular circular cambia in the sub-apical region of roots (Doku, 1969; Indira and Sinha, 1970; Hunt *et al.*, 1977; Izumi *et al.*, 1999; El-Sharkawy, 2003).

Several studies had different opinion on the time when thickened roots appeared in cassava during its growth and development (Doku, 1969; Wholey and Cock, 1974; Izumi *et al.*, 1999; Okogbenin and Fregene, 2002). For example, Izumi *et al.* (1999) showed that root bulking began about 3 months after planting (MAP) but rapid starch deposition does not occurred before 6 MAP. Doku (1969) concluded that root bulking of most genotypes began in the second month and produced reasonable fresh storage root yields by 6 MAP. Wholey and Cock (1974) concluded that thickened roots were present after 2 MAP and root bulking was increased with time

but, the rate of bulking differed between cultivars. They also confirmed that after three months, the number of thickened roots per plant remained quite constant for all cultivars except for one in which the thickened root number increased with time.

Wholey and Cock (1974), based on the fresh storage root mass (FSRM) accumulated by different genotypes at different times concluded that earliness was related to early onset of bulking, rapid bulking, or a combination of both factors. Comparable findings on accumulation of different amounts of FSRM at different times of harvest by different cultivars have been recorded in another place indicating existence of early bulking genotypes (Chang-Ho *et al.*, 2005, Kamau, 2006; Amenorpe *et al.*, 2007; Mtunda, 2009; Okogbenin *et al.*, 2013).

The study conducted by Suja *et al.* (2009) showed that high and low yielding cultivars of cassava differed in their bulking rate and the period at which they exhibited highest bulking rate. They also pointed that short duration cultivars recorded maximum bulking rate during their early growing stage.

Wholey and Cock (1974) indicated that SRN was generally determined early in the crop growth cycle even though some cultivars appeared to continue in producing new storage roots up to 7 MAP. Similarly, Ekanayake *et al.* (1997) reported that the storage root number (SRN) in cassava crop was assayed during the first 3 MAP.

Yield is defined as mass of produce harvested from a single crop plant or the quantity of produce harvested per unit of land area. In cassava, it is obtained in terms of marketable storage root yield along with leaves, stems or even seeds (Hershey, 2012).

2.5 COMBINING ABILITY AND HETEROSIS

General combining ability (GCA) evaluated additive portion of the genetic effects (Falconer and Mackay 1996; Sleper and Poehlman, 2006). Sleper and Poehlman (2006) confirmed that GCA of parental lines was the average contribution that made to the hybrid performance in a series of hybrid combinations in comparison to the contribution of other parental lines to hybrid performance in the same series of hybrid combinations.

Specific combining ability (SCA) was the contribution of a parental line to hybrid performance in a cross with another parental line, in relation to its contributions in crosses with an array of specified parental lines. It also evaluated the non-additive gene action and was utilized to identify the cross combinations with superior performance (Falconer and Mackay, 1996; Sleper and Poehlman, 2006).

Assay of inheritance of agronomic characters in cassava by utilising combining ability had gained importance with many of this work carried out by CIAT and by the national research institutes of countries in the world where cassava consumed as a staple crop and it related to study carried out by (Perez *et al.*, 2005; Jaramillo *et al.*, 2005; Calle *et al.*, 2005; Cach *et al.*, 2006; Kamau, 2006; Munga, 2008; Owolade *et al.*, 2009; Mtunda, 2009; Zacarias and Labuschagne, 2010; Kulembeka *et al.*, 2012 and Parkes *et al.*, 2013).

2.6 GENE ACTION

In cassava, gene action and gene effects had been broadly studied. The gene action is an important criterion in evaluating the type of cultivar, breeding methods to develop cultivars and in the interpretation of quantitative genetic experiments. Through GCA and SCA effects the gene action could be studied (Griffing, 1956; Sprague, 1966; Falconer and Mackay, 1996; Brown and Caligari, 2009). In populations, the additive vs. dominant gene action could be represented by GCA and

SCA. Additive, dominance, epistatic, and over-dominance were the four different types of gene action (Sleper and Poehlman, 2006; Acquaah, 2009).

2.7 CASSAVA BREEDING

In agricultural research, the plant breeding gained the maximum rates of return amongst the investments from which Cassava has also been profited. In most of the Cassava breeding projects, the important breeding objective was to produce high and stable fresh storage roots. The study was also confirmed that the crop productivity played a major role in industrial uses of cassava for starch, animal feed or for bio-ethanol, while stability of production was basic in the places where cassava was the main survival crop. (Sleper and Poehlman, 2006, Kawano, 2003; Ceballos *et al.*, 2004; 2012)

Conventional breeding in Cassava involved selection of superior parental genotypes for the interested characters, hybridizing them and conducting multi-stage offspring selections (Kawano *et al.*, 1998; Jennings and Iglesias, 2002; Ceballos *et al.*, 2004). Fukuda *et al.* (2002) confirmed that breeding methods in cassava were basically confirmed by the method of its reproduction, genetic variability and breeding objectives.

According to Ojulong *et al.* (2008) the high frequencies of genes for specific desirable characteristics, included with yield parameters, storage root quality, pests and disease resistance, tolerance to adverse soil and climatic conditions and ability to produce in the different environmental conditions were increasingly accomodated through recurrent selection. Dixon *et al.* (2008) confirmed that the F^1 generation resulted from each recombination cycle were studied and selections were recombined to develop a new population. They also opined that a period of 8 - 10 years required for the development of an improved cultivars.

2.8.1 Cassava selection cycle

The cassava selection cycle took five to six years from the seed germination to the last regional trial stage where several locations were integrated (Jennings and Iglesias, 2002; Ceballos *et al.*, 2004).

Table 2: Illustration of estimation and selection stages used in the International Center for Tropical Agriculture (CIAT) for cassava breeding programme as per Ceballos *et al.* (2012)

Time (months)	Stage of the crop	Plants per plot	Repetitions	Locations	Genotypes evaluated
18	Crossing blocks	-	-	-.	
19 - 30	F_1	1	1	1	2500
31 - 42	Clonal evaluation trial (CET)	6 - 8	1	1	1500-3000
43- 54	Preliminary yield trial (PYT)	10	3	1	100-300
55 - 56	Advanced yield trial (AYT)	20	3	1 - 2	75-150
67 - 78	Regional trial (RT) - I	25	3	2 - 6	20-40
79 - 90	Regional trial (RT) - II	25	3	5 - 10	20-40

2.8.2 Breeding and selection for early bulking

Reports by Kamau (2006), Mtunda (2009), Chikoti (2011), Tumuhimbise *et al.* (2012) and Bassey and Gamaliel (2013) stated that there was an increased trend in developing early bulking cassava in relation to increased demand for early bulking cultivars from farming community. Sleper and Poehlman (2006) studied the comparative maturity of cultivars and reported that they were expressed in different ways, some of the common ones were time period for heading in small grains and silking in maize.

So far Physiologists did not recognized particular stages of development in cassava, as they were commonly defined for crops where seeds were common product. In most of the plant's life, leaves and storage roots were developed

concurrently as a result there were no clear shoot characters to demonstrate when cassava plants started accumulating the starch in roots, apart from harvesting the storage roots (CIAT, 1972; Kawano, 1987).

Indirect methods were carried in the absence of direct plant shoot characters to follow in selection of early bulking. Kawano (1987) utilized the FSRY as the key factor for estimating early bulking. Hershey (2012) experimented on early bulking at CIAT and concluded that genotypes with the highest FSRY at an early harvest time were also tend to be highest yielding genotypes at later stages and that therefore.

Similar studies were conducted by Okogbenin *et al.* (2013) and reported that productivity at 12 MAP could be used as a key component to screen for early bulking since early fresh storage root yielders were the high yielders at later stages of FSRY assessment. Okogbenin and Fregene (2002) investigated the characters associated with early bulking and they found that dry storage root yield significantly correlated with starch initiation time, storage root diameter, plant height, harvest index, dry foliage mass, number of storage roots and plant vigour. Hence they confirmed that those factors were components that triggered early yield as a complex character.

At the initial stage of root bulking, storage root diameter appeared to be the key factor while harvest index and foliage considered as the most prominent factors at the later stage of storage root development during the evaluation time and the same has been reported by Kawano (1990), Ojulong *et al.* (2010) and Hershey (2012).

2.9 MOLECULAR MARKERS

Many DNA based markers revealed that polymorphism at DNA level had been developed for measuring similarity in agricultural crops and proved to be essential in the evaluation of genetic variation within population and between population and to confirm the genetic relationships among adapted crop cultivars for the required trait of interest (Lee, 1995; Karp *et al.,* 1996 and Kumar *et al.,* 2000).

2.9.1 The Polymorphism technique

Saiki *et al.* (1988) expressed that Polymorphism Chain response (PCR) known for the reason for developing the scope of new systems for the examination of the genome in light of the specific intensification of genomic DNA pieces. The utilization of PCR with short oligonucleotide preliminaries of self-assertive (arbitrary) arrangement to produce markers and Williams *et al.* (1990) detailed the premise of RAPD. Essentially, Welsh and McClelland (1990) considered on discretionary prepared polymerase chain response (AP-PCR) though, DAF announced by Caetano-Anolles *et al.* (1991). Hu and Quiros (1991) opined that he presentation of the PCR strategy revolutionarised standard sub-atomic procedures and allowed for the production of new apparatuses for distinguishing DNA polymorphism.

Points of interest connected to molecular markers made a broad commitment to hereditary qualities and reproducing of cassava and furthermore in the improvement of genetic maps (Fregene *et al.*,1997; Mba *et al.*, 2001 and Okogbenin *et al.*, 2006), genetic diversity assessment (Beeching *et al.*, 1993; Lefevre and Charrier, 1993; Bonierbale *et al.*, 1997; Mignouna and Dixon, 1997 and Fregene *et al.*, 2000) and studies on taxonomy (Second *et al.*, 1997).

According to Thottappilly *et al.* (2000), molecular markers were grouped into four types which were firstly, Hybridization based DNA markers namely RFLP and oligonucleotide fingerprinting. Secondly, Polymerase chain reaction (PCR) based DNA markers; RAPDs, which they have also been converted into SCARs, simple sequence repeats or microsatellites (SSRs), sequence-tagged sites (STS), amplified fragment length polymorphisms (AFLPs), inter simple sequence repeats amplification (ISA), cleaved amplified polymorphic sequences (CAPs) and amplicon length polymorphism (ALPs) thirdly, DNA chip and sequencing based DNA markers like single nucleotide polymorphisms (SNPs) and lastly, derivatives group included

Microsatellite primed polymerase chain reaction (MP-PCR), arbitrarily primed PCR (AP-PCR), allele specific PCR (AS-PCR) and DNA amplification fingerprinting (DAF) were proven to be useful in the detection of polymorphism.

2.9.1.1 Simple sequence repeats (SSR) or Microsatellite

They are generally tandem repeats of short (2 - 6) sequences like (GT) or (TA,CA,GTG,TAA,GATA). Hamada *et al.* (1982) verified the huge number and widespread occurrence of short tandem repeats in eukaryotic genomes. These results were confirmed by Tautz and Renz (1984).

For classification of cassava varieties, the SSR technique was used (Mkumbira *et al.*, 2001 and Peroni *et al.*, 2007). Vieira *et al.* (2007) used for the evaluation of genetic distances and for construction of genetic maps by Okogbenin *et al.* (2007).

2.10 VARIABILITY STUDIES

Crop improvement programme mainly depends on the magnitude of genetic variability and the extent to which the desirable characters are heritable in the crop concerned. The influence of environment in expression of economic traits taken into consideration. No doubt that the efficiency of selection depends mainly on the extent of genetic variability present in the population. However, studies of Burton (1952) suggested that co-efficient of variability together with the heritability estimate will give good picture of amount of genetic advance to be expected from selection.

2.10.1 Morphological variability

Among the 64 clones screened, Vijaya and Jos, (1981) observed that 44 clones did not show any pubescence on apical leaves. 15 clones showed scattered pubescence, while 5 accessions had dense pubescence.

Jos *et al.* (1987) reported that the colour of apical leaves varies from green to purple and frequently the purple colour was found interspersed with green. The green types are more among the indigenous clones.

Raghu *et al.* (2007) studied 58 cassava genotypes and observed wide range of genetic variation for young stem colour (green, yellowish green, dark green, green with red, purple colour and green with red ribs) flower bud colour, (green coloured buds, buds with coloured base and green scale and buds with green base and coloured scale and fully coloured buds) colour of young leaves (green, light green and purple), shape of the leaf lobe (normal shape, wide and rounded with two widening points), colour of petiole (green, yellowish green, vivid red, purple red and purple, outer skin colour of the tuber (grey, brown skin) tuber inner skin colour (rose and white) and pulp colour (white and yellow pulp).

Richardson, (2011) observed wide range of variation in the characteristics of three cassava varieties. He reported that the varieties "Blue Mountain" and "Cuban white stick" have green-purple colour of first fully expanded leaves, whereas "John LaMottehad" had dark green colour of fully expanded leaves and the colour of apical leaves of all the three varieties showed green-purple colour. He characterized 'John LaMotte' as a white fleshed tuber with a dark brown storage root surface colour with cream coloured root cortex.

The study was conducted by Mamba-Mbayi *et al.* (2014) to determine genetic relatedness and morpho-agronomic differentiation among Congolese cassava collections at two different locations and reported that the morphological and agronomic traits were highly variable among the accessions. There was variability for leaf, petiole, and stem color as well as for lobe shape and leaf petiole orientation. Roots were cylindrical, conical - cylindrical, or irregular.

Babu Rao *et al.* (2015) characterized 18 genotypes of cassava for morphological variability and reported high variation among genotypes.

2.10.2 Quantitative variability

Fokunang *et al.* (2000) evaluated 36 cassava genotypes and found significant differences in yield components like plant stand, tuber number, fresh root weight and dry matter of tuber among the genotypes. The highest mean number of tubers per plot was observed on genotypes 30572 and 91/00418 (73.0 and 67.3, respectively) whereas the least tuber number was observed on genotype 91/00431 (15.5). Tuber yield was recorded highest on genotypes 91/00430 and 91/00435 (57.1 and 45.7 kg/plot, respectively). The lowest mean tuber weight of 2.6 kg/plot was recorded on genotype 88/02555. Maximum and minimum tuber dry matter percentage was recorded for the genotypes 91/00434 and 91/00458 with a value of 36.0 % & 24.2 % respectively.

Pillai *et al.* (2000) conducted an experiment to identify genetic stocks for different economic characters in 73 accessions of cassava germplasm and reported that the population showed wide variability for all the 8 characters studied. Tuber yield per plant, harvest index, cyanogenic content and starch content showed significant differences among all genotypes.

Surya kumari and Anuradha (2000) conducted a study on 17 indigenous cultivars of cassava and reported that the genotypes significantly differed for the characters like height of the plant, leaves number, stem girth, tuber length, tubers plant^{-1} and yield of the tuber.

Chitiyo and Kasele (2004) evaluated 18 newly released cassava varieties at 2 different regions of Zimbabwe and found that root yield was significantly varied and they also concluded that the variety 91/02822 recorded the highest number of roots with maximum dry matter content (46.0%).

Chavez *et al.* (2005) recorded high variation for root dry matter content in cassava with a range of 10.7 to 57.2%.

Raghu *et al.* (2007) evaluated 53 cassava accessions with 29 different morphological traits and reported that cassava accessions exhibited greater degree of genetic variation for both quantitative and qualitative traits. Among the nine quantitative traits studied by them, tuber length, tuber girth, number of tubers plant^{-1} and tuber yield plant^{-1} were found to possess high level of variability in germplasm.

Sankaran *et al.* (2008) evaluated 8 high yielding cassava varieties under upland conditions of Tripura, and reported that the plant height (3.9 m) number of nodes (110.33), petiole length (35.33 cm) and leaf length (56.00 cm) were found to be more in H -1687.

Sheela *et al.* (2008) conducted a study on variation in crude protein, dry matter and starch content in inbred and backcross lines of cassava and reported that the clones exhibited high variation for crude protein in the tubers with a range of 1.11 to 10.40% and 0.37 to 2.74% on dry weight and fresh weight basis respectively. They observed a high variation in root dry matter (7.57 - 49.60%) and starch content (4.01 - 42.00%) among the inbred lines of cassava clones.

Odedina *et al.* (2009) conducted an experiment on variability studies in growth parameters of 10 cassava varieties and reported that the important characters like height of the plant, internodes length, number of nodes 25 cm^{-1} cutting and leaf area showed significant variability.

Aina *et al.* (2010) conducted a study on effect of G×E interactions on crop yield and yield components of land races and improved genotypes and reported that high variability was observed for the number of roots harvested and fresh root yield.

Ntawuruhunga and Dixon (2010) conducted a study to investigate inter relationship between factors that control cassava yield and significant differences were observed for all yield traits.

Adeniji *et al.* (2011) studied genetic relationship and selection criterion for root yield of 19 improved cassava clones and found significant differences for plant height, length of petiole, root width, root length, stem girth, number of leaves per plant, number of tubers per plant, number of tubers per plant, height of first branching, per cent dry matter and per cent starch.

Bhagath *et al.* (2011) conducted a field experiment to identify the short duration, high yielding variety suitable for Konkan conditions. Among the cassava varieties studied, H-119 produced highest mean tuber yield (34.59 t/ha) with 35.2 % starch and its duration is 225 to 240 days.

Nageswari and Palaniswamy (2011) evaluated 15 cassava genotypes and observed significant differences among the genotypes with respect to tuber length, tuber girth, tuber yield, starch content and cassava mosaic virus incidence.

A study was carried out by Mulualem and Ayenew (2012) on thirty five accessions of Cassava at Jimma agricultural research centre to examine the relationship between yield characters and their contribution of fresh root yield. They observed highly significant differences between accessions for fresh root yield and its component characters *viz.,* plant height, stem girth, number of roots per plant, length of root, root diameter, fresh weight of root and root dry weight.

Mamba -Mbayi *et al.* (2014) observed significant genotype x location interactions for storage root yield. The maximum storage root yield plant^{-1} was recorded in the genetically improved variety "Mbankana" (10.11 kg) at first location (Mpiana) and the variety Zizila recorded highest storage root yield (21.08 kg) at second location (INERA) while the local variety Luenyi was recorded the lowest storage root yield at both the locations 1.87 kg & 3.37 kg, respectively.

Kundy *et al.* (2015) examined 12 genotypes of cassava for the assessment of genetic variability of root yield and its components at 3 different locations on 6 important characters like number of branches plant^{-1}, plant height, shoot weight root number, root weight, and root dry matter and they also statedted that genotype ×

location interaction was found to be significant for all the characters that were studied indicated significant effect of the environment on the expression of the characters.

Adjebeng-Danquah *et al.* (2016) evaluated the agronomic performance from a collection of local and exotic cassava genotypes and indicated that for all the growth and yield characters measured, there was a great significant effect by genotype.

Patrick *et al.* (2016) experimented on 16 Cassava genotypes which were comprised introductions, local landraces and improved genotypes examined for 2 seasons to study the response of cassava genotypes to CMD and agronomic characters showed that there was a significant differences among the genotypes across the seasons for the biotic and agronomic related traits.

2.11 PCV, GCV, HERITABILITY AND GENETIC ADVANCE

Genotype and environment influenced the ideal phenotype of an individual in which it grows. Success of a breeder in changing and improving the heredity of a character depends upon the degree of correspondence between phenotypic and genotypic values. Heritability is a measure that provides this information (Dabholkar, 1992). Heritability in broad sense or degree of genetic determination is amount of total hereditary variance to phenotypic variance. The more useful estimate i.e. narrow sense heritability or degree of resemblance between relatives is ratio of additive genetic variance to phenotypic variance (Falconer, 1989).

Heritable variation can be determined with greater accuracy, when heritability is studied along with genetic advance (Swarup and Chaughale, 1962). High heritability with high genetic gain is associated with additive gene effects (Panse, 1957). On the contrary, non-additive gene effect (dominance or epistasis) is associated with characters exhibiting high heritability and low genetic advance.

Naskar *et al.* (1991) observed high GCV for all the characters ranged from (24.14 % to 98.36 %) among 91 genotypes of Cassava. They reported that plant

height (59.57 %), stem diameter (24.14 %), Petiole length (98.36 %), number of tubers (73.22 %), girth of the tuber (73.80 %) and tuber yield (70.99 %) showed high GCV. Their heritability estimates varied from 68.87 – 95.12 %. Mahungu *et al.* (1984) observed moderate heritability (50.0 %) for root yield.

Surya kumari and Anuradha (2000) conducted a study on 17 genotypes of Cassava and found that GCV was ranged from 12.8 - 44.5 %. In their studies plant height (21.2 %) and number of branches (44.5 %) were recorded high GCV whereas number of leaves (13.8 %), stem girth (13.4 %), number of tubers per plant (16.35 %), tuber yield (13.9 %) and tuber length (12.8 %) recorded moderate GCV. They also recorded high heritability coupled with high genetic advance as percent of mean for the characters *viz.*, plant height (98.03 % and 43.7 %), number of leaves (99.80 % and 28.30 %), number of branches (72.70 % and 90.80 %), stem girth (84.90 % and 28.3 %), tuber length (96.20 % and 26.3 %) , tubers per plant (92.40 % and 33.50 %) and tuber yield (97.80 % and 28.80 %) .

Studies conducted on 6 genotypes of cassava by Ntui *et al.* (2006) revealed that the phenotypic coefficient of variation were higher than the genotypic coefficient of variation for all the traits studied.

Aina *et al.* (2007) reported that high PCV and GCV were recorded for shoot weight (36.46 % and 22.57 %) followed by tuber yield (20.20 % and 16.08 %) and number of roots per plant (16.65 % and 14.42 %) while low PCV and GCV were observed for number of nodes 4.27 % and 3.17 % respectively. High heritability was recorded for plant height (73.20 %), number of roots (75.00 %), yield per hectare (63.34 %) and root dry matter (79.83 %) and low heritability was observed for height of first branching (29.86 %) among 20 genotypes of Cassava.

Nageswari and Palaniswamy (2007) observed high PCV (52.85 %) and GCV (38.19 %) for yield per hectare followed by CMV incidence (41.52 %and 41.08 %) respectively , whereas starch content recorded low PCV (11.84 %) and number of tubers per plant was recorded low GCV (8.53 %). They also reported that the heritability estimates of 15 genotypes ranged from 13.22 % - 97.89 % and CMV

incidence (97.89 %), tuber girth (96.20 %), tuber length (92.94 %), and starch content (70.86 %) recorded high heritability, while number of tubers per plant recorded low heritability (13.22 %).

Akinwale *et al.* (2010) evaluated 43 cassava genotypes and reported that the magnitude of phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) in all the characters in his evaluation. Cassava Mosaic disease severity was recorded the maximum GCV and PCV (38.52 % and 42.10 %) respectively. Heritability estimates of his study ranges from 25.0 to 95.0 %.

Ntawuruhunga and Dixon (2010) conducted a study on 10 cassava genotypes and observed high PCV and GCV for fresh storage root yield per plant (31.4 % and 23.1 %) followed by root size (28.1 % and 18.6 %), storage root number (26.8 % and 16.9 %) and moderate PCV and GCV were observed for petiole length (19.9 % and 18.1 %) and leaf area (19.1 % and 14.7 %) where as low PCV and GCV were observed for dry matter content (4.3 % and 3.2 %). Their heritability estimates (h^2) were high for petiole length (81.1 %) and moderate for leaf area (59.2 %) followed by dry matter content (56.1 %), fresh storage root yield plant^{-1} (43.5 %) and number of storage root (39.9 %).

Adeniji *et al.* (2011) conducted a study on 12 cassava genotypes and found that estimates of phenotypic variation were greater in magnitude than their corresponding genotypic variance. They also observed high heritability estimates for plant height (92 %), petiole length (95 %), root length (82 %), stem girth (88 %), tubers per plant (97 %), plant dry matter (99 %), dry matter yield (99 %), percent starch (97 %), number of leaves (98 %) and height of first branching (95 %).

Babu Rao *et al.* (2016) evaluated 18 cassava genotypes and reported that high magnitude of PCV and GCV were recorded for number of leaves per plant (40.86 & 40.63), total leaf area (57.09 & 55.94), height of first branching (26.94 & 26.87), HCN content (51.26 & 39.28). High heritability estimated more than 60 per cent

along with high estimates from genetic gain as more than twenty percent ninety nine mean leaves, 96.01 of total leaf area, 99.45cm of first branching height, 98.03cm stem diameter, tuber dry matter content (100), starch content (99.00) and tuber yield hectare^{-1} (82.50) revealed that the heritability was mainly due to influence of additive gene effect.

Adjebeng-Danquah *et al.* (2016) observed phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) for all the traits. The lowest PCV (13.1 %) was observed for root dry matter content whereas cassava mosaic disease (CMD) severity showed the highest (73.48 %).

2.12 CORRELATION STUDIES

Here, generally more than one trait is measured on progenies estimated either for a specific character in cyclical selection programmes or in applied breeding programmes which required a combination of characters to be satisfied the growers. It was measured by a correlation coefficient and very crucial for plant breeding. The reason for correlation could be either genetic or environmental. Pleiotropism and/or linkage disequilibrium were attributed to the genetic causes. While genes were not closely linked, linkage disequilibrium was not a vital cause of correlation between characters in random mating populations. Under such circumstances, the origin of genetic correlations was probably due to pleiotropism (Hallauer and Miranda, 1982).

Sreekumari and Abraham (1991) carried out an experiment on correlation studies on shade and open grown cassava and reported that the significant correlations were observed for all the characters. In shade grown cassava, there was a significant positive correlation with the plant height and total leaves (0.519) similarly, for stem girth with tuber yield (0.866), tuber girth with tuber yield (0.666). There was a significant positive correlation of total leaves, tuber length and tuber girth with tuber yield (0.654), (0.580) and (0.547) respectively in open grown Cassava.

Rubaihayo *et al.* (2001) reported on the positive direct effect of number of roots per plant on tuber yield of Cassava.

Sankaran *et al.* (2008) reported on the plant height, number of internodes $stem^{-1}$ and stem diameter, length of petiole, leaf area and leaf length had positive correlation with tuber yield while number of branches and number of tubers per plant has negative correlation with tuber yield and also there was inverse relationship between the length of internodes and tuber yield.

Sheela *et al.* (2008) observed highly significant and positive correlation between dry matter percentage and starch in the clonal populations. Crude protein content of cassava had high significant negative correlations of with dry matter and starch content.

Padma *et al.* (2009) carried out correlation studies on twelve Cassava germplasm lines at Horticulture Research Station, Pandirimamidi, Andhra Pradesh and revealed that tuber yield was positively and significantly correlated with plant height, tuber weight and tuber width

Akinwale *et al.* (2010) studied the phenotypic and genotypic correlation coefficients for eight agronomic traits across locations and found that genotypic correlation coefficients were higher than their corresponding phenotypic correlation coefficients. They concluded that root number was positive and significantly correlated with root weight, shoot weight, cassava mosaic disease and sprouting and root weight had positive correlation with root number and sprouting.

Ntawuruhunga and Dixon (2010) conducted correlation studies in cassava and reported that there was a significant positive correlation with plant height, root number, root diameter, stem diameter, dry matter content and negatively correlated with leaf area.

Character associations studied by Aina *et al.* (2010) revealed that significant positive correlation with fresh root yield and number of roots, root weight, harvest index, shoot weight and cyanogenic potential .They also said that number of roots had positive correlation with root weight, cyanogenic potential and harvest index.

Adeniji *et al.* (2011) revealed that width of leaf had positive correlation with root yield however CMD had a negative correlation with root yield. They also observed negative and significant correlation between percent of starch and tuber yield.

Babu Rao *et al.* (2015) reported that number of leaves per plant, total leaf area, stem diameter, tuber diameter, plant dry matter content, starch content, HCN content had significant and positive association with tuber yield hectare^{-1} at both phenotypic and genotypic levels.

Djirabaye *et al.* (2016) observed highly significant and positive correlations between leaflets characters and the weight of fresh roots was positively correlated to root characters. Harvesting index was positively correlated with fresh root weight while negatively for fresh aboveground biomass.

Patrick *et al.* (2016) conducted correlation studies in 16 cassava genotypes and reported that fresh root yield was significantly and positively correlated with total fresh biomass (0.93), harvest index (0.29) leaf retention (0.23), root size (0.26) similarly for harvest index and root size (0.13).

2.13 CASSAVA MOSAIC DISEASE (CMD)

Cassava mosaic disease (CMD) was first time reported in 1894 from Tanzania and was later noticed in many other African countries. Currently the disease known to occurred in all cassava-growing countries of Africa and adjacent islands which formed the main constriction to produce the Cassava on this continent. Losses attributed to susceptible varieties was 88% and for tolerant verities, 50%. (Edison, 2002; Obonyo *et al.*, 2007). Jose *et al.* (2011) reported the yield loss of 10-90% due to the attack of disease.

Calvert and Thresh (2002) documented that disease incidence was ranged from 21% to 84% in thirteen African countries and highest incidence was occurred in Kenya.

Hillocks and Thresh (2000) reported the disease incidence in India and Sri Lanka and Edison (2002) stated that the disease was slowly reached in the cassava growing states then caused losses as like in Africa. Calvert and Thresh (2002) stated that the overall incidence of CMD was highest in Kerala (23%) and Tamil Nadu (30%) where Cassava was largely grown in India and however, the disease was also appeared in Andhra Pradesh (<1%) and Karnataka (5%).

Hillocks and Thresh (2000) described the symptoms of CMD as chlorotic areas were intermixed with normal green tissues and formed a mosaic pattern then caused leaf distortion, defoliation and resulted in severe stunting of the plant. In severe cases, leaves are reduced in size, twisted, and deformed. Edison (2002) mentioned that the symptoms were masked under hot dry period and made the diseased plants impossible to identify and the disease was spread by infected planting materials, grafting and sucking insect like whitefly.

CMD caused by a virus which belonged to Begomoviridae. East African cassava mosaic virus of Uganda (EACMV-Ug) was known to be a virulent recombinant strain that was associated with the severe CMD pandemic in Africa during 1990s (Edison, 2002; Obonyo *et al.*, 2007) and they have also documented the African cassava mosaic virus (ACMV), Indian cassava mosaic virus (ICMV) South African cassava mosaic virus (SACMV) and East African cassava mosaic virus (EACMV).

Ariyo *et al.* (2005) conducted a study in Nigeria on molecular variability and the distribution of CMV and estimated that ACMV as the dominant virus formed 80 % of all the samples and EACMCV was detected in single and mixed infections (2 and 18%) respectively with the ACMV.

Alabi *et al.* (2008) considered that ACMV and East African cassava mosaic Cameroon virus (EACMCV) was detected by multiplex PCR. Busogoro *et al.* (2008) investigated that a multiple infections with begomoviruses in the same plant led to the evolution of important viral species.

2.14 MOLECULAR CHARACTERIZATION FOR CMD

The main source of resistance to the CMD was known to be polygenic which required assessment in different environmental conditions to distinguish resistant genotypes. Currently, few landraces were identified which exhibited high levels of resistance to CMD. Molecular markers associated with resistance to CMD were identified in resistant landrace. Fregene *et al.* (1997) confirmed that with the utilization of single marker analysis (SMA), 4 CMD resistance markers were found out namely SSRY28, SSRY235, SSRY44 and NS136. SSRY28 and SSRY235 were placed on linkage group G and SSRY44 and NS136 on linkage group P of cassava genetic map.

Akano *et al.* (2002) utilised landraces for the identification of SSR marker which was linked to a dominant CMD-resistance gene and so far CMD-resistance genes like CMD1 and CMD2 were placed on the map (Akano *et al.*, 2002; Fregene *et al.*, 2001).

Lokko *et al.* (2005) used Marker-trait associated detection technique and revealed that the marker estimated for 57.41% of total phenotypic variation for resistance and they also stated that another SSRY106-207 and E-ACC/M-CTC-225 estimated for 35.59 and 22.5 % respectively for resistance.

Kunkeaw *et al.* (2009) opined that the genetic linkage map used for genetic studies in cassava especially for the identification of genetic markers which were linked to interested characters. Melaku Gedil *et al.* (2010) reported that SSR markers, NS158 and SSRY28 and SCAR marker, RME-1 were earlier linked to a CMD2 gene.

As per Okogbenin *et al.* (2012) the molecular markers for CMD2 gene were utilized for the introgression of CMD resistance into Latin American cassava genotypes and the innovation of a new QTL (CMD3) for CMD resistance in TMS 97/2205 offered new chances for pyramiding CMD genes for increased stability of CMD resistance in plant.

Olasanmi *et al.*, (2014) reported 9 SSR markers *viz.*, SSRY 106, (ESTs) SSRY 292, SSRY 239, (ESTs)SSRY 7, NS 194, (ESTs)SSRY 47, SSRY 63, SSRY 250, and NS 323 and were closely linked to early bulking (EB) in six of nine hybrid populations then 7 of the markers with ten per cent or more coefficient of determination (R2) were supposed to be linked to major quantitative trait loci which was found to be associated with EB in cassava. Parkes *et al.* (2015) stated that multiple marker analysis was utilized to screen the *CMD2* gene.

MATERIAL AND METHODS

3. MATERIALS AND METHODS

The present study is entitled with "Introgression of mosaic disease resistance in to popular short duration cassava varieties of Kerala through marker assisted breeding" with objective of introgression of cassava mosaic disease resistance to short duration varieties of cassava through marker assisted selection and to study the inheritance of early bulking nature. The materials utilized and the methodologies adopted in the present study are described below.

3.1 GENERAL DESCRIPTION

3.1.1 Experimental Site

The experiment was conducted in Division of Crop Improvement, ICAR - CTCRI, Sreekariyam, Trivandrum, Kerala during the period 2014- 2017.

3.1.2. Experimental Location

The experimental site was located at 8° 29′ N latitude, 76° 57′ E longitude, at an altitude of 64 m above mean sea level. Dominating soil of the test site was all around depleted well-drained acid Ultisol with p^H 4.35.

The experiment was conducted in four different experiments.

Experiment I: Raising of pollination block and hybrid seed production

Experiment II: Screening of seedlings for resistance and early bulking nature.

a) Field evaluation
b) Marker aided selection of CMD resistant clones

Experiment III: Evaluation of early bulking clones with resistance to CMD

Experiment IV: Bulk segregant analysis

3.2 EXPERIMENT I: RAISING OF POLLINATION BLOCK AND HYBRID SEED PRODUCTION

3.2.1 Materials

The experimental materials comprised of Five early bulking high yielding lines *viz,* Sree Jaya, Sree Vijaya, Vellayani Hraswa, CI 889 and 9S 75 and three testers *viz,* CR 54A3, IMS2-5 and CI 273 with resistance to cassava mosaic disease (Table 1).

3.2.2 Hybridization Programme

Hybridization and selection type of breeding method was employed; the parental crosses were made by adopting line x tester (LxT) analysis (Kempthorne, 1957) to produce 15 F_1 combinations (Table 4). The plants with male and female blooms which are available on the same stretched panicle, with female blossoms at the base and male blossoms toward the tip had been chosen. Female flowers are selected and bagged in the evening (4.00-5.00 pm) hours of the previous day with the pollination bags (red colour) and next day morning (10.00-12.00am) male flowers, which are more numerous are selected and cross was made by hand emasculation and the inflorescence was covered with white pollination bag and labeled it to have a clear picture of flower selection for the further emasculation. The pollination bags were retained till the fruit formation and maturity. The fruits after maturation were collected and the seeds were extracted manually from all the 15 crosses separately. The extracted seeds were kept labeled separately for the further experiments.

3.3 EXPERIMENT II: SCREENING OF SEEDLINGS FOR RESISTANCE AND EARLY BULKING NATURE.

The seeds were sown in the poly bags and maintained proper care of watering and weeding under shade net in the nursery for 40 days and the healthy seedlings were transplanted to the main field.

Plate 1. View of pollination block established to produce hybrids

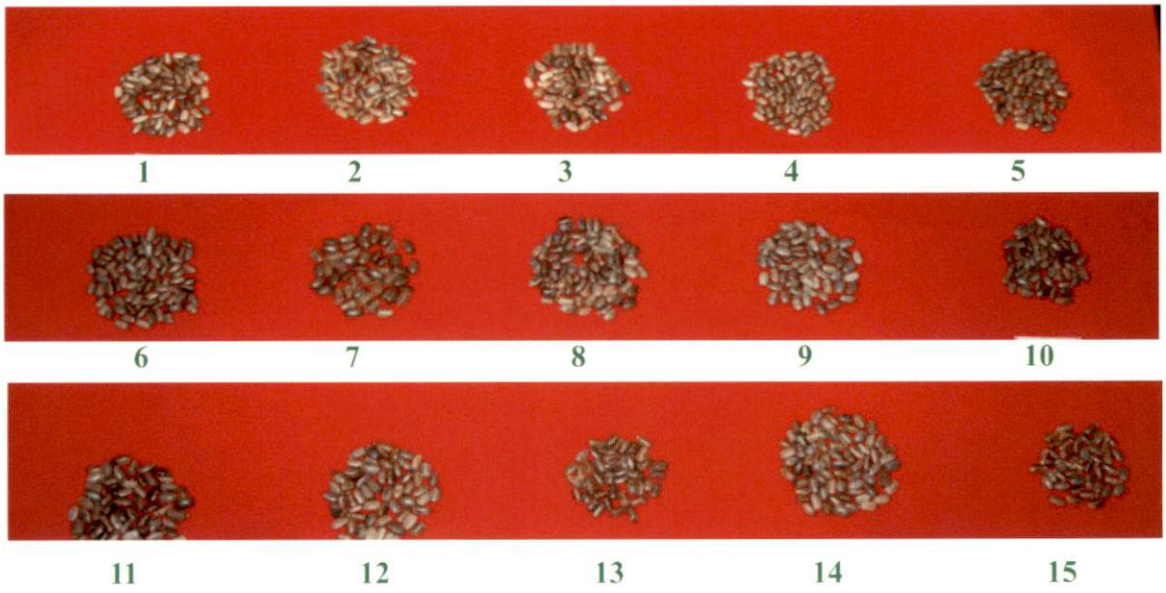
Table 3. List of parents and their details used in the experiment.

No.	Name	Source	Traits
LINES			
L1	Sree Jaya	CTCRI	Early maturing(6-7 months), Yield (26-30 t ha^{-1})
L2	Sree Vijaya	-do-	Early maturing (6-7 months), Yield (25-28 t ha^{-1})
L3	Vellayani Hraswa	KAU	Early maturing(5-6 months), Short plant stature, high branching; high yield (40-45 t ha^{-1})
L4	CI 889	CTCRI	Early maturing (6-7 months), Yield (20-25 t ha^{-1})
L5	9S 75	-do-	Early maturing (6-7 months), Yield (20-25 t ha^{-1})
TESTERS			
T1	CR 54 A3	-do-	CMD resistant
T2	IMS2 -5	-do-	CMD resistant
T3	CI 273	-do-	CMD resistant

The selected parents were planted in a pollination block and crossed to produce fifteen F_1 combinations.

Table 4. Details of F_1 combinations

No.	Crosses	
1	Sree Jaya x CR 54 A3	L1xT1
2	Sree Jaya x IMS2 -5	L1xT2
3	Sree Jaya x CI 273	L1xT3
4	Sree Vijaya x CR54 A3	L2xT1
5	Sree Vijaya x IMS2-5	L2xT2
6	Sree Vijaya x CI 273	L2xT3
7	Vellayani Hraswa x CR 54 A3	L3xT1
8	Vellayani Hraswa x IMS2 -5	L3xT2
9	Vellayani Hraswa x CI 273	L3xT3
10	CI 889 x CR54 A3	L4xT1
11	CI 889 x IMS2-5	L4xT2
12	CI 889 x CI 273	L4xT3
13	9S75 x CR54 A3	L5xT1
14	9S75 x IMS2- 5	L5xT2
15	9S75 x CI 273	L5xT3



1-Sree Jaya X CR 54 A3, **2**- Sree Jaya X IMS2-5, **3**- Sree Jaya X CI 273, **4**- Sree Vijaya X CR 54 A3
5- Sree Vijaya X IMS2-5, **6**- Sree Vijaya X CI 273, **7**- V. Hraswa X CR 54 A3, **8**- V. Hraswa X IMS2-5
9- V. Hraswa X CI 273, **10**- CI 889 X CR 54 A3, **11**- CI 889 X IMS2-5, **12**- CI 889 X CI 273,
13- 9S 75 X CR 54 A3, **14**- 9S 75X IMS2-5, **15**- 9S 75X CI 273

Plate 2. Hybrid Seeds of All 15 Crosses Produced

Plate 3. Field View of Experiment II

3.3.1 Field Evaluation

3.3.1.1 Materials

The experimental materials comprised of hybrids along with the parents. The list of parental lines and F_1 hybrids were given in table 3 & 4.

3.3.1.2 Methods

3.3.1.2.1 Design and Layout

The trial was spread out in a randomized block design with 23 replication (15 F_1s and 8 parents) with three replications. Transplanting and other agronomic practices were done as per KAU, 2011. Forty days old seedlings were transplanted into the fundamental field at a separating of 90 x 90 cm. The mound method of planting along with the drip irrigation was used. FYM at 12.5 t ha−1 was connected at the season of planting, N: P: K at 100: 50: 100 kg ha−1. The entire measurement of P2O5 and a large portion of the dosages of N and K were connected after 30 DAT. Following multi month the rest of the amounts of N and K were connected alongside weeding and earthing up.

3.3.1.2.2 Biometric Observations

All the observations were recorded from average of 5 plants in each replication.

3.3.1.2.2.1 Plant Height (cm)

It was measured in centimetres from the base of the stem to tip of the growing point with the measuring scale.

3.3.1.2.2.2 Stem Girth (cm)

Girth of the stem was measured with a tape at mid portion of the stem and expressed in centimetres.

3.3.1.2.2.3 Number of Tubers plant^{-1}

The number of storage tubers in the plant was counted and expressed as number of tubers plant^{-1}.

3.3.1.2.2.4 Mean Weight of tuber (kg)

The average weight of the total number of storage tubers in the plant was measured and expressed in kilograms.

3.3.1.2.2.5 Length of tuber (cm)

The length of the storage tubers was measured with the scale and the mean was expressed in centimeter.

3.3.1.2.2.6 Girth of tuber (cm)

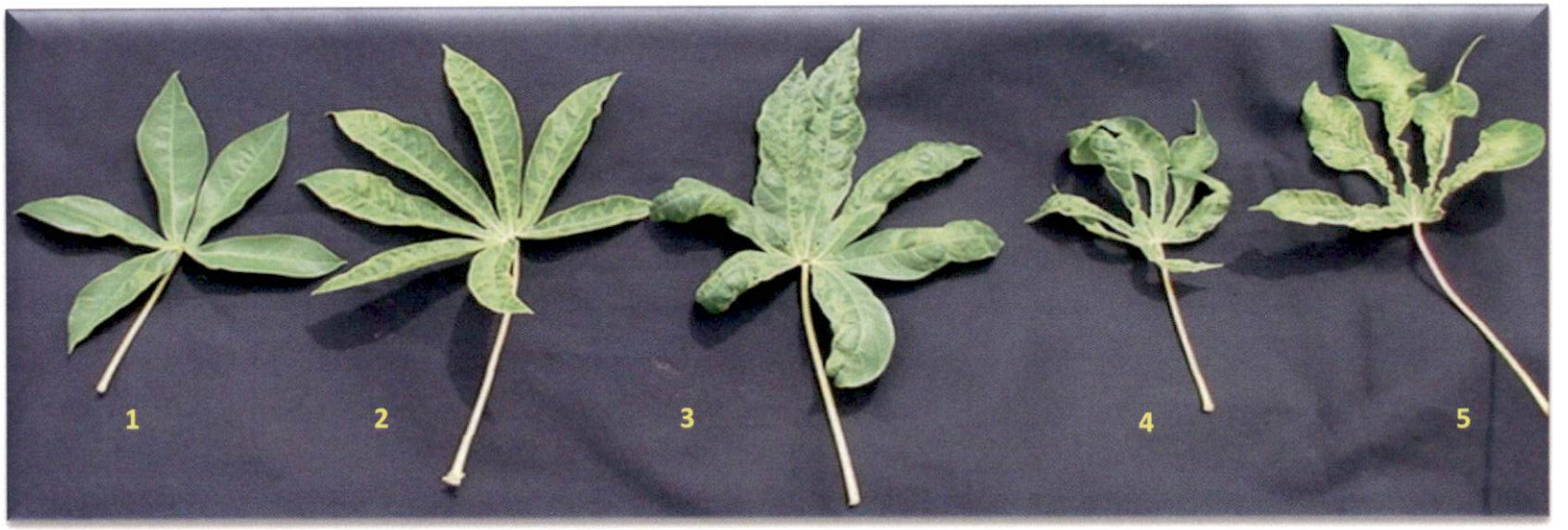
The maximum diameter of the tuber was measured at middle part of the tuber for 5 tubers per plant with the twine and scale and expressed in centimetres.

3.3.1.2.2.7 Tuber Yield (kg plant^{-1})

The total number of storage tubers in the plant was weighed and expressed as tuber yield in kilogram per plant.

3.3.1.2.2.8 CMD disease scoring (1-5 scale)

Different grades of CMD infection were scores from 1 to 5 scales (Hahn, 1980) (Plate 4).



Grade 1 – no symptom

Grade 2 – mild chlorosis mild distortions at bases of most leaves

Grade 3 – pronounced mosaic pattern on most leaves, narrowing and distortion of the lower one-third of the leaflets

Grade 4 – severe mosaic distortion of two thirds of most leaves and general reduction of leaf size and stunting of shoots

Grade 5- very severe mosaic symptoms on all leaves, distortion, twisting, misshapen and severe leaf reduction of most leaves accompanied by severe stunting of plants.

Plate 4. CMD Disease Scoring (1-5 Scale) Chart

3.3.1.2.3 Statistical Analysis

The data collected on the agronomic traits were subjected to statistical analysis and the different statistical parameters were worked out.

3.3.1.2.3.1 Analysis of Variance (ANOVA)

Analysis of variance was carried out separately for each character as per RBD design.

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Replications	t-1	SSR	MSR	MSR/MSE
Treatment	r-1	SST	MST	MST/MSE
Error	(r-1)(t-1)	SSE	MSE	
Total	rt-1			

Where,

r = number of replications

t = number of treatments

MSR=mean squares for replication

SSR=sum of squares for replications

MST=mean squares for treatments

SSR=sum of squares for treatments

MSE=mean squares for error

SSR=sum of squares for error

$$\text{Critical difference (CD)} = t\alpha \sqrt{\frac{2 \times MSE}{r}}$$

Where,

$t\alpha$ is the table value of students' t distribution at error degrees of freedom and α is the level of significance (5 % or 1%), (Panse and Sukhatme, 1985)

3.3.1.2.3.2 Correlation

The correlation co-efficient among all possible traits combinations at phenotypic, r_{xy} (p) and genotypic, r_{xy} (g) level were estimated employing formula.

$$\text{Phenotypic correlation} = r_{xy}(p) = \frac{Cov_{xy}(p)}{\sqrt{V_x(p) \times V_y(p)}}$$

$$\text{Genotypic correlation} = r_{xy}(g) = \frac{Cov_{xy}(g)}{\sqrt{V_x(g) \times V_y(g)}}$$

Where,

$Cov_{xy}(G)$	=	Genotypic covariance between x and y
$Cov_{xy}(P)$	=	Phenotypic covariance between x and y
$V_x(G)$	=	Genotypic variance of character 'x'
$V_x(P)$	=	Phenotypic variance of character 'x'
$V_y(G)$	=	Genotypic variance of character 'y'
$V_y(P)$	=	Phenotypic variance of character 'y'

The test of significance for association between characters was done by comparing table 'r' values at n-2 error degrees of freedom for phenotypic and genotypic correlations with estimated values, respectively.

3.3.2 Marker aided selection of CMD resistant clones.

The seedlings without CMD visual symptoms were subjected to multiplex PCR and real time quantitative estimation to study the presence of virus in field tolerant plants. The resistances of the selected clones were further verified by using SSR markers linked to CMD resistance.

3.3.2.1 Isolation of Total Genomic DNA

DNA was extracted from fresh and tender young leaves of cassava using the CTAB (Cetyl Trimethyl Ammonium Bromide) method.

3.3.2.1.1 Procedure of CTAB Method

Tender leaf samples were collected from the field, 100 mg samples weighed and homogenized to a fine powder using liquid nitrogen. Pre-warmed CTAB extraction buffer (2ml) was added to the samples and ground once more. The samples were transferred to 2 ml microfuge tubes and vortexed. The samples were incubated at 65 ℃ for 30 minutes with frequent swirling in water bath. Samples were centrifuged at 12000 rpm for 10 minutes at 4 ℃ and the supernatant was collected to a fresh microfuge tube. Equal volume of Chloroform: Isoamyl alcohol (24:1) was added and mixed by gentle inversion. Again samples were centrifuged at 12000 rpm for 10 minutes at 4 ℃. The supernatant was collected to a fresh microfuge tube and 5 µl of RNase were added and incubated at 37 ℃ for 1 hour. After incubation, equal volume of chloroform: Isoamyl alcohol (24:1) was added into the sample. Samples were centrifuged at 12000 rpm for 10 minutes at 4 ℃. Supernatant was collected in fresh microfuge tube and to the collected supernatant added 0.8 volume propanol and centrifuged at 12000 rpm for 10 minutes at 4 ℃. Supernatant was discarded and saved the pellet. The DNA pellet was washed with 70% ethanol. Air dried the pellet until the ethanol evaporated and dissolved in appropriate amount of 1x TE buffer (100-150 µl). All samples were checked for DNA in 1% agarose gel and confirmed.

3.3.2.2 Analysis of the Extracted DNA

3.3.2.2.1 Agarose Gel Electrophoresis

Agarose gel electrophoresis is used for separating and analysing DNA molecules in which DNA molecules were separated based on the charge by giving an electric field to the electrophoretic apparatus. The gel can be used to look at the DNA

in order to quantify the amount of DNA or to isolate a particular band of interest. By the addition of Ethidium Bromide, the intercalating agent along with agarose solution, the DNA can be visualized when exposed on UV. 1% agarose gel was used to check the quality and integrity of the extracted DNA. 1% agarose solution was prepared by weighing out 1 gm agarose in a conical flask and dissolving it using 1X TBE buffer. Agarose was dissolved by heating and after that the flask was allow`ed to cool and when the temperature of the flask became bearable, about 1.5 µl of Ethidium Bromide (EtBr) was added directly to the molten gel and gentle mixing was done. Casting tray was prepared with combs so that gel was poured into the tray and allowed to solidify. 6 µl of isolated sample mixed with 2 µl of 1X loading dye was loaded into the wells of prepared gel.

Horizontal gel electrophoresis unit was used to run the gel. The gel was run for about 30 minutes at 80V. The run was stopped after the dye front reached 3/4th of the gel. Then it was visualized under ultraviolet light using a gel documentation system.

3.3.2.2.2. Quantification of DNA

The Thermo Scientific NanoDrop^{TM} ND- 1000 spectrophotometer was used to quantify the isolated DNA. It helped to assess the yield and purity with high accuracy and reproducibility. TE buffer in which DNA was dissolved was used to calibrate the machine to blank *i.e.,* zero absorbance. The advantage of Nanodrop is that it requires only 1 µl sample to measure its quantity and quality. The quantity of DNA was determined at OD 260 and the purity was determined by OD 260/OD 280 ratio. According to the better absorbance value/ OD value samples were selected.

3.3.2.2.3 Dilution of Samples

Samples were diluted to 10 ng/ µl concentration using nuclease free water.

3.3.2.3 Multiplex PCR For Virus Detection.

To distinguish between ICMV and SLCMV, PCR amplification was carried out using designed primers to specifically amplify ICMV fragment of about 900bp and SLCMV fragment of about 600bp of DNA A.

The primers used were:

1. **MULTI_IC_A_F:**(5'-GCT GAT TCC GC ATT TGT AN-3')
2. **MULTI_SL_A_F:**(5'-TGT AAT TCT CAA AAG TTA GAG TCN-3')
3. **MULTI_IC_SL_A_R:** (5'-ATA TGG ACC ACA TCG TGT TGT CN-3')

The amplification was performed in a total volume of 20µl PCR mix containing Emerald master mix: 10 µl, primer1: 0.5 µl, primer2: 0.5 µl, primer3: 0.5 µl, SDW: 3.5 µl, DNA: 5.0 µl.

The PCR was carried out in Veriflex PCR. The cycling for 30 cycles program was:

Initial temperature	: 94°C (2 minutes)
Denaturation	: 94°C (1 minute)
Annealing	: 55°C (1 minute)
Extension	: 72°C (2 minutes)
Final extension	: 72°C (5 minutes)

The whole programme is of 30 cycles and the holding temperature is at 4°C.

The PCR products were electrophoresed in 2% agarose at 75V in TBE (Tris Borate EDTA) buffer of pH 8. Gene ruler 100bp plus DNA ladder was used as standard. The gel was observed and photographed using UV Transilluminator gel documentation system, Alpha Imager.

3.3.2.4 Real Time quantitative estimation to study the presence of virus in field tolerant plants.

The real time PCR assay (q-PCR) was done as per the method described by Deepthi (2017), which as follows:

Primers used for qPCR

For absolute quantification of DNA A was done using primers in qPCR.

Sy-SLCMV A_ F (5′ TTCATCCATCCATATCTT 3′),

Sy-SLCMV A _R (5′ CCATATAGGTAAGGTCAT 3′).

All primers were tested using DNA extracted from cassava leaves affected with SLCMV and total DNA from non-infected plants as negative controls.

PCR Set Up

Working solutions of primers, DNA samples from plants and plasmid dilutions were stored at -20°C and not thawed more than twice prior to use. Master mixes and water were kept in small aliquots at 4°C. To avoid DNA contamination and carry over, DNA extraction, preparation of master mixes and set up of PCR was done in separate rooms. All buffers and solutions for qPCR were pipetted using sterile filter tips and composed on ice to avoid errors from evaporation during pipetting. For each sample, triplicate qPCR reactions were composed in 96-well microplate formats and microplates were sealed immediately after loading. Samples, standards, non-template controls (NTC) and negative water controls were run on each plate to minimize variations between plates.

Standard real time PCR mix (25 µl)

Maxima® SYBR Green qPCR Master Mix (2X)	12.5 µl
Forward Primer	0.3 µM
Reverse Primer	0.3 µM
Template DNA	≤100µg
Water, nuclease-free	to 25 µl
Total volume	25 µl

Real time PCR assays for sensitive detection and quantitation of SLCMV DNA in cassava using SYBR green.

A Sybr Green qPCR assay for SLCMV was established and conducted during the growing season to quantify the viral DNA. Absolute quantification of DNA A and DNA B of SLCMV was done using primers Sy-SLCMV A_ F, Sy-SLCMV A _R. For each DNA sample, three technical replicate reactions were prepared. A reaction master mix was prepared by adding 12.5 µl of Maxima® SYBR Green qPCR Master Mix (2X), 0.3 µM forward and reverse primer each, ≤100ng of template DNA and nuclease free sterile water to a volume of 25 µl. Cycling parameters were as follows: 1 cycle at 95°C for 3 min (DNA polymerase activation), and 40 cycles, each consisting of 95°C for 15 seconds (denaturation) 53°C for 20 seconds(annealing) and 72°C for 20 seconds (extension). All real time PCR assays were performed in an Eppendorf realplex Mastercycler (Eppendorf, Germany).

To determine copy numbers of DNA-A molecules the qPCR was calibrated. Standard curves were prepared with plasmids harbouring a full-length copy of DNA-A component of SLCMV in pDrive. The molecular weight of each plasmid was determined from plasmid and vector sizes and plasmid DNA dilutions were prepared to generate standard curves from which copy numbers of the genomic components could be calculated. The conversion from mass to molecules was done assuming an average molecular weight of a deoxyribonucleotide (330 kDa) and the number of base pairs in the DNA using the formula:

(X g/µl DNA / [plasmid length in basepairs x 660]) x Avogadro's number x 10^{-12}).

The calculation of the standard curves was done using the Eppendorf realplex software (version 2.2) using the CT values obtained from qPCR as a function of the amount (copies) of nucleic acid target.

The standard curve chart displays the following information:

Slope	Increase in standard curve
Y-Intercept	Point at which standard curve intersects with Y-axis (based on amount = 1 [unit])
Efficiency	Efficiency of PCR , E= 10[-1/slope]-1
Where a slope (S)	-3.3 (E=1) represents 100% efficiency
R^2	Correlation coefficient of standard curve

For absolute quantification, the number of DNA A molecules was determined from standard curves generated from plasmid containing DNA A genome components. The standard curves reproduced a linear relationship between the C_T value and the amount of total input DNA. Thus from the fitted line of regression, the target concentrations in the samples (genome copies) were interpolated from the appropriate standard curve. The amount of virus was also calculated by a linear regression analysis of the C_T value of each sample over the log of the total DNA concentration.

Statistical analysis

Results from qPCR were investigated as for standard bends and measure target fixation in tests. Duplicates of tests were examined to figure standard deviation of C_T esteems. Standard deviation between tests was broke down utilizing log of the sum (duplicates). From the standard bends the duplicate quantities of DNA-A genome parts were controlled by interjection. To factually assess all qualities acquired in partitioned qPCR responses, information were displayed graphically in box and bristle plots. These plots depend on computations for the primary middle from all information; the upper middle, for information > middle and; for the lower middle, from information < middle. In a graphical show middle qualities speak to the upper (75% of information) and lower limits (25% of information) of the case, with a cross segment of the fundamental middle. Bristles reach out to the information's littlest and most noteworthy qualities mirroring the extremes. A box plot (figure 1)

along these lines graphically shows the variety of the test information and is less affected by outrageous qualities.

3.3.2.5 SSR Primer Screening

SSR reaction mixture was prepared as follow,

Ingredients	Stock concentration	Required concentration	Required volume for one reaction (20 µl)
Taq Buffer	10X	1X	2.0 µl
dNTP	10 mM each	0.2 mM each	0.4 µl
Primer (F)	10 µM	0.25 µM	0.5 µl
Primer (R)	10 µM	0.25 µM	0.5 µl
Taq DNA polymerase	5U/ µl	1U	0.2 µl
Template DNA	10 ng/ µl	20ng	2.0 µl
SDW	----	----	14.4 µl

3.3.2.5.1 SSR PCR conditions

PCR was carried out in Proflex Thermocycler programmed for an initial denaturation at 95 °C for 5 minutes followed by 35 cycles with denaturation at 95 °C for 40 seconds, primer annealing at 56 °C for 1 minute and extension at 72 °C for 1 minute. The final extension was performed at 72 °C for 10 minutes followed by its holding at 4 °C. The amplified products were resolved in a 2% agarose gel using 100 bp ladder for checking amplification, to identify molecular weight of obtained bands and for polymorphism studies.

3.3.2.5.2 List of the SSR Markers Linked to CMD Resistance

The resistance of selected clones was verified using following SSR markers linked to CMD resistance.

Table 5. List of the SSR Markers Linked to CMD Resistance

Genes	Nature of resistance	Markers associated	Linkage group	Reference
CMD 1	Recessive	SSRY 40	LG-D	Fragene (2000)
CMD 2	Dominant	SSRY 28,NS138, RME-1	LG-G	Akano *et al.*, (2002)
--------		SSRY 28 SSRY 106	LG-G	Lokko *et al.*, (2005)
--------	Polygenic (QTL), recessive	SSRY 28, SSRY 235 – (G) SSRY 44, NS 136 –(P)	LG-G,P	Mohan *et al.*, (2013)

Among the above listed markers 5 SSR markers were selected based on bulk segregant analysis (BSA) and were further used for the identification of CMD resistant hybrids.

Table 6. List of SSR markers used for identification of CMD resistance

Locus	**Forward primer (5'-3') and Reverse primer**	**Product size (bp)**
SSRY40	GCATCATGGTCCACTCACT CATTCTTTTTCGGCATTCCAT	293
SSRY28	TTGACATGAGTGATATTTTCTTGAG GCTGCGTGCAAAACTAAAAT	180
RME1	ATGTTAATGTAATGAAAGAGC AGAAGAGGGTAGGAGTTATGT	700
SSRY106	GGAAACTGCTTGCACAAAGA CAGCAAGACCATCACCAGTTT	270
NS 136	CGACTGCATCAGAACAATGC AGCATGTCATTGCACCAAAC	296
SSRY44	GGTTCAAGCATTCACCTTGC GACTATTTGTGATGAAGGCTTGC	194
SSRY235	CAGCTTTGCCATCCAATTTT CAGCAAAATGACATGAGTGTATCTC	166
NS 138	CGCTTACAAACCACCTTCA GCTTGATCTCAGCCATGTCA	268

3.3.2.5.2 Agarose Gel Electrophoresis for SSR Analysis

Gel electrophoresis was performed with 4% agarose.

3.3.2.5.2.1 Gel Preparation

Four percent of agarose was used to resolve the amplicons obtained after the PCR. For preparing a total of 120 ml of 4% gel, 4.8 g of agarose was weighed out and dissolved in 120 ml of 1X TBE buffer. The flask was undisturbed and using a microwave oven, the gel was melted completely. Added approximately 1.2 µl EtBr into the conical flask containing the melted agarose. Then gently and gradually rotated the conical flask for the uniform distribution of EtBr. After proper mixing it was poured on to the casting tray and allowed to solidify for about 30 minutes.

3.3.2.5.2.1 **Gel loading and running**

The PCR products were arranged in a tray according to the sample number for loading. About 2 µl of gel loading dye was added directly to PCR tubes. Mixed well and a short spin using centrifuge was done. The combs from the solidified gel was removed slowly without disturbing the wells and the samples were loaded in the same order along with 100 bp DNA ladders

The electrophoresis apparatus was connected to a power pack and the voltage was set at 80V. The movement of the tracking dye was noted and the run was stopped when the dye reached the bottom of the tray.

3.3.2.5.2.2 **PCR product detection**

The gel including the tray was taken out and was viewed under the ultraviolet light in gel documentation system. The image was taken under appropriate exposure and saved in JPEG format for scoring.

3.3.2.5.2.3 Analysis of Molecular Marker Data

All the images of resolved PCR products were taken. Clear and reproducible bands were taken for scoring. Binary scoring was carried out by assigning "1" for the presence of a specific band and "0" for the absence of a band.

3.4 EXPERIMENT III: EVALUATION OF EARLY BULKING CLONES WITH RESISTANCE TO CMD

The CMD resistant clones with short duration were multiplied through miniset technology and evaluated along with parents for the confirmation of early bulking nature. Harvesting was done at six months after planting.

3.4.1 Materials, methods, design and layout

As given in *3.3.1.1.*, *3.3.1.2* and *3.3.1.2.1* respectively.

3.4.1.2 Biometric Observations

3.4.1.2.1 Height at First Branching (cm)

Height at first branching was measured in centimetres from the base of the stem to the point of primary branching with the measuring scale.

3.4.1.2.2 Nature of Branching

The pattern of branching habit was observed at the first branching on the main stem and recorded the most frequent occurrence as top branching (TB), non-branching (NB), spreading (SP) and semi spreading (SSP).

3.4.1.2.3 Plant Height (cm)

It was recorded in centimetres from stem base to tip of the growing point with the measuring scale.

3.4.1.2.4 Stem Girth (cm)

Girth of the stem was measured with a tape at mid portion of the stem and expressed in centimetres.

3.4.1.2.5 Leaf Retention (1-5 scale)

Visually score for leaf retention using 1 - 5 scale based on the presence of leaves in the plant were recorded.

Scale	Description
1	Very poor retention
2	Less than average retention
3	Average leaf retention
4	Better than average retention
5	Outstanding leaf retention

3.4.1.2.6 Internodal Distance (cm)

Distance between the two internodes from the mid part of the stem is measured and expressed in centimetres

3.4.1.2.7 Number of Tubers Plant^{-1}

The number of storage tubers in the plant was counted and expressed as number of tubers plant^{-1}.

3.4.1.2.8 Mean Weight of Tuber (kg)

The average weight of the total number of storage tubers in the plant was measured and expressed in kilograms.

3.4.1.2.9 Length of Tuber (cm)

The length of the storage tubers was measured with the scale and the mean was expressed in centimeter.

3.4.1.2.10 Girth of Tuber (cm)

The maximum diameter of the tuber was measured at middle part of the tuber for 5 tubers per plant with the twine and scale and expressed in centimetres.

3.4.1.2.11 Weight of Foliage (kg plant^{-1})

Total leaves from the plant were separated from the main stem are weighed and expressed in kilogram per plant.

3.4.1.2.12 Tuber Yield (kg plant^{-1})

The total number of storage tubers weighed and expressed as tuber yield in kilogram per plant.

3.4.1.2.13 Harvest Index

It is calculated by using following formula

Harvest index (H.I) = Tuber yield (kg) / Foliage weight (kg)

3.4.1.2.14 Dry Matter Content (%)

Immediately after tuber harvesting samples were chopped into small pieces. 50 g were kept at 65°C for 72 hr, in hot air oven and the dry weight was recorded and percentage was estimated using the following formula.

$$\text{Dry matter percentage} = \frac{\text{Dry weight (DW)}}{\text{Fresh weight (FW)}} \text{X } 100$$

The samples were crushed and stored in plastic bottles. The flour is further used for starch estimation.

3.4.1.2.15 Starch (%)

a) Principle

Treatment with 80% ethanol extracts the sugars present in the tuber sample. The extract is filtered and the filtrate is used for the analysis of sugars. The residue is acid hydrolyzed for the analysis of starch. The starch is completely hydrolyzed by treatment of with 2N Hydrochloric acid (HCl), while the non-reducing sugars are converted to reducing ones, using Conc. HCl. Analysis of both of the components is done in a similar manner, based on the number of reducing groups.

b) Reagents

a) Potassium ferricyanide reagent (1%)

Dissolve exactly 1.0 g potassium ferricyanide (AnalaR) in 100 ml distilled water and store in a brown bottle.

b) Sodium hydroxide (2.5N)

Dissolve 10.0 g sodium hydroxide pellets in 100 ml distilled water.

c) Hydrochloric acid (2.0 N)

Dilute concentrated hydrochloric acid six times by adding 250 ml distilled water to 50ml Con. HCl.

d) Methylene blue (*aq*) staining solution

Diluted methylene blue indicator supplied by M / s Qualigens Fine Chemicals, Mumbai

e) Glucose standard

Dissolve 100 mg D-glucose (AR grade) in 100 ml distilled H_2O.

f) 80 per cent ethanol

Dilute 80 ml absolute ethanol to 100 ml using distilled H_2O.

c) Preparation of samples

Powdered samples were used for starch estimation. One gram of well dried sample was taken into a 100 ml conical flask, to which 20 ml of 80% alcohol was added and kept overnight, to extract sugar content. Similarly leaf samples are used for the estimation of starch, in which 80% of acetone is used to remove pigments. Dried leaf powder can be used for starch estimation.

After incubation filter the samples using Whatman No. 1 filter paper and the filtrate is used for the sugar estimation. Residue on the filter paper was washed with 2 lots (10 ml each) of distilled water to remove adhering sugar molecule and filtrate is added to original solution. To this flask 20 ml of 2N HCl was added and boiled for half an hour till the colour changes to brown and the flask is cooled and made up to 100 ml using distilled water. This supernatant was directly used for starch estimation.

The alcoholic sugar filtrate is treated with 1.0 ml concentrated HCl and heated for 30 minutes on a hot plate at 100^0 C. the volume of the sugar extract is raised to 50 ml and used for titration.

d) Titrimetric Assay

Pipette out 10 ml of Potassium ferricyanide into 100 ml Erlenmeyer flasks. To this, 5.0 ml NaOH (2.5N) is added. Mix the contents thoroughly. The flasks are then kept over the flame for boiling. When the reagent begins to boil, the flame is lowered and 3 drops of dilute methylene blue is added. The solution immediately turns blue-green. The starch hydrolysate is taken in a 2.0 ml blow pipette during starch estimation and added drop by drop to the boiling reagent, while for sugar estimation, the sugar extract is taken in a 10.0 ml blow pipette (since the titre value generally will be around 5.0-8.0 ml). The nearing of the end point is indicated by change of colour from blue-green to violet. A few more drops are added carefully, to reach the end

point, which is indicated by the rapid disappearance of the violet colour. At this stage, the titre reading is noted. Titrations are repeated for each of the aliquots.

e) Calculations

Each lot of potassium ferricyanide is calibrated using standard glucose solution and the relation:

10 mg of glucose = 10 ml of potassium ferricyanide is to be established.

The starch content of the sample is calculated from the formula,

$$\text{Starch (g/100g fresh weight)} = \frac{10^a \times 100^b \times 0.9^c \times 100}{T \times 2^d \times 1000}$$

Where,

a – Titre obtained for ferricyanide reagent, while calibrating against std. glucose solution (if a value different than 10 is obtained, e.g., 9.2, 10.4 etc. this has to be used)

b – Total volume of starch hydrolysate

c – 0.9 is the Morris factor, for converting sugar to starch

d – Weight of tuber sample (g) used for analysis

T – Titre value for starch hydrolysate

For expressing starch on dry matter basis, the dry matter of the tuber can be determined by

$$\text{Dry Matter (\%)} = \frac{\text{Dry weight of the tuber} \times 100}{\text{Fresh weight of the tuber}}$$

$$\text{Starch (g/100g DM)} = \frac{\text{Starch content (g/100g fresh weight)} \times 100}{\text{DM (\%)}}$$

For calculating the total (reducing + non - reducing) sugar content the following formula is used:

$$\text{Sugar (g/100g fresh weight)} = \frac{10a \times 50b \times 100}{T \times 2c \times 1000}$$

Where,

a – titre obtained for ferricyanide reagent, while calibrating against std. glucose solution

b – Total volume of hydrolysate

c – weight of tuber sample (g) used for analysis

T – titre value for hydrolysate

$$\text{Sugar (g/100g DM)} = \frac{\text{Sugar (\% in FW basis)} \times 100}{\text{DM \%}}$$

3.4.1.2.16 Sugar (%)

Procedure is given in *3.3.1.2.2.15*

3.4.1.2.17 Cooking Quality

Freshly harvested mature tubers were cleaned and cooked. Scoring was given,

Excellent - 5, Very good - 4, Good - 3, Not Cooked - 2, Waxy -1

3.4.1.2.18 CMD Disease Scoring (1-5 scale)

Different grades of CMD infection were scores from one to five scales (No symptom to Very severe mosaic symptoms) as per Hahn, 1980.

3.4.1.2.19 Number of Whiteflies

The count was taken among the top leaves. The number of nymphs per leaf was rated for the consideration of sampling.

3.4.1.2.20 Measurement of Photosynthesis Rate

The rate of photosynthesis in leaves was measured using portable photosynthetic CO_2 analyser.

3.4.1.3 Statistical Analysis

The data collected on the agronomic characters were subjected to statistical analysis and the different statistical parameters were worked out.

The data obtained on the above characters were subjected to the analysis to estimate the following parameters.

1. Analysis of variance
2. LxT analysis, combining ability analysis and gene action involved
    a. Estimation of general combining ability effects.
    b. Estimation of specific combining ability effects.
    c. Estimation of gca and sca variances.
3. Estimation of heterosis
    a. Estimation of heterosis over the mid parental value.
    b. Estimation of heterosis over the better parental value.
    c. Estimation of standard heterosis.
4. Association analysis of phenotypic and molecular data.

3.4.1.3.1 Analysis of Variance (ANOVA)

Analysis was carried out separately for each trait as per RBD design.

Sources of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Replications	t-1	SSR	MSR	MSR/MSE
Treatment	r-1	SST	MST	MST/MSE
Error	(r-1)(t-1)	SSE	MSE	

Where,

r = number of replications t = number of treatments

MSR=mean squares for replication SSR=sum of squares for replications

MST=mean squares for treatments SSR=sum of squares for treatments

MSE=mean squares for error SSR=sum of squares for error

$$\text{Critical difference (CD)} = t\alpha \sqrt{\frac{2 \times MSE}{r}}$$

Where,

$t\alpha$ is the table value of students' t distribution at error degrees of freedom and α is the level of significance (5 % or 1%), (Panse and sukhatme, 1985)

3.4.1.3.2 Combining Ability Analysis

The analysis with parents and crosses was calculated for different characters using the L x T model as,

Mathematical model for combining ability analysis

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + r_k + e_{ijk}$$

Where,

Y_{ijk} = Any measurable character of the cross ixj in the k^{th} replication

μ= population mean

g_i = gca effect of the female patent

g_j= gca effect of the male parent

s_{ij}= sca effect of the cross.

r_k = Effect due to k^{th} replication

e_{ijk} = Environmental effect on (ijk)th individual.

Table 7. ANOVA of L x T mating design for combining ability

Source	Df	MSS	Expected MSS
Replications	(r-1)		
Lines	(s-1)	M_4	$\sigma^2+r\sigma^2s+rt\sigma^2f$
Testers	(t-1)	M_3	$\sigma^2+r\sigma^2s+rs\sigma^2m$
Line x testers	(s-1) (t-1)	M_2	$\sigma^2+r\sigma^2s$
Error	(r-1) (st-1)	M_1	σ^2

Where,

r = number of replications

s = number of male parents

t = number of female parents

σ^2 = random error

σ^2s = variance of interaction between lines and testers.

σ^2f = variance due to lines.

σ^2m = variance due to testers.

3.4.1.3.2.1 Estimation of Combining Ability Effects

(i) gca effect of line and tester

$$\text{Line } g_i = \frac{X_i\ldots}{tr} - \frac{X\ldots}{str}$$

(ii) sca effect of cross

$$S_{ij} = \frac{X_{ij}\ldots}{r} - \frac{Xi\ldots}{tr} - \frac{Xi\ldots}{sr} + \frac{X\ldots}{str}$$

where,

X = Grand total

X_i = Total of i^{ih} line over replicates and testers

X_j = Total of j^{th} tester over replicates and lines

X_{ij} = Total of j^{th} cross over replicates.

3.4.1.3.2.2 Standard Errors of Estimates

$$S.E\ (g_i) = [M_1/rt]^{1/2}$$

$$S.E\ (g_i) = [M_1/rs]^{1/2}$$

$$S.E\ (s_{ij}) = [M_1/r]^{1/2}$$

Where,

r = Number of replications

s = Number of female parents

t = Number of female parents

M_1= MSS due to error

3.4.1.3.2.3 Estimation of Genetic Components of Variation

The estimates of variance components were obtained from the algebraic manipulation of mean squares in the ANOVA of LxT mating design for combining ability as follows:

Since $\sigma^2 f = \sigma^2$ m in the absence of maternal effects, the line and tester mean squares were pooled mean as:

Pooled mean squares of lines and testers (M_o)

(i) $$M_0 = \frac{(s-1)\,M_4 + (t-1)\,M_3}{S + t - 2}$$

(ii) $$\sigma^2 f = \sigma^2 m = \frac{(M_0 - M_1)\quad (s + t - 2)}{r\,[t(s-1) + (t-1)\,]}$$

(iii) $$\sigma^2 s = \frac{(M_3 - M_4)}{r}$$

The genetic components of variation were estimated by relating to variance components to covariance of half sibs (Co v. HS) and full sibs (Co v. FS) as:

(i) $\sigma^2 f = \sigma^2 m = Co\ v.\ HS$

(ii) $\sigma^2 s = Co\ v.\ FS - 2\ Co\ v.\ HS$

(iii) $\sigma^2 gca = Co\ v.\ HS = 1/2\ \sigma^2 A$

(iv) $\sigma^2 sca = Co\ v.\ FS - 2\ Co\ v.\ HS = \sigma^2 D$

Where,

$\sigma^2 gca$ = General combining ability variance

$\sigma^2 sca$ = Specific combining ability variance

3.4.1.3.3 Estimation and Testing of Heterosis

The heterotic effects were measured as deviation of F_1 mean from mid parent (relative heterosis), the better parent (heterobeltiosis) mean and mean value of standard check.

3.4.1.3.3.1 Heterosis over the Mid-Parent

Heterosis was expressed as percent increase or decrease in the value of F_1 over the mid parent as per the formula.

$$\text{Heterosis over mid parent} = \frac{\text{Mean of } F_1 - \text{Mean of parents}}{\text{Mean of parents}} \times 100$$

3.4.1.3.3.2 Heterobeltiosis

Heterobeltiosis was expressed as percent increase or decrease in the value of F_1 over the better parent (B.P) as per the formula,

$$\text{Heterobeltiosis} = \frac{\text{Mean of } F_1 - \text{Mean of BP}}{\text{Mean of B.P}} \times 100$$

3.4.1.3.3.3 Standard Heterosis

Standard heterosis was expressed as percent increase or decrease in the F_1 value over the high yielding standard check.

$$\text{Standard heterosis} = \frac{\text{Mean of } F_1 - \text{Mean of Std. check}}{\text{Mean of Std. check}} \times 100$$

Heterosis was considered significant if the difference between F_1 and parental means used for comparison was found significant. To test the significance of heterosis following formulas were used.

$$\text{Heterosis, } t = \frac{F_1 - MP}{\sqrt{2MSE/r}}$$

$$\text{Heterobeltiosis, } t = \frac{F_1 - B.P}{\sqrt{2MSE/r}}$$

Where,

MSE=mean squares for error

r= Number of replication

The calculated 't' value was compared with table 't' values at the error degrees of freedom.

3.5 EXPERIMENT IV: BULK SEGREGANT ANALYSIS

DNA from the early bulking clones was isolated and pooled. Similarly DNA from the late bulking clones was isolated and pooled. Using molecular marker (SSR) analysis pooled DNA from the early and late bulking segregants was compared.

Table 8. List of Early bulking SSR primers used

Name	Left primer Right primer	Product size	Annealing temperature(°C)
SSRY 250	GATCGGATGTCTGAGGAGGA AATTGGAAGGGAAAGCCAAA	197	60.16
(ESTs)SSRY47	GTGTCGCTGCTAGATCTTGACT TCATCAGATCACCACCATCAA	236	55
SSRY63	TCAGAATCATCTACCTTGGCA AAGACAATCATTTTGTGCTCCA	290	55
(ESTs)SSRY292	TCTCTCTTCCTTGAACTCTCC CATCATAAACTGGTGAGACG	294	55
SSRY 239	TGCATTTCCCTGGGTGTAAG TTTCTCAATAGACAGACGAGCA	208	60.88

RESULTS

4. RESULTS

In an experiment with objective of introgression of cassava mosaic disease resistance to short duration varieties of cassava through marker assisted selection and to study the inheritance of early bulking nature, the results obtained are depicted as follows.

4.1 EXPERIMENT I: RAISING OF POLLINATION BLOCK AND HYBRID SEED PRODUCTION

The experimental materials comprising of Five early bulking high yielding lines *viz,* S. Jaya, S. Vijaya, V. Hraswa, CI 889 and 9S 75 and three testers *viz,* CR 54A3, IMS2-5 and CI 273 with resistance to cassava mosaic disease were crossed to produce fifteen F_1 combinations. (Table 9)

4.2 EXPERIMENT II: SCREENING OF SEEDLINGS FOR RESISTANCE AND EARLY BULKING NATURE

4.2.1 Field Evaluation

The ANOVA uncovered exceedingly significant contrast among the genotypes for all the quantitative attributes considered, defending the choice of parents for the investigation. The mean sum of squares because of crosses was profoundly significant, demonstrating the various executions of various cross combinations. The mean total of squares because of parents versus crosses was exceptionally significant for all qualities uncovering the nearness of heterosis because of the critical distinction in the mean execution of hybrids and parents. Huge contrasts were likewise watched for all qualities contemplated in testers (male) and lines (females) showing the predominance of non-additive gene action. The hybrids showed exceptionally critical variety for every one of the characters (Table10 &11).

Table 9. Details of the F_1 combinations produced.

CROSSES	No. of flowers crossed	No. of Seeds obtained	No. of seeds germinated	No. of seedlings transplanted
S. JAYA X CR 54 A3	40	66	32	14
S. JAYA X IMS2-5	35	65	30	17
S. JAYA X CI 273	28	55	30	22
S. VIJAYA X CR 54 A3	36	71	42	20
S. VIJAYA X IMS2-5	42	67	40	30
S. VIJAYA X CI 273	43	81	49	21
V. HRASWA X CR 54 A3	29	48	31	25
V. HRASWA X IMS2-5	60	122	80	42
V. HRASWA X CI 273	40	63	42	29
CI 889 X CR 54 A3	22	54	30	25
CI 889X IMS2-5	36	59	38	23
CI 889X CI 273	30	66	36	12
9S 75 X CR 54 A3	26	47	29	21
9S 75X IMS2-5	29	75	42	27
9S 75X CI 273	34	55	29	32
TOTAL	530	1240	580	360

Table 10. Mean table of F_1 seedlings in Experiment II (a)

CROSSES	PH	SG	NOT	MWT	LOT	GOT	TY	CMD
S. JAYA X CR 54 A3	149.00	7.50	6.88	185.00	30.00	13.90	2.81	1
S. JAYA X IMS2-5	165.20	10.60	7.47	216.67	28.87	13.00	1.23	5
S. JAYA X CI 273	168.46	7.92	9.30	371.50	35.15	15.75	2.43	3
S. VIJAYA X CR 54 A3	185.58	9.47	8.53	233.16	24.79	13.16	1.98	4
S. VIJAYA X IMS2-5	181.31	8.65	6.81	292.69	29.38	22.31	1.73	4
S. VIJAYA X CI 273	157.67	7.87	8.27	254.67	26.93	16.67	1.60	1
V. HRASWA X CR 54 A3	151.09	8.05	7.82	328.64	32.27	15.00	2.73	1
V. HRASWA X IMS2-5	179.80	7.96	7.36	288.20	22.24	13.72	2.52	2
V. HRASWA X CI 273	170.68	8.00	7.21	157.37	24.53	11.84	0.81	1
CI 889 X CR 54 A3	179.18	7.82	9.86	209.55	28.50	13.41	1.70	5
CI 889X IMS2-5	175.24	8.00	8.38	210.38	31.00	14.76	1.77	1
CI 889X CI 273	208.25	8.13	5.63	203.75	24.00	9.44	1.06	1
9S 75 X CR 54 A3	152.35	7.06	5.24	146.47	18.47	8.71	0.98	2
9S 75X IMS2-5	249.30	8.70	6.30	158.90	24.70	8.50	0.95	1
9S 75X CI 273	158.78	7.00	7.00	217.78	27.78	12.44	1.05	4
Mean	175.46	8.18	7.47	231.65	27.24	13.51	1.69	2.40
Max	249.30	10.60	9.86	371.50	35.15	22.31	2.81	5.00
Min	149.00	7.00	5.24	146.47	18.47	8.50	0.81	1.00

Where, PH- Plant Height, SG- Stem Girth, NOT- No. of Tubers, MWT- Mean Weight of Tuber, LOT- Length of Tuber, GOT- Girth of Tuber, TY- Tuber Yield, CMD- Cassava Mosaic Disease

Table 10(a). Descriptive statistics table for field evaluation of screening of seedlings in Experiment II (a)

Character	Mean	Standard Deviation	Standard Error
PH	175.461	39.635	2.992
SG	8.176	1.811	0.633
NOT	7.469	1.884	0.689
MWT	231.642	81.022	5.323
LOT	27.242	6.727	1.289
GOT	13.507	4.346	1.182
TY	1.693	17.356	5.634
CMD	2.401	0.455	0.331

Where, PH- Plant Height, SG- Stem Girth, NOT- No. of Tubers, MWT- Mean Weight of Tuber, LOT- Length of Tuber, GOT- Girth of Tuber, TY- Tuber Yield, CMD- Cassava Mosaic Disease

Table 11. Correlation coefficients for the morphological characters, yield and diseasein Experiment II (a)

	PH	SG	NOT	MWT	LOT	GOT	TY	CMD
PH	1							
SG	0.761**	1						
NOT	0.492**	0.685**	1					
MWT	0.347*	0.538**	0.715**	1				
LOT	0.503**	0.704**	0.840**	0.780**	1			
GOT	0.311*	0.576**	0.685**	0.785**	0.769**	1		
TY	0.045NS	0.104NS	0.360*	0.748**	0.373*	0.383*	1	
CMD	0.788**	0.754**	0.695**	0.452**	0.573**	0.583**	-0.536**	1

* Significant at 5% level ** Significant at 1% level

Where, PH- Plant Height, SG- Stem Girth, NOT- No. of Tubers, MWT- Mean Weight of Tuber, LOT- Length of Tuber, GOT- Girth of Tuber, TY- Tuber Yield, CMD- Cassava Mosaic Disease

4.2.1.1 Plant Height (cm)

The mean value was ranged from 249.30 cm (9S 75 x Sree Vijaya) to 149.00cm (Sree Jaya x CR 54A3). The trait exhibited significant and positive correlation for all the characters except tuber yield.

4.2.1.2 Stem Girth (cm)

The trait expressed maximum value of 1.60 cm with Sree Jaya x IMS2-5 and minimum of 7.00 cm with 9S 75 x CI 273. The trait exhibited significant and positive correlation for all the characters except tuber yield.

4.2.1.3 Number of Tubers Plant^{-1}

The Maximum value for the character was 9.86 (CI 889 x CR 54A3) and minimum of 5.24 (9S 75 x Sree Jaya). The trait exhibited significant and positive correlation for all the characters studied.

4.2.1.4 Mean Weight of Tuber (gm)

The highest mean value for the trait is 371.50 gm (Sree Jaya x CI 273) and 146.47 gm (9S 75 x Sree Jaya) lowest. The trait exhibited significant and positive correlation for all the characters studied and also highest positive significance with tuber yield.

4.2.1.5 Length of Tuber (cm)

The highest mean value for the trait is 35.15 cm (Sree Jaya x CI 273) and 18.47 cm (9S 75 x Sree Jaya) lowest. The trait exhibited significant and positive correlation for all the characters studied.

4.2.1.6 Girth of Tuber (cm)

The trait expressed maximum mean value of 22.31 cm with Sree Vijaya x IMS2-5 and minimum of 8.50 cm with 9S 75 x IMS2-5. The trait exhibited significant and positive correlation for all the characters studied and also highest positive significance with mean weight of tuber.

4.2.1.7 Tuber Yield (kg)

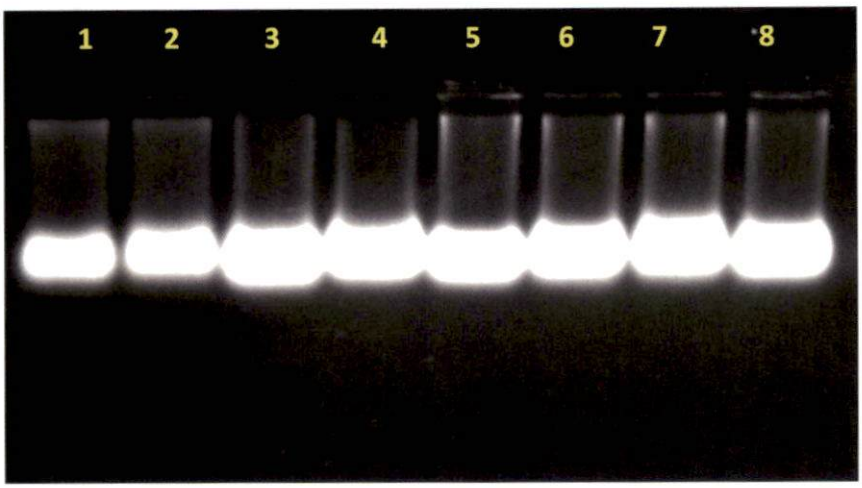
The mean tuber yield was ranged from 2.81 kg (Sree Jaya x CR 54A3) to 0.81 kg (Vellayani Hraswa x CI 273). The trait showed positive and non-significant correlation with plant height and stem girth but negative and high significance with CMD disease scoring.

4.2.1.8 CMD Disease Scoring (1-5 scale)

Among the hybrids studied, Sree Jaya x IMS2-5 and CI 889 x CR 54A3 expressed disease scoring of 5 (Very severe mosaic symptom). Disease scoring of 1 (No Symptoms) was expressed among the hybrids of Sree Jaya x CR 54A3, Sree Vijaya x IMS2-5, Vellayani Hraswa x CR 54A3, Vellayani Hraswa x CI 273, CI 889 x IMS2-5, CI 889 x CI 273 and 9S 75 x Sree Vijaya. The trait exhibited significant and positive correlation for all the characters studied and also highest negative significance with tuber yield, indicating yield and disease correlation for the hybrid seedlings studied.

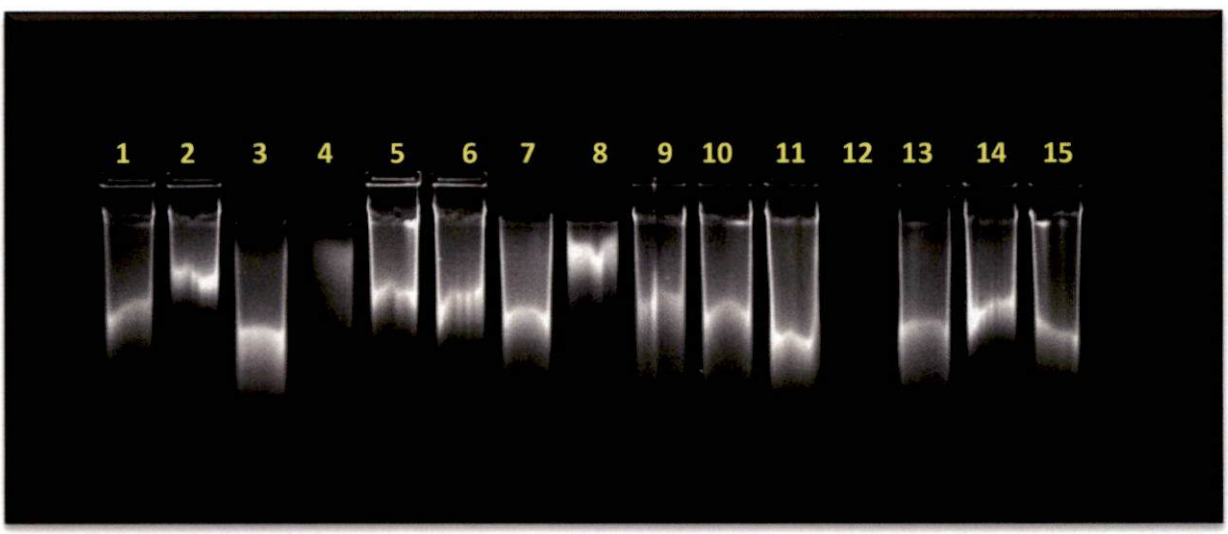
4.2.2 Marker Aided Selection of CMD Resistant Clones

The seedlings without CMD visual symptoms were subjected to multiplex PCR and real time quantitative estimation to study the presence of virus in field tolerant plants. The resistance of the selected clones were further verified by using SSR markers associated with resistance to CMD. The quality and integrity of



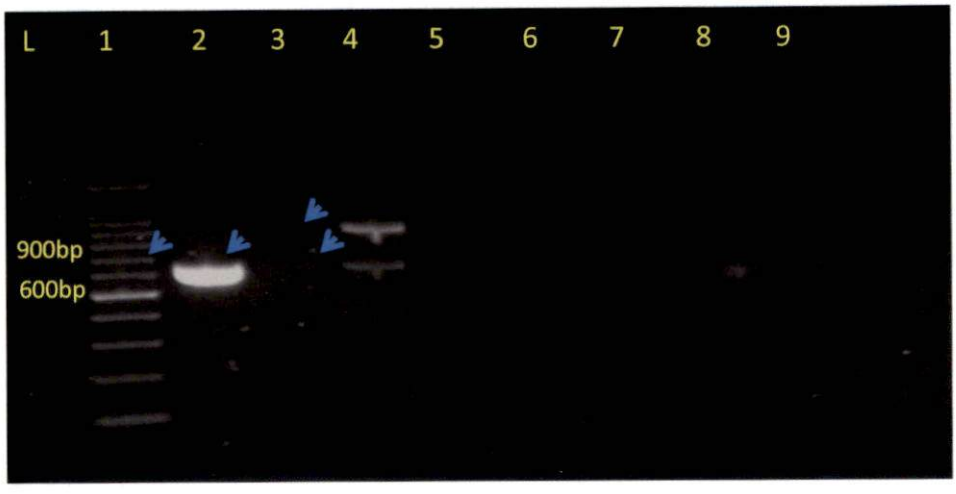
1- Sree Jaya, 2- Sree Vijaya, 3- V. Hraswa, 4- CI 889, 5- 9S75
6-CR 54A3, 7- IMS2-5, 8-CI 273

Plate 5. DNA Check of Parents(Lines and Testers)



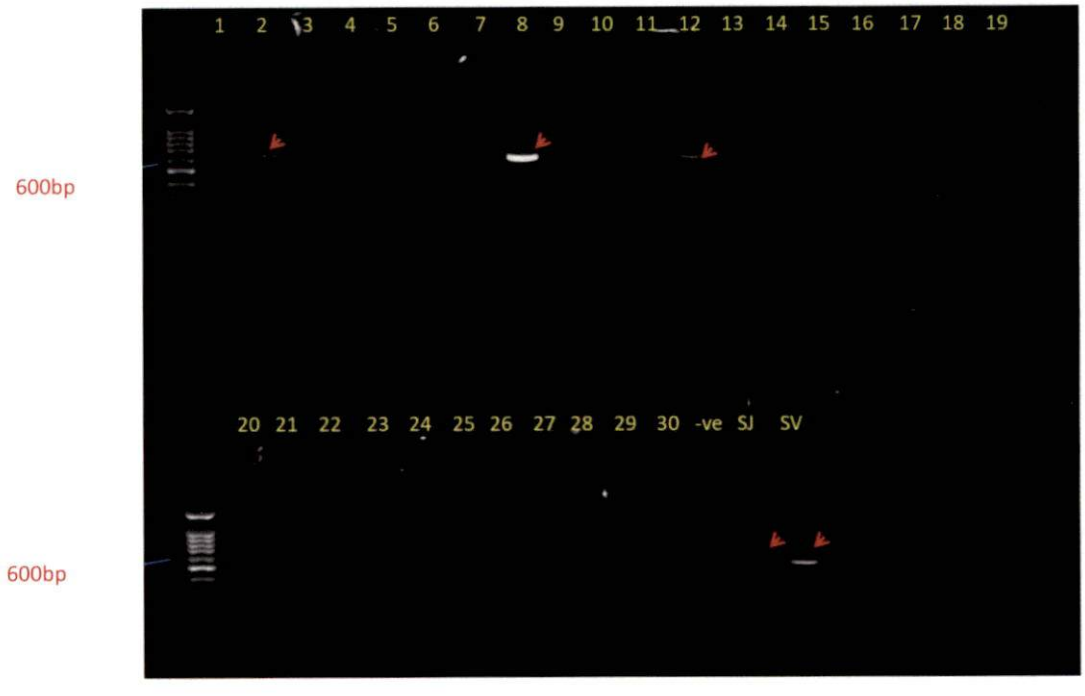
1-Sree Jaya X CR 54 A3, **2**- Sree Jaya X IMS2-5, **3**- Sree Jaya X CI 273, **4**- Sree Vijaya X CR 54 A3
5- Sree Vijaya X IMS2-5, **6**- Sree Vijaya X CI 273, **7**- V. Hraswa X CR 54 A3, **8**- V. Hraswa X IMS2-5
9- V. Hraswa X CI 273, **10**- CI 889 X CR 54 A3, **11**- CI 889 X IMS2-5, **12**- CI 889 X CI 273,
13- 9S 75 X CR 54 A3, **14**- 9S 75X IMS2-5, **15**- 9S 75X CI 273

Plate 6. DNA Check of Crosses (Lines X Testers)



L- 100bp ladder, 1- Sree Jaya, 2- Sree Vijaya, 3- V. Hraswa, 4- CI 889, 5- 9S75
6-CR 54A3, 7- IMS2-5, 8-CI 273, 9- Negative control

Plate 7. Multiplex PCR for Parents



1-Sree Jaya X CR 54 A3, **2**- Sree Jaya X IMS2-5, **3**- Sree Jaya X CI 273, **4**- Sree Vijaya X CR 54 A3
5- Sree Vijaya X IMS2-5, **6**- Sree Vijaya X CI 273, **7**- V. Hraswa X CR 54 A3, **8**- V. Hraswa X IMS2-5
9- V. Hraswa X CI 273, **10**- CI 889 X CR 54 A3, **11**- CI 889 X IMS2-5, **12**- CI 889 X CI 273,
13- 9S 75 X CR 54 A3, **14**- 9S 75X IMS2-5, **15**- 9S 75X CI 273
16 to 30- 1 to 15 respectively

Plate 8. Multiplex PCR for F_1Seedlings

extracted DNA (Parents and Crosses) was checked using agarose gel electrophoresis using 1% agarose (Plate 5 & 6).

4.2.2.1 Multiplex PCR

To distinguish between Indian Cassava Mosaic virus (ICMV) and Sri Lankan Cassava Mosaic virus (SLCMV), PCR amplification was carried out using designed primers to specifically amplify ICMV fragment of about 900bp and SLCMV fragment of about 600bp of DNA A of both parents (Plate 7) and hybrids (Plate 8).

Among parents studied, the isolated DNA expressed amplification of 600bp in Sree Jaya and 600bp as well as 900bp for the presence of ICMV and SLCMV by Vellayani Hraswa.

Through multiplex PCR in F_1s keeping Sree Jaya and Sree Vijaya as positive control and some highly infected CMD line (MVD1) as negative, it was noticed that the presence of SLCMV with 600bp was confined to hybrid seedlings of Sree Jaya x IMS2-5, Vellayani Hraswa x IMS2-5 and CI 889 x CI 273, implies SLCMV is causing cassava mosaic disease in the most.

4.2.2.2 Real Time PCR

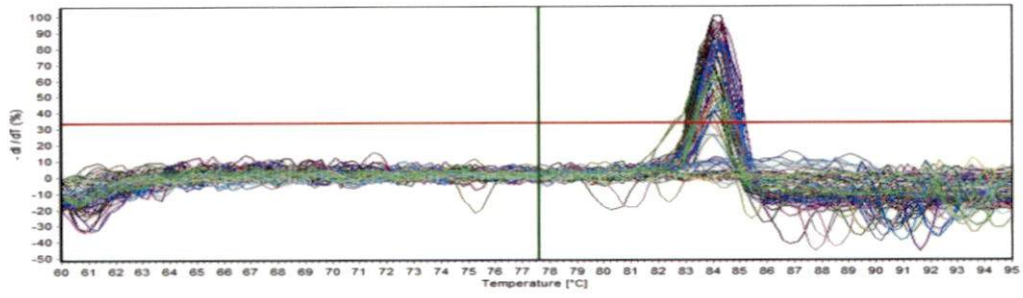
A Sybr Green qPCR assay for SLCMV was established and conducted during the growing season to quantify the viral DNA. Absolute quantification of DNA A and B of SLCMV has been carried out by primers Sy-SLCMV A_ F, Sy-SLCMV A _R. For each DNA sample, three technical replicate reactions were prepared. Results from qPCR were analysed with respect to standard curves and quantify target concentration in samples. From the standard curves the copy no. of DNA-A genome components were found out by interpolation. To estimate all data attained in separate qPCR reactions, data were depicted graphically in box plot and whisker plots. Whiskers extend to the values smaller and higher reflecting the extremes. A box plot

Table 12. Quantification of virus titre in Parents, F_1 seedlings and F_1clones using Real time PCR assay

	F_1 SEEDLING	F_1 CLONES
PARENTS	VIRAL LOAD	VIRAL LOAD
SREE JAYA	$1.7x10^3$	$1.4x10^6$
SREE VIJAYA	$1.2x10^3$	$1.5x10^5$
VELLAYANI HRASWA	$1.0x10^2$	$2.0x10^8$
CI 889	316	3165
9S 75	184	210
CR 54A3	144	307
IMS2-5	200	324
CI 273	0	373
CROSSES	VIRAL LOAD	VIRAL LOAD
S. JAYA X CR 54 A3	0	0
S. JAYA X IMS2-5	0	$7.6x10^6$
S. JAYA X CI 273	241	549
S. VIJAYA X CR 54 A3	$2.1x10^3$	$7.9x10^7$
S. VIJAYA X IMS2-5	$2.8x10^2$	$9.7x10^7$
S. VIJAYA X CI 273	1507	16073
V. HRASWA X CR 54 A3	1234	17904
V. HRASWA X IMS2-5	$1.2x10^3$	$2.4x10^6$
V. HRASWA X CI 273	$2.9x10^5$	$3.1x10^6$
CI 889 X CR 54 A3	$1.1x10^2$	$3.5x10^5$
CI 889X IMS2-5	$1.1x10^2$	$3.6x10^7$
CI 889X CI 273	$1.1x10^3$	$3.7x10^7$
9S 75 X CR 54 A3	0	540
9S 75X IMS2-5	0	3373
9S 75X CI 273	0	7746

Where,

VIRAL LOAD SCALE	
<1000	HIGHLY RESISTANT
10^3 TO 10^5	FIELD TOLERENT
$>10^5$	SUSCEPTIBLE



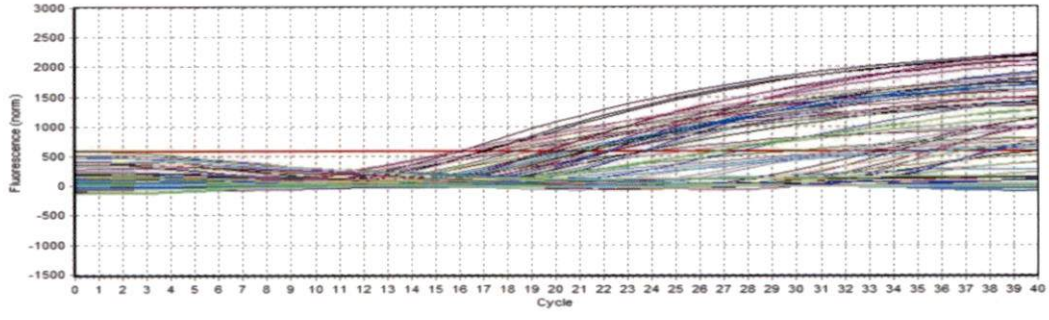


Figure 1.Standard curve for real time quantitative (RT PCR) estimation of virus titre in F_1 seedlings

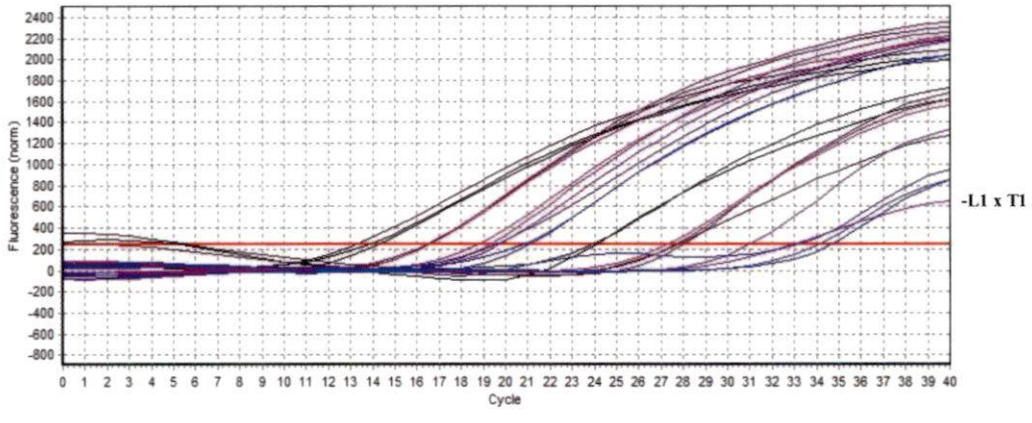


Figure 2.Standard curve for real time quantitative (RT PCR) estimation of virus titre in F_1 clones

thus graphically shows the variation of investigational values and is less influenced by extreme values.

4.2.2.2.1 Real time PCR for F_1 Seedlings

The viral load scale (Table 12) was formulated in grouping seedlings into highly resistant, field tolerant and susceptible based on which among lines, CI 889 (316) and 9S 75 (184) recorded to be highly resistant. Sree Jaya ($1.7x10^3$), Sree Vijaya ($1.2x10^3$) and Vellayani Hraswa ($1.0x10^2$) grouped to be field tolerant. Among crosses S. Jaya x CR 54A3 (0), S. Jaya x IMS2-5 (0), S. Jaya x CI 273 (241), 9S 75 x CR 54A3 (0), 9S 75 x IMS2-5 (0) and 9S 75 x CI 273 (0) grouped to be highly resistant seedlings. S. Vijaya x CR 54A3 ($2.1x10^3$), S. Vijaya x IMS2-5 ($2.8x10^2$), S. Vijaya x CI 273 (1507), V. Hraswa x CR 54A3 (1234), V. Hraswa x IMS2-5 ($1.2x10^3$), CI 889 x CR 54A3 ($1.1x10^2$), CI 889 x IMS2-5 ($1.1x10^3$) and CI 889 x CI 273 ($1.1x10^3$) grouped to be field tolerant. Among the crosses V. Hraswa x CI 273 ($2.9x10^5$) found to record with highest viral load. A picture as a consequence graphically displayed in depicting the variations of the investigational data and is less inclined by extreme end values. A standard curve with No. of cycles v/s Florescence traits has been used to calculate virus titre values (Figure 1).

4.2.2.2.2 Real time PCR for F_1 Clones

Among the lines only 9S 75 (210) grouped to be highly resistant, CI 889 (3165) as field tolerant and rest of the lines, Sree Jaya ($1.4x10^6$), Sree Vijaya ($1.5x10^5$) and V. Hraswa ($2x10^8$) bounded to be susceptible to CMD virus. Viral load scale among crosses, S. Jaya x CR 54A3 (0), S. Jaya x CI 273 (549) and 9S 75 x CR 54A3 (540) grouped as highly tolerant. Crosses, 9S 75 x IMS2-5 (3373) and 9S 75 x CI 273 (7746) found to be field tolerant. S. Jaya x IMS2-5 ($7.6x10^6$), S. Vijaya x CR 54A3 ($7.9x10^7$), S. Vijaya x CI 273 (16073), V. Hraswa x CR 54A3 (17904), V. Hraswa x IMS2-5 ($2.4x10^6$), V. Hraswa x CI 273 ($3.1x10^6$), CI 889 x CR 54A3

($3.5x10^6$), CI 889 x IMS2-5 ($3.6x10^7$) and CI 889 x CI 273 ($3.7x10^7$) were recorded to be susceptible. Among the crosses, S. Vijaya x IMS2-5 ($9.7x10^7$) had a highest viral load and highly susceptible to CMD. A Standard curve with No. of cycles v/s Florescence has been used to calculate virus titre values indicating S. Jaya x CR 54A3 (L1 x T1) with zero viral load (Figure 2).

4.2.2.3 *Molecular Characterisation to the Resistance of Selected Clones by using SSR Markers Linked to CMD Resistant*

The isolation of DNA from fresh leaf samples was carried out using the CTAB method. The genomic DNA was isolated and quality was analysed by Agarose gel electrophoresis (1% agarose) and pure DNA was obtained by RNase treatment (Plate 5 & 6). The quantification of isolated DNA samples was carried out using Nanodrop spectrophotometer.

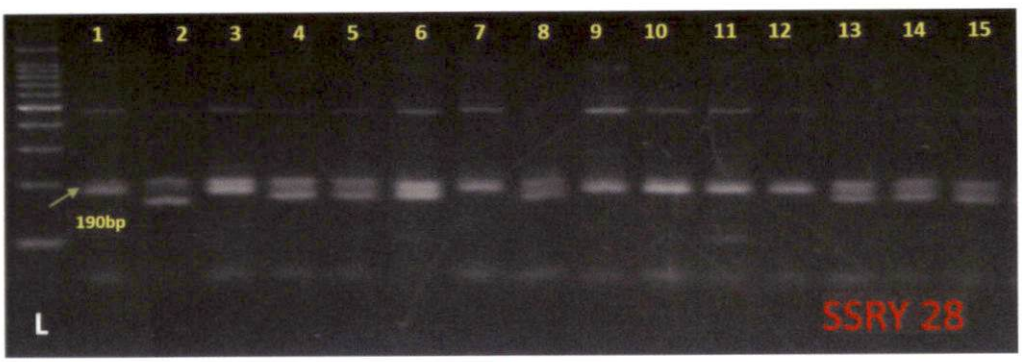
The different SSR primers selected based on review of literature (Table 5) were screened by Bulk Segregant Analysis. DNA of lines, testers and crosses were used for the study. Out of SSR markers screened, five markers (SSRY 28, SSRY 40, SSRY 44, SSRY 106 and SSRY 235) showed association with cassava mosaic disease resistance and were selected for marker assisted selection for the identification of CMD resistance among the clones.

Five SSR markers were selected based on bulk segregant analysis and were used for the identification of CMD resistant hybrids. All the 5 lines, 3 testers and 15 L x T crosses DNA were selected and PCR was performed using the selected SSR markers linked to cassava mosaic disease.

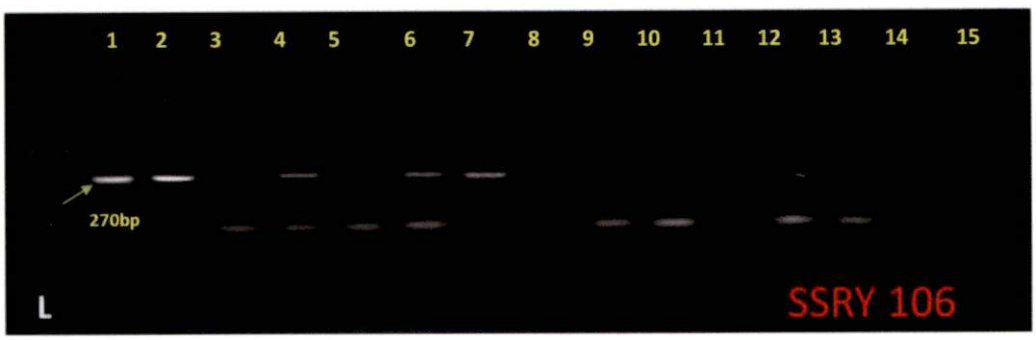
The marker SSRY 28 resulted in four bands (Plate 9.A) ranging from 170bp to 216 bp and all are found to be polymorphic. One allele (190 bp) was found to be present only in resistant parent (CI 273) and absent in the susceptible. Few clones from crosses *viz.* S. Jaya x CR 54A3, S. Vijaya x CR 54A3, CI 889 x CR 54A3, CI

Table 13. SSR markers profile for CMD in parents and crosses along with disease scores

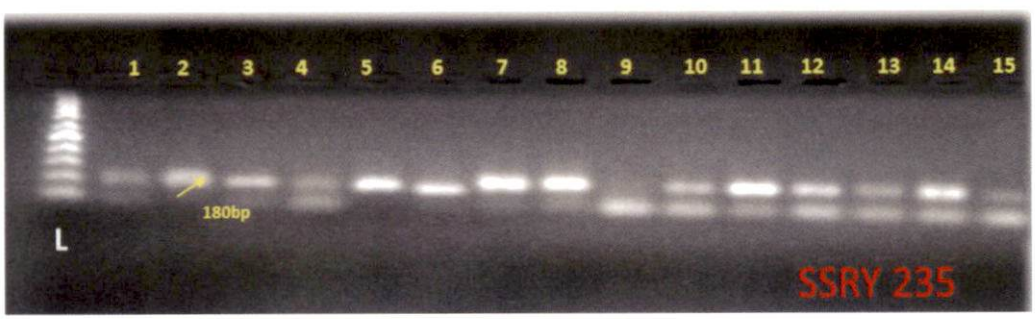
	CMD(1-5)scale	SSRY 28	SSRY 40	SSRY 44	SSRY 106	SSRY 235
PARENTS						
SREE JAYA	2	1	1	0	0	0
SREE VIJAYA	2	0	1	0	0	0
VELLAYANI HRASWA	1	1	1	0	0	1
CI 889	1	0	1	0	0	1
9S 75	1	1	1	0	0	0
CR 54A3	1	1	1	0	0	0
IMS2-5	1	1	0	0	0	1
CI 273	3	1	0	1	0	1
CROSSES						
S. JAYA X CR 54 A3	1	1	1	1	0	0
S. JAYA X IMS2-5	1	0	1	0	0	0
S. JAYA X CI 273	1	1	1	0	0	0
S. VIJAYA X CR 54 A3	4	1	0	0	1	0
S. VIJAYA X IMS2-5	2	1	1	0	0	1
S. VIJAYA X CI 273	2	0	1	0	0	0
V. HRASWA X CR 54 A3	1	1	0	0	1	1
V. HRASWA X IMS2-5	2	0	0	0	0	1
V. HRASWA X CI 273	2	1	1	0	1	0
CI 889 X CR 54 A3	3	1	0	0	0	0
CI 889X IMS2-5	2	1	0	0	0	0
CI 889X CI 273	4	1	0	0	0	0
9S 75 X CR 54 A3	3	1	0	0	0	0
9S 75X IMS2-5	1	1	0	0	0	0
9S 75X CI 273	2	1	0	0	0	0



(a)

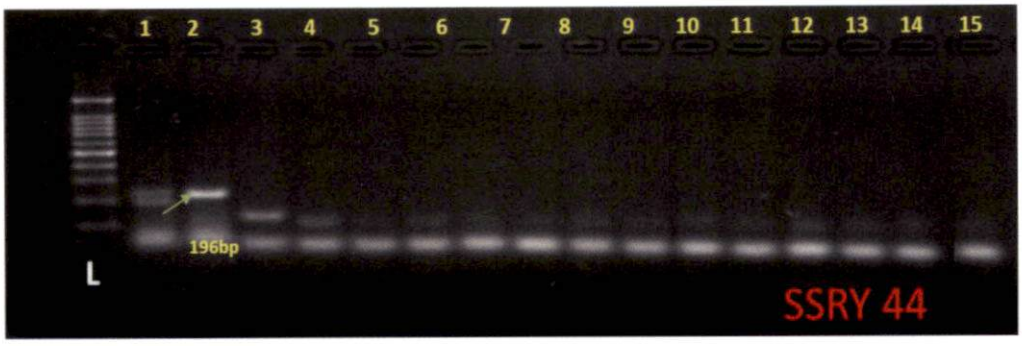


(b)

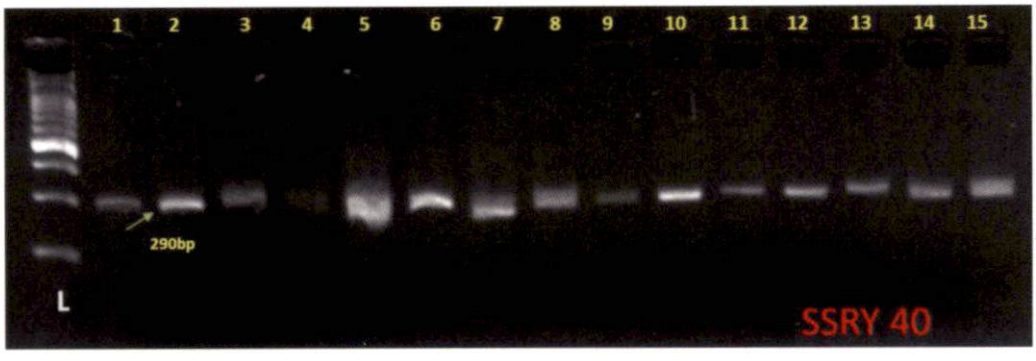


(c)

1-Sree Jaya X CR 54 A3, **2**- Sree Jaya X IMS2-5, **3**- Sree Jaya X CI 273, **4**- Sree Vijaya X CR 54 A3
5- Sree Vijaya X IMS2-5, **6**- Sree Vijaya X CI 273, **7**- V. Hraswa X CR 54 A3, **8**- V. Hraswa X IMS2-5
9- V. Hraswa X CI 273, **10**- CI 889 X CR 54 A3, **11**- CI 889 X IMS2-5, **12**- CI 889 X CI 273,
13- 9S 75 X CR 54 A3, **14**- 9S 75X IMS2-5, **15**- 9S 75X CI 273, **L-** Ladder 100bp



(d)



(e)

1-Sree Jaya X CR 54 A3, **2**- Sree Jaya X IMS2-5, **3**- Sree Jaya X CI 273, **4**- Sree Vijaya X CR 54 A3 **5**- Sree Vijaya X IMS2-5, **6**- Sree Vijaya X CI 273, **7**- V. Hraswa X CR 54 A3, **8**- V. Hraswa X IMS2-5 **9**- V. Hraswa X CI 273, **10**- CI 889 X CR 54 A3, **11**- CI 889 X IMS2-5, **12**- CI 889 X CI 273, **13**- 9S 75 X CR 54 A3, **14**- 9S 75X IMS2-5, **15**- 9S 75X CI 273, **L**- Ladder 100bp

Plate 9. Gel Images of SSR Markers Linked to CMD Resistance

(a) **Image of SSRY 28 Marker**
(b) **Image of SSRY 106Marker**
(c) **Image of SSRY 235Marker**
(d) **Image of SSRY 44Marker**
(e) **Image of SSRY 40Marker**

889 x CI 273 and 9S 75 x CR 54A3 also found to amplify this band (190bp) and are identified as genetically resistant clones.

The marker SSRY106 amplified five alleles (Plate 9.B) and it ranged from 260 bp to 320 bp of which only one allele (270 bp) was found to be polymorphic and this allele was present in resistant testers used (CR 54A3 and CI 273). Crosses, S. Jaya x CR 54A3, S. Vijaya x CR 54A3, V. Hraswa x CI 273 were found to be linked and rest crosses found to be genetically susceptible.

The marker SSRY 235 amplified five alleles (Plate 9.C), ranged from 120 to 190bp and found to be polymorphic. The unique allele 180bp occurred in the resistant genotypes *viz,* IMS2-5 and CI 273. The same unique allele occurred in the crosses *viz,* S. Jaya x IMS2-5, CI 889 x IM2-5 and S. Vijaya x IMS2-5 and found to be genetically resistant clones.

The SSRY44 amplified six alleles (Plate 9.D), ranged from 119bp to 212bp and were found to be polymorphic. The resistant allele of 196bp was found to be rare alleles, was present in parent CI 273 and cross S. Jaya x CR 54A3.

The SSRY40 amplified eight alleles (Plate 9.E), ranged from 273bp to 297bp. The resistant allele of 290bp was found to be rare alleles, was present only in parent CR 54A3 and among crosses S. Jaya x CR 54A3, S. Jaya x IMS2-5, S. Jaya x CI 273 and S. Vijaya x IMS2-5 were identified as genetically resistant clones to CMD.

Among the SSR markers studied, the maximum polymorphism was elucidated by SSRY 28, SSRY 44 and followed by SSRY 235. These polymorphic markers are found to be linked to CMD and hence can be used in marker assisted selection in cassava.

SSR markers profile for CMD in parents and crosses along with disease scoring (Table 13) revealed that the cross combination S. Jaya x CR 54A3, S.Vijaya x

Plate 10. Field View of Experiment III

IMS2-5, V. Hraswa x CR 54A3 and V. Hraswa x CI 273 three of five SSR markers found associated with CMD 2 gene.

4.3 EXPERIMENT III: EVALUATION OF EARLY BULKING CLONES WITH RESISTANCE TO CMD

The CMD resistant clones obtained through L x T crosses with short duration were multiplied through miniset technology and evaluated along with parents for the confirmation of early bulking nature. Harvesting was done at six months after planting.

4.3.1 Line x Tester Analysis

4.3.1.1 Analysis of Variance

The ANOVA uncovered profoundly huge contrast among the genotypes for all the quantitative attributes contemplated (Table 17). The mean total of squares because of crosses was exceptionally huge, demonstrating the various executions of various crosses for the attributes. The mean sum of squares because of parents versus crosses was exceedingly critical for all characters contemplated. Huge contrasts were additionally watched for all qualities in testers (male) and lines (females) demonstrating the predominance of non-additive gene action. The hybrids exhibited highly significant variation for all characters studied.

4.3.1.2 Mean Performance of Parents and Hybrids

The mean performance of lines, testers (Table 14) and crosses (Table 15) obtained for different characters are explained in ensuing paragraphs.

Table 14. Mean performance of Parents (Lines and Testers) for different morphological characters.

	Height at first branching	Plant height	Stem girth	Leaf retention	Inter nodal distance	Number of tubers	Mean weight of tubers	Length of tuber	Girth of tubers	Weight of foliage	Tuber yield
LINES											
SREE JAYA	0.000	208.667	10.333	3.000	3.333	8.333	0.267	29.00	16.000	1.100	2.100
SREE VIJAYA	100.333	243.667	8.667	2.667	3.333	12.00	0.180	27.667	13.667	0.933	2.167
VELLAYANI HRASWA	68.000	228.333	9.333	2.667	3.333	7.667	0.167	29.333	15.667	0.800	1.300
CI 889	92.333	250.00	10.667	2.000	3.000	9.333	0.230	37.00	13.00	1.533	2.167
9S 75	89.000	208.00	10.00	3.667	3.667	8.667	0.470	35.00	20.00	1.433	4.000
TESTERS											
CR 54A3	22.667	151	12.333	3.000	2.667	10.00	0.183	41.667	13.667	1.267	1.801
IMS2-5	74.667	280.333	10.667	4.000	3.00	10.00	0.167	28.667	16.000	1.333	1.567
CI 273	52.000	261.667	12.333	3.333	3.00	14.00	0.210	31.667	16.000	1.667	2.933

	Harvest Index	Dry matter content	Starch	Sugar	Cooking quality	CMD
LINES						
SREE JAYA	4.653	24.833	24.80	0.773	4.00	2.00
SREE VIJAYA	2.917	33.967	25.033	1.17	4.00	3.00
VELLAYANI HRASWA	2.153	36.600	27.533	1.677	4.00	3.00
CI 889	3.11	28.933	27.50	1.463	4.00	2.00
9S 75	6.71	24.700	30.20	0.840	4.00	1.00
TESTERS						
CR 54A3	2.633	34.10	26.167	0.893	4.00	1.00
IMS2-5	2.033	34.40	28.433	1.017	3.00	1.00
CI 273	3.81	36.667	28.633	0.980	4.00	1.00

Table 15. Mean performance of Line x Tester crosses for different morphological characters.

Character	Height At First Branch (cm)	Plant Height (cm)	Stem Girth (cm)	Leaf Retention (1-5 Score)	Internodal Distance	Number of Tubers Per Plant	Mean Weight of Tuber (kg)	Length of Tuber (cm)	Girth of Tuber (cm)	Weight of Foliage (kg/Plt)	Tuber Yield (kg/Plt)	Harvest Index	Dry Matter Content (%)	Starch (%)	Sugar (%)	Cooking Quality	CMD Disease Scoring (1-5 Scale)
S. JAYA X CR 54 A3	156.00	236.33	12.67	3.67	4.00	16.00	0.30	37.00	15.33	2.30	4.83	4.88	27.23	25.67	0.80	3.00	1.00
S. JAYA X IMS2-5	122.00	301.00	12.33	3.00	4.00	10.33	0.17	33.33	11.00	1.93	1.73	2.28	31.13	27.50	0.81	2.00	1.33
S. JAYA X CI 273	85.00	209.00	7.67	3.33	3.33	13.33	0.34	29.33	11.00	1.10	4.37	8.36	35.67	24.50	0.88	3.00	1.00
S. VIJAYA X CR 54 A3	0.00	207.00	9.33	2.33	2.67	10.67	0.26	27.67	11.00	1.10	2.63	2.16	41.07	29.00	0.97	2.00	3.67
S. VIJAYA X IMS2-5	66.67	223.67	11.00	3.00	3.33	9.33	0.27	28.67	14.00	0.93	2.50	6.98	40.70	27.13	1.04	3.00	1.67
S. VIJAYA X CI 273	25.00	257.00	8.33	3.33	2.67	8.33	0.31	30.33	12.67	1.73	2.53	1.60	35.13	27.27	0.88	2.00	2.00
V. HRASWA X CR 54 A3	61.00	261.33	10.67	3.00	3.33	11.67	0.24	28.00	12.00	1.23	2.80	3.00	27.97	29.13	1.29	3.00	1.33
V. HRASWA X IMS2-5	80.67	232.67	11.67	3.00	3.00	11.00	0.20	23.00	12.33	1.00	2.07	3.41	26.13	28.07	1.08	4.00	1.33
V. HRASWA X CI 273	0.00	259.00	10.67	3.00	3.67	9.67	0.11	23.00	10.00	1.07	1.07	1.18	27.63	23.77	0.86	3.00	2.33
CI 889 X CR 54 A3	0.00	182.00	9.33	2.33	3.00	7.67	0.12	21.67	10.00	0.87	0.93	1.36	32.53	29.83	0.89	3.00	1.67
CI 889X IMS2-5	84.67	323.00	12.33	4.00	4.00	8.67	0.22	21.33	11.33	0.97	1.87	2.24	38.57	26.47	0.81	3.00	2.33
CI 889X CI 273	70.67	192.00	12.67	3.33	4.00	11.33	0.10	26.33	12.67	1.00	1.17	1.27	30.80	27.90	0.79	4.00	4.00
9S 75 X CR 54 A3	0.00	270.00	9.67	3.00	3.67	12.00	0.09	26.33	7.67	1.00	1.03	1.51	27.37	28.13	1.27	4.00	2.33
9S 75X IMS2-5	0.00	273.33	9.00	3.00	3.67	11.67	0.11	25.67	8.33	1.23	1.30	1.47	28.65	25.67	1.07	3.00	1.33
9S 75X CI 273	129.00	307.33	10.00	3.33	3.67	10.33	0.10	27.00	7.33	0.93	3.73	6.29	34.33	27.70	1.35	4.00	2.33
Mean	59.99	242.01	10.51	3.09	3.36	10.52	0.21	29.07	12.64	1.24	2.29	3.30	32.14	27.22	1.03	3.00	1.80
C.D. 5%	36.67	62.79	1.91	0.83	0.81	2.91	0.06	4.58	2.57	0.22	0.51	1.12	1.85	1.75	0.10	0.27	0.87

Table 16. Observation on Nature of Branching, Number of Whiteflies and Mean Photosynthetic rate among parents and crosses

	Nature of Branching	Number of Whiteflies	Mean Photosynthetic rate (CO_2 μmol^{-1} $m^2 s^{-1}$)
PARENTS			
SREE JAYA	NB	11	19.27±1.29
SREE VIJAYA	TB	13	22.70±1.24
VELLAYANI HRASWA	TB	8	20.01±1.55
CI 889	TB	16	16.06±3.01
9S 75	TB	10	22.59±3.24
CR 54A3	NB	11	27.29±3.61
IMS2-5	TB	9	25.31±1.98
CI 273	TB	5	24.49±1.74
CROSSES			
S. JAYA X CR 54 A3	TB	7	30.06±3.11
S. JAYA X IMS2-5	TB	13	16.06±3.01
S. JAYA X CI 273	TB	13	26.06±2.11
S. VIJAYA X CR 54 A3	NB	12	15.06±2.14
S. VIJAYA X IMS2-5	TB	22	14.06±2.04
S. VIJAYA X CI 273	TB	9	25.06±2.41
V. HRASWA X CR 54 A3	TB	9	23.06±3.01
V. HRASWA X IMS2-5	TB	11	20.06±1.01
V. HRASWA X CI 273	SSP	12	21.06±3.04
CI 889 X CR 54 A3	NB	9	16.06±2.03
CI 889X IMS2-5	TB	9	17.06±2.01
CI 889X CI 273	TB	10	17.03±2.21
9S 75 X CR 54 A3	SP	13	26.06±1.31
9S 75X IMS2-5	SSP	16	24.06±2.12
9S 75X CI 273	TB	15	25.06±2.01

Table 17: Analysis of variance (ANOVA) for various characters – RBD

Source of Variation	DF	Mean Squares									
		Height at first branching	Plant height	Stem girth	Leaf retention	Inter nodal distance	Number of tubers	Mean weight of tubers	Length of tuber	Girth of tubers	Weight of foliage
Replication	2	744.289	909.356	0.422	0.089	0.467	3.267	0.006	0.022	1.622	0.021
Lines	4	11,385.42	1,831.02	8.644	1.056	0.356	10.633	0.022	35.301*	16.722*	0.823
Testers	2	10,413.62	6,598.02	1.489	0.622	1.667	19.267	0.032	176.089	34.689	0.773
Lines x Testers	8	6,269.218**	6,848.61**	8.961**	0.289**	0.556	12.767*	0.026**	34.033**	9.106**	0.329**
Error	28	694.217	845.475	1.398	0.16	0.252	3.552	0.019	3.951	1.717	0.03

Source of Variation	DF	Mean Squares						
		Tuber yield	Harvest Index	Dry matter content	Starch	Sugar	Cooking Quality	CMD
Replication	2	1.052	3.579	2.719	0.016	0.003	0.09	0.36
Lines	4	2.721	17.954	59.755*	8.419	0.102*	3.06	3.47
Testers	2	9.307	34.578	106.209	2.733	0.095	0.09	2.02
Lines x Testers	8	4.202**	10.814**	76.977**	10.365**	0.115*	1.09**	1.97**
Error	28	1.858	7.615	0.892	1.412	0.004	0.04	0.31

* Significant at 5% level ** Significant at 1% level

Table 18.Heterosis (%) over mid-parent (RH), better-parent (HB) and standard commercial variety (SH) for various characters

Crosses	Height At First Branch (cm)			Plant height(cm)		
	RH	HB	SH	RH	HB	SH
S. JAYA X CR 54 A3	276.47**	588.24**	129.41**	31.42*	13.26	3.50
S. JAYA X IMS2-5	226.79**	63.39*	79.41**	23.11*	7.37	31.82*
S. JAYA X CI 273	226.92**	63.46*	25.00	-11.13	-20.13	-8.47
S. VIJAYA X CR 54 A3	-100.00**	-100.00**	-100.00**	4.90	-15.05	-9.34
S. VIJAYA X IMS2-5	-23.81	-33.55	-1.96	-14.63	-20.21	-2.04
S. VIJAYA X CI 273	-67.18**	-75.08**	-63.24*	1.72	-1.78	12.55
V. HRASWA X CR 54 A3	34.56	-10.29	-10.29	37.79*	14.45	14.45
V. HRASWA X IMS2-5	13.08	8.04	18.63	-8.52	-17.00	1.90
V. HRASWA X CI 273	-100.00**	-100.00**	-100.00**	5.71	-1.02	13.43
CI 889 X CR 54 A3	-100.00**	-100.00**	-100.00**	-9.23	-27.20*	-20.29
CI 889X IMS2-5	1.40	-8.30	24.51	21.81*	15.22	41.46**
CI 889X CI 273	-2.08	-23.47	3.92	-24.95*	-26.62*	-15.91
9S 75 X CR 54 A3	-100.00**	-100.00**	-100.00**	50.42**	29.81*	18.25
9S 75X IMS2-5	-100.00**	-100.0**0	-100.00**	11.95	-2.50	19.71
9S 75X CI 273	82.98**	44.94*	89.71**	30.87*	17.45	34.60
CD 5%	32.28	37.27	37.27	55.27	63.82	63.82

Crosses	Stem Girth (cm)			Leaf Retention (1-5 Score)		
	RH	HB	SH	RH	HB	SH
S. JAYA X CR 54 A3	11.76	2.70	35.71**	22.22	22.22	37.50*
S. JAYA X IMS2-5	17.46*	15.63	32.14**	-14.29	-25.00*	12.50
S. JAYA X CI 273	-32.35**	-37.84**	-17.86	5.26	0.00	25.00
S. VIJAYA X CR 54 A3	-11.11	-24.32**	0.00	-17.65	-22.22	-12.50
S. VIJAYA X IMS2-5	13.79	3.13	17.86	-10.00	-25.00*	12.50
S. VIJAYA X CI 273	-20.63*	-32.43**	-10.71	11.11	0.00	25.00
V. HRASWA X CR 54 A3	-1.54	-13.51	14.29	5.88	0.00	12.50
V. HRASWA X IMS2-5	16.67	9.38	25.00	-10.00	-25.00*	12.50
V. HRASWA X CI 273	-1.54	-13.51	14.29	0.00	-10.00	12.50
CI 889 X CR 54 A3	-18.84*	-24.32**	0.00	-6.67	-22.22	-12.50
CI 889X IMS2-5	15.63	15.63	32.14	33.33**	0.00	50.00**
CI 889X CI 273	10.14	2.70	35.71**	25.00	0.00	25.00
9S 75 X CR 54 A3	-13.43	-21.62**	3.57	-10.00	-18.18	12.50
9S 75X IMS2-5	-12.90	-15.63	-3.57	-21.74*	-25.00*	12.50
9S 75X CI 273	-10.45	-18.92	7.14	-4.76	-9.09	25.00
CD 5%	1.68	1.94	1.94	0.73	0.85	0.85

* Significant at 5% level ** Significant at 1% level

Table 18. (Continued)

Crosses	Internodal Distance(cm)			No. of Tubers per Plant		
	RH	HB	SH	RH	HB	SH
S. JAYA X CR 54 A3	33.33**	20.00	20.00	74.55**	60.00**	108.70**
S. JAYA X IMS2-5	26.32*	20.00	20.00	12.73	3.33	34.78
S. JAYA X CI 273	5.26	0.00	0.00	19.40	-4.76	73.91**
S. VIJAYA X CR 54 A3	-11.11	-20.00	-20.00	-3.03	-11.11	39.13*
S. VIJAYA X IMS2-5	5.26	0.00	0.00	-15.15	-22.22	21.74
S. VIJAYA X CI 273	-15.79	-20.00	-20.00	-35.90**	-40.48**	8.70
V. HRASWA X CR 54 A3	11.11	0.00	0.00	32.08*	16.67	52.17**
V. HRASWA X IMS2-5	-5.26	-10.00	-10.00	24.53	10.00	43.48*
V. HRASWA X CI 273	15.79	10.00	10.00	-10.77	-30.95**	26.09
CI 889 X CR 54 A3	5.88	0.00	-10.00	-20.69	-23.33	0.00
CI 889X IMS2-5	33.33**	33.33*	20.00	-10.34	-13.33	13.04
CI 889X CI 273	33.33**	33.33*	20.00	-2.86	-19.05	47.83*
9S 75 X CR 54 A3	15.79	0.00	10.00	28.57*	20.00	56.52**
9S 75X IMS2-5	10.00	0.00	10.00	25.00	16.67	52.17**
9S 75X CI 273	10.00	0.00	10.00	-8.82	-26.19*	34.78
CD 5%	0.72	0.83	0.83	2.56	2.96	2.96

Crosses	Mean weight of tubers(kg)			Length of tuber(cm)		
	RH	HB	SH	RH	HB	SH
S. JAYA X CR 54 A3	33.33**	12.50	80.00**	4.72	-11.20*	26.14**
S. JAYA X IMS2-5	-23.08	-37.50**	0.00	15.61*	14.94	13.64
S. JAYA X CI 273	41.26**	26.25*	102.00**	-3.30	-7.37	0.00
S. VIJAYA X CR 54 A3	44.95**	43.64*	58.00**	-20.19**	-33.60**	-5.68
S. VIJAYA X IMS2-5	53.85**	48.15**	60.00**	1.78	0.00	-2.27
S. VIJAYA X CI 273	57.26**	46.03**	84.00**	2.25	-4.21	3.41
V. HRASWA X CR 54 A3	37.14*	30.91	44.00*	-21.13**	-32.80**	-4.55
V. HRASWA X IMS2-5	18.00	18.00	18.00	-20.69**	-21.59**	-21.59**
V. HRASWA X CI 273	-41.59**	-47.62**	-34.00	-24.59**	-27.37**	-21.59**
CI 889 X CR 54 A3	-40.32**	-46.38**	-26.00	-44.92**	-48.00**	-26.14**
CI 889X IMS2-5	10.92	-4.35	32.00	-35.03**	-42.34**	-27.27**
CI 889X CI 273	-53.03**	-55.07**	-38.00*	-23.30**	-28.83**	-10.23
9S 75 X CR 54 A3	-72.45**	-80.85**	-46.00*	-31.30**	-36.80**	-10.23
9S 75X IMS2-5	-64.40**	-75.89**	-32.00	-19.37**	-26.67**	-12.50
9S 75X CI 273	-70.59**	-78.72**	-40.00*	-19.00**	-22.86**	-7.95
CD 5%	0.05	0.06	0.06	4.04	4.66	4.66

* Significant at 5% level ** Significant at 1% level

Table 18. (Continued)

Crosses	Girth of tuber (cm)			Weight of foliage (kg/plant)		
	RH	HB	SH	RH	HB	SH
S. JAYA X CR 54 A3	3.37	-4.17	-2.13	94.37**	81.58**	187.50**
S. JAYA X IMS2-5	-31.25**	-31.25**	-29.7**9	58.90**	45.00**	141.67**
S. JAYA X CI 273	-31.25**	-31.25**	-29.79**	-20.48**	-34.00**	37.50*
S. VIJAYA X CR 54 A3	-19.51*	-19.51*	-29.79**	0.00	-13.16	37.50*
S. VIJAYA X IMS2-5	-5.62	-12.50	-10.64	-17.65*	-30.00**	16.67
S. VIJAYA X CI 273	-14.61	-20.83*	-19.15*	33.33**	4.00	116.67**
V. HRASWA X CR 54 A3	-18.18*	-23.40**	-23.40**	19.35*	-2.63	54.17**
V. HRASWA X IMS2-5	-22.11**	-22.92**	-21.28*	-6.25	-25.00**	25.00
V. HRASWA X CI 273	-36.84**	-37.50**	-36.17**	-13.51	-36.00**	33.33*
CI 889 X CR 54 A3	-25.00**	-26.83**	-36.17**	-38.10**	-43.48**	8.33
CI 889X IMS2-5	-21.84**	-29.17**	-27.66**	-32.56**	-36.96**	20.83
CI 889X CI 273	-12.64	-20.83*	-19.15*	-37.50**	-40.00**	25.00
9S 75 X CR 54 A3	-54.46**	-61.67**	-51.06**	-25.93**	-30.23**	25.00
9S 75X IMS2-5	-53.70**	-58.33**	-46.81**	-10.84	-13.95	54.17**
9S 75X CI 273	-59.26**	-63.33**	-53.19**	-39.78**	-44.00**	16.67
CD 5%	2.26	2.61	2.61	0.20	0.23	0.23

Crosses	Tuber yield (kg/plant)			Harvest Index		
	RH	HB	SH	RH	HB	SH
S. JAYA X CR 54 A3	147.86**	130.16**	271.79**	33.94*	4.87	126.63**
S. JAYA X IMS2-5	-5.45	-17.46	33.33	-31.70*	-50.93**	6.04
S. JAYA X CI 273	73.51**	48.86**	235.90**	97.48**	79.58**	288.08**
S. VIJAYA X CR 54 A3	32.77**	21.54	102.56**	-22.04	-25.83	0.46
S. VIJAYA X IMS2-5	33.93**	15.38	92.31**	182.15**	139.43**	224.30**
S. VIJAYA X CI 273	-0.65	-13.64	94.87**	-52.43**	-58.01**	-25.70
V. HRASWA X CR 54 A3	80.65**	55.56**	115.38**	25.49	14.05	39.47
V. HRASWA X IMS2-5	44.19**	31.91	58.97**	62.74**	58.20**	58.20*
V. HRASWA X CI 273	-49.61**	-63.64**	-17.95	-60.54**	-69.12**	-45.36
CI 889 X CR 54 A3	-52.94**	-56.92**	-28.21	-52.64**	-56.27**	-36.84
CI 889X IMS2-5	0.00	-13.85	43.59*	-12.90	-27.97	4.02
CI 889X CI 273	-54.25**	-60.23**	-10.26	-63.29**	-66.67**	-41.02
9S 75 X CR 54 A3	-64.37**	-74.17**	-20.51	-67.75**	-77.55**	-30.03
9S 75X IMS2-5	-53.29**	-67.50**	0.00	-66.45**	-78.14**	-31.89
9S 75X CI 273	7.69	-6.67	187.18**	19.58*	-6.26	192.11**
CD 5%	0.45	0.52	0.52	0.98	1.13	1.13

* Significant at 5% level ** Significant at 1% level

4.3.1.2.1 Height at First Branching (cm)

The mean height at first branching ranged from 0cm (L1) to 100cm (L2) among the lines, among testers 22.67 cm (T1) to 74.67 cm (T2). The cross L1 x T1 with 156.00cm had maximum, L2 x T1, L3 x T3, L5 x T1 and L5 x T2 expressed zero.

4.3.1.2.2 Plant Height (cm)

The value ranged from 250 cm (L4) to 208 cm (L5) among the lines, 151 cm (T1) to 280.33 cm (T2) among testers and 323 cm (L4 x T2) to 182 cm (L4 x T2) among the hybrid.

4.3.1.2.3 Stem Girth (cm)

The values ranged from 8.67 cm (L2) to 10.33 cm (L1) among the lines, 10.67 cm (T2) to 12.33 cm (T1 &T2) among testers and 12.67 cm (L1 x T1) to 7.67 cm (L1 x T3) among the crosses.

4.3.1.2.4 Leaf Retention (1-5 scale)

The leaf retention was high in L5 (4.00) followed by L1 (3.00) among lines, tester T2 (4.00) had high followed by both T1 (3.00) and T3 (3.00) for lesser retention value.

4.3.1.2.5 Internodal Distance (cm)

The value ranged from 3.00 cm (L4) to 3.67 (L5) among the lines, 2.67 cm (T1) to 3.00 cm (T2 & T3) among testers and 4.00 cm (L1 x T1, L1 x T2, L4 x T2 & L4 x T3) to 2.67 cm (L2 x T1 and L2 x T3).

4.3.1.2.6 Number of Tubers Plant[1]

The largest value was observed with L2 (12.00) followed by L4 (9.33) among lines, T3 (14.00) followed by T1 (10.00) & T2 (10.00) among testers. Among hybrids, L1 x T1 (16.00) with highest and L4 x T1 (7.67) lowest values.

4.3.1.2.7 Mean Weight of Tuber (kg)

The highest value among lines was recorded with L5 (0.47 kg) and lowest with L3 (0.17 kg), among testers T3 (0.21 kg) recorded higher value and among hybrids L1 x T3 (0.34 kg) recorded highest and L5 x T1 (0.09 kg) lowest values.

4.3.1.2.8 Length of Tuber (cm)

The maximum value for this trait among lines was recorded with L4 (37.00 cm) and minimum with L2 (27.67 cm), among testers T1 (41.67 cm) recorded the highest and among hybrids L1 x T1 (37.00 cm), L4 x T2 (21.33) recorded highest and lowest respectively.

4.3.1.2.9 Girth of Tuber (cm)

Among lines maximum girth was achieved by L5 (20.00 cm) followed by L1 (16.00 cm) and minimum with L4 (13.00 cm), among testers T1 (13.67 cm) had lesser value comparatively than that of T2 & T3 (16.00 cm each), among hybrids L1 x T1 (15.53 cm), L5 x T3 (7.33 cm) achieved maximum and minimum values respectively.

4.3.1.2.10 Weight of Foliage (kg per plant)

The maximum value for this trait among lines was recorded with L4 (1.53 kg/ plt) and minimum with L3 (0.80 kg/ plt), among testers T3 (1.67 kg/ plt) recorded the

highest and among hybrids maximum value with L1 x T1 (2.30 kg/ plt) and L4 x T1 with (0.87 kg/ plt) minimum values.

4.3.1.2.11 Tuber Yield (kg per plant)

The maximum value for this trait among lines was recorded with L5 (4.00 kg/plt) and minimum with L3 (1.30 kg/plt), among testers T3 (2.93 kg/plt) recorded the highest and among hybrids maximum value with L1 x T1 (4.83 kg/ plt) and L4 x T1 with (0.93 kg/ plt) minimum values.

4.3.1.2.12 Harvest Index

The maximum value for this trait among lines was recorded with L5 (6.71) and minimum with L3 (2.15), among testers T3 (3.81) recorded the highest and among hybrids L1 x T1 (8.36) recorded highest and L3 x T3 (1.18) with the lowest.

4.3.1.2.13 Dry Matter Content (%)

The values ranged from 24.70 % (L5) to 36.60 % (L3) among the lines, 34.10% (T1) to 36.67 % (T3) among testers and 41.07 % (L2 x T1) to 26.13 % (L3 x T3) among the crosses.

4.3.1.2.14 Starch (%)

The starch values ranged from 24.80 % (L1) to 30.20 % (L5) among the lines, 26.17% (T1) to 28.63 % (T3) among testers and among hybrids from 29.83 % (L4 x T1) to 23.77 % (L3 x T3).

4.3.1.2.15 Sugar (%)

The sugar content ranged from 0.77 % (L1) to 1.68 % (L3) among the lines, 0.89% (T1) to 1.02 % (T2) among testers and among hybrids 1.35 % (L5 x T3) to 0.79 % (L4 x T3).

4.3.1.2.16 Cooking Quality

The cooking quality score value (4.00) among all lines and testers was observed to be same, except T2 (3.00) and among hybrids higher score value (4.00) was recorded by L3 x T2, L4 x T3, L5 x T1 and L5 x T3.

4.3.1.2.17 CMD Disease Scoring (1-5 scale)

The scale value indicating 1.00 (no symptoms) was observed in L5 among lines and all the three testers. Among hybrids L1 x T1 and L1 x T3 recorded no symptoms of CMD and L4 x T3 was found to highly susceptible.

4.3.2 Heterosis

The mean execution of lines, testers (Table 14) and L x T crosses (Table 15) acquired for various characters were contrasted and the relating mid-parent (MP), better parent (BP) and standard check Vellayani Hraswa for the estimation of heterosis and the distinctions are being communicated according to per cent heterosis (Table 18) and the outcomes got are exhibited underneath.

4.3.2.1 Height at First Branching (cm)

The pertinent data on heterosis revealed that 4 crosses over the mid-parent, 3 over better parent and three over commercial check had shown significant and positive heterosis for the height at first branching. The values of heterosis ranged from -100 (L2 x T1, L3 x T3, L4 x T1, L5 x T1 and L5 x T3) to 276.47 per cent (L1 x T1), from -100 (L2 x T1, L3 x T3, L4 x T1, L5 x T1 and L5 x T3) to 588.24 per cent (L1 x T1), from -100 (L2 x T1, L3 x T3, L4 x T1, L5 x T1 and L5 x T3) to 129.41 per cent (L1 x T1) respectively with mid-parent, better parent and commercial check. The highest heterosis value over standard check was seen in the cross L1 x T1 (129.41 per cent) and lowest in L5 x T3 (89.71 per cent).

Table 18. (Continued)

Crosses	Dry matter content (%)			Starch (%)		
	RH	HB	SH	RH	HB	SH
S. JAYA X CR 54 A3	-7.58**	-20.14**	-25.59**	0.72	-1.91	-6.78*
S. JAYA X IMS2-5	5.12	-9.50**	-14.94**	3.32	-3.28	-0.12
S. JAYA X CI 273	15.99**	-2.73	-2.55	-8.30**	-14.44**	-11.02**
S. VIJAYA X CR 54 A3	20.67**	20.43**	12.20**	13.28**	10.83**	5.33
S. VIJAYA X IMS2-5	19.06**	18.31**	11.20**	1.50	-4.57	-1.45
S. VIJAYA X CI 273	-0.52	-4.18	-4.01	1.61	-4.77	-0.97
V. HRASWA X CR 54 A3	-20.89**	-23.59**	-23.59**	8.50**	5.81	5.81
V. HRASWA X IMS2-5	-26.38**	-28.60**	-28.60**	0.30	-1.29	1.94
V. HRASWA X CI 273	-24.57**	-24.64**	-24.50**	-15.37**	-17.00**	-13.68**
CI 889 X CR 54 A3	3.23	-4.59	-11.11**	11.18**	8.48*	8.35*
CI 889X IMS2-5	21.79**	12.11**	5.37*	-5.36	-6.92*	-3.87
CI 889X CI 273	-6.10*	-16.00**	-15.8**5	-0.59	-2.56	1.33
9S 75 X CR 54 A3	-6.92*	-19.75**	-25.23**	-0.18	-6.84*	2.18
9S 75X IMS2-5	-3.05	-16.72**	-21.72**	-12.45**	-15.01**	-6.78*
9S 75X CI 273	11.90**	-6.36*	-6.19*	-5.84*	-8.28**	0.61
CD 5%	1.63	1.88	1.88	1.54	1.78	1.78

Crosses	Sugar (%)			Cooking Quality		
	RH	HB	SH	RH	HB	SH
S. JAYA X CR 54 A3	-4.00	-10.45	-52.29**	-25.00**	-25.00**	-25.00**
S. JAYA X IMS2-5	-9.87	-20.66**	-51.89**	-42.86**	-50.00**	-50.00**
S. JAYA X CI 273	0.38	-10.20*	-47.51**	-25.00**	-25.00**	-25.00**
S. VIJAYA X CR 54 A3	-5.98	-17.09**	-42.15**	-50.00**	-50.00**	-50.00**
S. VIJAYA X IMS2-5	-4.57	-10.83*	-37.77**	-14.29**	-25.00**	-25.00**
S. VIJAYA X CI 273	-18.45**	-25.07**	-47.71**	-50.00**	-50.00**	-50.00**
V. HRASWA X CR 54 A3	0.39	-23.06**	-23.06**	-25.00**	-25.00**	-25.00**
V. HRASWA X IMS2-5	-20.05**	-35.79**	-35.79**	14.29**	0.00	0.00
V. HRASWA X CI 273	-35.01**	-48.51**	-48.51**	-25.00**	-25.00**	-25.00**
CI 889 X CR 54 A3	-24.19**	-38.95**	-46.72**	-16.67**	-16.67**	-16.67**
CI 889X IMS2-5	-34.68**	-44.65**	-51.69**	-4.76	-16.67**	-16.67**
CI 889X CI 273	-35.33**	-46.01**	-52.88**	0.00	0.00	0.00
9S 75 X CR 54 A3	46.15**	41.79**	-24.45**	0.00	0.00	0.00
9S 75X IMS2-5	15.26**	5.25	-36.18**	-14.29**	-25.00**	-25.00**
9S 75X CI 273	48.35**	37.76**	-19.48**	0.00	0.00	0.00
CD 5%	0.09	0.10	0.10	0.24	0.28	0.28

* Significant at 5% level ** Significant at 1% level

Table 18. (Continued)

Crosses	CMD Disease Score (1-5 Scale)		
	RH	HB	SH
S. JAYA X CR 54 A3	-33.33	-50.00*	-25.00
S. JAYA X IMS2-5	-11.11	-33.33	0.00
S. JAYA X CI 273	-50.00*	-50.00*	-25.00
S. VIJAYA X CR 54 A3	144.44**	83.33**	175.00**
S. VIJAYA X IMS2-5	11.11	-16.67	25.00
S. VIJAYA X CI 273	0.00	0.00	50.00
V. HRASWA X CR 54 A3	14.29	0.00	0.00
V. HRASWA X IMS2-5	14.29	0.00	0.00
V. HRASWA X CI 273	40.00	16.67	75.00*
CI 889 X CR 54 A3	42.86	25.00	25.00
CI 889X IMS2-5	100.00**	75.00*	75.00*
CI 889X CI 273	140.00**	100.00**	200.00**
9S 75 X CR 54 A3	133.33**	133.33**	75.00*
9S 75X IMS2-5	33.33	33.33	0.00
9S 75X CI 273	55.56*	16.67	75.00*
CD 5%	0.77	0.88	0.88

* Significant at 5% level ** Significant at 1% level

4.3.2.2 Plant Height (cm)

The data on heterosis revealed that 6 hybrids over mid-parent, none of the cross over the better parent and only two crosses over the commercial check had significant and positive heterosis for the plant height. The magnitude of heterosis varied from -24.95 (L4 x T3) to 50.42 per cent (L5 x T1), from -27.20 (L4 x T1) to 17.45 per cent (L5 x T3), from -20.29 (L4 x T1) to 41.46 per cent (L4 x T2) respectively with mid-parent, better parent and commercial check variety. The highest value of heterosis over standard check was revealed in the cross combination of L4 x T2 (41.46 per cent).

4.3.2.3 Stem Girth (cm)

The heterosis over mid parent ranged between -32.35 (L1 x T3) to17.46 per cent (L1 x T2). Six of the crosses exhibited significant negative heterosis over better parent. The heterosis over better parent ranged from -37.84 (L1 x T3) to 15.63 per cent (L1 x T2 and L4 x T2 each). The magnitude of standard heterosis varied from -17.86 (L1 x T3) to 35.71 per cent (L1 x T1 and L4 x T3). Out of 15 crosses, 5 hybrids exhibited significant positive heterosis over commercial check.

4.3.2.4 Leaf Retention (1-5 scale)

Only one hybrid expressed significant negative heterosis over mid parent, heterosis ranged from -21.74 (L5 x T2) to 33.33 per cent (L4 x T2). The four hybrids that revealed negative significant heterosis over the better parent for leaf retention were L1 x T2, L2 x T2, L3 x T2 and L5 x T2 (-25.00 each). Two hybrids exhibited desired positive heterosis for trait over commercial check. The estimates of standard heterosis over the check varied from -12.50 (L2 x T1) to 50.00 per cent (L4 x T2).

4.3.2.5 Internodal Distance (cm)

The heterosis over mid parent ranged between -15.79 (L2 x T3) to 33.33 per cent (L1 x T1, L4 x T2 and L4 T3). Only two crosses L4 x T2 and L4 x T3 (33.33 % each) expressed positive and significant for heterosis over better parent. The magnitude of standard heterosis varied from -20.00 (L2 x T1 and L2 x T3) to 20.00 per cent (L1 x T1, L1 x T2, L4 x T2 and L4 x T3). Out of 15 crosses, none of the cross combination showed the significant positive heterosis values over commercial check, V. Hraswa.

4.3.2.6 Number of Tubers Plant[1]

Estimates of relative heterosis revealed that out of 15 hybrids, 3 hybrids revealed positively significant heterosis over the mid parent. The magnitude of relative heterosis ranged from -35.90 (L2 x T3) to 74.55 % (L1 x T1). For heterobeltiosis, three hybrids showed significant and negative heterosis over better parent. The magnitude of heterobeltiosis varied from -40.48 (L2 x T3) to 60.00 % (L1 x T1). For standard heterosis, eight hybrids showed significant and positive heterosis over check V. Hraswa. The magnitude of standard heterosis varied from zero (L4 x T1) to 108.70 per cent (L1 x T1). Maximum standard heterosis for this trait was depicted by hybrid L1 x T1 (108.70 %).

4.3.2.7 Mean Weight of Tuber (kg)

The expression of significant heterosis over the mid parent in a desired positive direction was revealed in 6 crosses. Per cent heterosis over mid parent ranged from -72.45 per cent (L5 x T1) to 57.26 per cent (L2 x T3). Heterosis in F_1 hybrids over their respective better parent value ranged from -80.85 per cent (L5 x T1) to 48.15 per cent (L2 x T2). Expression of heterosis over better parent was in positive direction in four crosses. Six crosses manifested significant positive

heterosis over commercial check. The cross L1 x T3 (102 %) exhibited significantly higher positive heterosis over commercial check.

4.3.2.8 Length of Tuber (cm)

The range of relative heterosis for length of tuber was from -44.92 per cent (L4 x T1) to 15.61 per cent (L1 x T2). Significantly negative heterosis was exhibited by ten of crosses. Heterobeltiosis values ranged -48.00 per cent (L4 x T1) to 14.94 per cent (L1 x T2). Heterobeltiosis values were significantly positive in one of the cross. The standard heterosis ranged from -27.27 per cent (L4 x T2) to 26.14 per cent (L1 x T1). Standard heterosis was significantly negative in four crosses.

4.3.2.9 Girth of Tuber (cm)

Evaluations of relative heterosis uncovered that out of 15 crosses, 11 crosses indicated noteworthy negative heterosis over mid parent. The degree of relative heterosis ran from - 59.26 (L5 x T3) to 3.37 % (L1 x T1). For heterobeltiosis, thirteen hybrids indicated critical and negative heterosis over the better parent. The size of heterobeltiosis differed from - 63.33 (L5 x T3) to - 4.17 % (L1 x T1). For standard heterosis, thirteen hybrids demonstrated noteworthy and negative heterosis over check. The extent of standard heterosis changed from - 53.19 (L5 x T3) to - 2.13 % (L1 x T1). Greatest standard heterosis for this quality was delineated by cross L5 x T3 (- 53.19%).

4.3.2.10 Weight of Foliage (kg per plant)

The heterosis over mid parent ranged between -39.78 (L5 x T3) to 94.37 per cent (L1 x T1). Top two crosses for heterosis over mid parent were L1 x T1 (94.37 %) and L1 x T2 (58.90 %). Two crosses exhibited significant positive heterosis over the better parent. Heterosis over better parent ran from -44.00 (L5 x T3) to 81.58 per cent (L1 x T1). The magnitude of standard heterosis varied from 8.33 (L4 x T1) to

187.50 per cent (L1 x T1). Out of 15 crosses, 8 hybrids exhibited significant positive over commercial check values.

4.3.2.11 Tuber Yield (kg plant^{-1})

Heterosis over mid parent ranged between -64.37 (L5 x T1) to 147.86 per cent (L1 x T1). Among 15 crosses, three showed positively significant heterosis over better parent. The heterosis over better parent ran from -74.17 (L5 x T1) to 130.16 per cent (L1 x T1). The magnitude of standard heterosis varied from -28.21 (L4 x T1) to 271.79 per cent (L1 x T1). Out of 15 crosses, 9 hybrids exhibited significant positive heterosis over commercial check.

4.3.2.12 Harvest Index

The heterosis over mid parent ranged between - 67.76 (L5 x T1) to 182.15 per cent (L2 x T2). Top two crosses for heterosis over mid parent were L2 x T2 (182.15 %) and L1 x T3 (97.48 %). Out of 15, two traverses the heterosis over the better parent, positively and significantly. The heterosis over better parent extended from -78.14 (L5 x T2) to 139.43 per cent (L2 x T2). The magnitude of standard heterosis varied from -45.36 (L3 x T3) to 288.08 per cent (L1 x T3). Out of 15 crosses, 5 hybrids exhibited significant positive heterosis over commercial check.

4.3.2.13 Dry Matter Content (%)

The outflow of noteworthy heterosis over mid parent wanted positive way was uncovered in 5 crosses. Per cent heterosis over mid parent ranged from -26.38 per cent (L3 x T2) to 21.79 per cent (L4 x T2). Heterosis in F_1 hybrids over their respective better parent value ranged from -28.60 per cent (L3 x T2) to 20.43 per cent (L2 x T1). Expression of heterosis over better parent was in positive direction in 3 crosses. Ten crosses manifested significant negative heterosis over commercial

check. The cross L3 x T2 (12.20%) exhibited significantly higher positive heterosis over commercial check.

4.3.2.14 Starch (%)

The range of relative heterosis for starch was from -15.37 per cent (L3 x T3) to 13.28 per cent (L2 x T1). Significantly negative heterosis was exhibited by four of crosses over mid parent. Heterobeltiosis values ranged -17.00 per cent (L3 x T3) to 10.83 per cent (L2 x T1). Heterobeltiosis values were significantly positive in two of the crosses. The standard heterosis ranged from -13.68 per cent (L3 x T3) to 8.35 per cent (L4 x T1). Standard heterosis was significantly negative in four crosses.

4.3.2.15 Sugar (%)

An aggregate of 3 hybrids communicated significant positive heterosis over mid parent, which ranged from -35.33 (L4 x T3) to 48.35 per cent (L5 x T3). The three hybrids that showed significant positive heterosis for trait were L5 X T1 (46.15%), L5 x T2 (15.26 %) and L5 x T3 (48.35%) over mid parent. The degree of heterosis showed by the F_1 hybrids over their better parent ran from -48.51per cent (L3 x T3) to 41.79 per cent (L5 x T1). All the 15 hybrids exhibited desired negative heterosis for trait over commercial check. The estimates of standard heterosis over the check V. Hraswa varied from -52.88% (L4 x T3) to -19.48% (L5 x T3).

4.3.2.16 Cooking Quality

An aggregate of 4 cross hybrids communicated critical positive heterosis over mid parent, which ran from - 50.00 (L2 x T1 and L2 x T3) to 14.29 percent (L3 x T2). The degree of heterosis showed by the F_1 hybrids over their better parent ran from -50.00 (L1 x T2 and L2 x T1) to zero (L3 X T2, L4 x T3, L5 x T1 and L5 x T3). Eleven hybrids displayed negative heterosis for the cooking quality over the commercial check. The appraisals of standard heterosis over the check differed from -

50.00 (L1 x T2, L2 x T1 and L2 x T3) to zero percent (L3 x T2, L4 x T3, L5 x T1 and L5 x T3).

4.3.2.17 CMD Disease Scoring (1-5 scale)

For the trait under consideration negative heterosis is desirable. Heterosis over mid parent value ranged from -50.00 per cent (L1 x T3) to 144.44 per cent (L2 x T1), only one cross exhibited significantly negative heterosis over mid parent. The extent of heterosis over better parent ranged from -50.00 (L1 x T1 and L1 x T3) to 133.33 per cent (L5 x T1). Two crosses showed significantly negative heterosis over the better parent for CMD disease scoring. Similarly, high amount of economic heterosis was observed in crosses L4 x T3 (200.00%) and followed by L1 x T1 and L1 x T3 (-25.00 each) for lowest magnitude heterosis over commercial check.

4.3.3 Combining Ability Analysis

4.3.3.1 Combining Ability Variances

In the present study, for all characters sca variance was higher than gca variance as evidenced by ratio being less than one, suggesting significant role of non-additive gene action (Table 23) like dominance, epistasis and other interaction effects in expression of these characters. When non-additive genes govern the characters this suggest that there is constraint of improvement of these traits by using different selection methods as well as to go for hybrid breeding programme for exploitation of heterosis.

4.3.3.2. Estimation of Combining Ability (gca and sca) Effects.

The gca effects estimated for both lines and testers and the sca effects of hybrids for different traits studied are depicted in Tables 20 and 21 respectively. The salient features are presented as under,

Table 19. ANOVA for combining ability analysis

	DF	Height At First Branch (cm)	Plant Height (cm)	Stem Girth (cm)	Leaf Retention (1-5 Score)	Internodal Distance	Number of Tubers Per Plant	Mean Weight of Tuber (kg)	Length of Tuber (cm)	Girth of Tuber (cm)	Weight of Foliage (kg/Plt)
Replicates	2.0	479.23	329.75	2.80	0.04	0.28	5.52	0.00	10.28	6.97	0.00
Treatments	22.0	6506.71**	5248.80**	6.57**	0.73**	0.57**	12.93**	0.03**	77.85**	27.85**	0.42**
Parents	7.0	3773.57**	4841.66**	5.04**	1.18**	0.29	13.05**	0.03**	73.52**	14.57**	0.27**
Parents (Line)	4.0	5012.90**	1127.23	1.93	1.10**	0.17	8.43*	0.05**	51.07**	22.50**	0.30**
Parents (Testers)	2.0	2039.11*	1461.33**	2.78	0.78	0.11	16.00*	0.00	139.00**	5.44	0.14**
Parents (L vs T)	1.0	2285.14*	60.03	22.00**	2.34**	1.11*	25.60**	0.03**	32.40*	1.11	0.39**
Parents vs Crosses	1.0	210.12	6273.05*	0.04	0.08	1.41*	10.02	0.02**	432.33**	301.50**	0.02
Crosses	14.0	8323.04**	5379.21**	7.80**	0.56*	0.66**	13.09**	0.02**	54.69**	14.94**	0.53**
Line Effect	4.0	11473.98	4200.91	6.92	0.28	1.19	23.58	0.05*	141.74*	34.06*	0.97
Tester Effect	2.0	2932.16	6011.49	7.62	0.69	0.27	7.80	0.00	11.29	1.76	0.07
Line * Tester Eff.	8.0	8095.29**	5810.29**	8.29**	0.66*	0.49	9.16*	0.01**	22.01*	8.67**	0.43**
Error	44.0	496.66	1456.09	1.34	0.26	0.25	3.13	0.00	7.76	2.44	0.02

	DF	Tuber Yield (kg/Plt)	harvest Index	Dry Matter Content (%)	Starch (%)	Sugar (%)	Cooking Quality	CMD (1-5 Scale)
Replicates	2.0	0.05	0.13	0.58	0.24	0.00	0.06	0.19
Treatments	22.0	3.57**	12.72**	72.00**	8.86**	0.18**	1.49**	1.93**
Parents	7.0	2.20**	7.26**	73.13**	10.39**	0.30**	0.38**	0.66*
Parents (Line)	4.0	2.97**	9.83**	86.27**	14.61**	0.46**	0.36	0.60
Parents (Testers)	2.0	1.60**	2.45**	5.91*	5.63*	0.01*	1.00**	1.00*
Parents (L vs T)	1.0	0.34	6.60**	154.97**	3.01	0.28**	0.63**	0.22
Parents vs Crosses	1.0	0.04	1.44	4.78	0.17	0.21**	9.13**	4.22**
Crosses	14.0	4.51**	16.25**	76.23**	8.72**	0.11**	1.51**	2.40**
Line Effect	4.0	6.77	15.73	176.53*	6.47	0.26*	3.06	3.47
Tester Effect	2.0	1.96	5.08	13.87	17.48	0.04	0.09	2.02
Line * Tester Eff.	8.0	4.01**	19.30**	41.68**	7.65**	0.05**	1.09**	1.97**
Error	44.0	0.10	0.46	1.26	1.14	0.00	0.03	0.28

* Significant at 5% level ** Significant at 1% level

Table 20. General Combining Ability (gca) effects of parentsfor all the characters

	Height at first branching	Plant height	Stem girth	Leaf retention	Inter nodal distance	Number of tubers	Mean weight of tubers	Length of tuber	Girth of tubers	Weight of foliage
LINES										
SREE JAYA	62.29**	-0.20	0.40	0.22**	0.31	2.42**	0.08**	5.98**	1.33**	0.55**
SREE VIJAYA	-28.15**	-19.75**	-0.93**	-0.22**	-0.58**	-1.36**	0.08**	1.65**	1.44**	0.03
VELLAYANI HRASWA	-11.49**	2.02	0.51	-0.11	-0.13	-0.02	-0.02	-2.65**	0.33	-0.13**
CI 889	-6.94	-16.64	0.96**	0.11	0.20	-1.58**	-0.05**	-4.14**	0.22	-0.28**
9S 75	-15.71**	34.58**	-0.93**	-0.02	0.20	0.53	-0.09**	-0.91**	-3.33**	-0.18**
CD (5%)	**15.22**	**26.05**	**0.79**	**0.35**	**0.34**	**1.21**	**0.02**	**1.90**	**1.07**	**0.09**
TESTERS										
CR 54A3	-15.31**	-17.64**	-0.16	-0.24**	-0.13	0.80	0.01	0.89**	0.09	0.08**
IMS2-5	12.09**	21.76**	0.78**	0.09	0.13	-0.60	-0.01	-0.84**	0.29	-0.02
CI 273	-3.23	-4.11	-0.62**	0.16	0.02	-0.20	-0.01	-0.04	-0.38	-0.06
CD (5%)	**11.79**	**20.18**	**0.61**	**0.27**	**0.26**	**0.94**	**0.02**	**1.47**	**0.83**	**0.07**

	Tuber Yield(kg)	Harvest Index	Dry Matter Content (%)	Starch (%)	Sugar (%)	Cooking Quality	CMD (1-5 Scale)
LINES							
SREE JAYA	1.34**	1.97**	-0.99**	-1.29**	-0.16**	-0.44**	-0.87**
SREE VIJAYA	0.25**	0.38	6.64**	0.62	-0.02	-0.78**	0.47**
VELLAYANI HRASWA	-0.33**	-0.67**	-5.08**	-0.19	0.09**	0.22**	-0.311**
CI 889	-0.98**	-1.58**	1.64**	0.89**	0.15**	0.44**	0.69**
9S 75	-0.28**	-0.11	-2.21**	-0.02**	0.24**	0.55**	0.02
CD (5%)	**0.21**	**0.46**	**0.77**	**0.73**	**0.04**	**0.11**	**0.36**
TESTERS							
CR 54A3	0.14	-0.62**	-1.09**	1.17**	0.06**	-0.04	0.02
IMS2-5	-0.41**	0.07	0.71**	-0.22	-0.02	-0.04	-0.38**
CI 273	0.26**	0.53**	0.39	-0.96**	-0.04**	0.09**	0.36**
CD (5%)	**0.16**	**0.36**	**0.59**	**0.56**	**0.03**	**0.09**	**0.28**

*Significant at 5% ** Significant at 1%

Table 21: Specific Combining Ability (sca) effects of crosses

CROSSES	Height at first branching (cm)	Plant height (cm)	Stem girth (cm)	Leaf retention (1-5 scale)	Inter nodal distance (cm)	Number of tubers/plant	Mean weight of tubers(kg)	Length of tuber(cm)	Girth of tuber(cm)
S. JAYA X CR 54 A3	50.311**	5.200	1.933**	0.578	0.356	1.978	0.025	2.889	2.800**
S. JAYA X IMS2-5	-11.089	30.467	0.667	-0.422	0.089	-2.289*	-0.098**	0.956	-1.733
S. JAYA X CI 273	-39.222**	-35.667	-2.600**	-0.156	-0.444	0.311	0.073**	-3.844*	-1.067
S. VIJAYA X CR 54 A3	-15.244	-4.578	-0.067	-0.311	-0.089	0.422	-0.023	-2.111	-1.644
S. VIJAYA X IMS2-5	24.022	-27.311	0.667	0.022	0.311	0.489	-0.009	0.622	1.156
S. VIJAYA X CI 273	-8.778	31.889	-0.600	0.289	-0.222	-0.911	0.032	1.489	0.489
V. HRASWA X CR 54 A3	29.089*	27.978	-0.178	0.244	0.133	0.089	0.050*	2.444	0.467
V. HRASWA X IMS2-5	21.356	-40.089	-0.111	-0.089	-0.467	0.822	0.018**	-0.822	0.600
V. HRASWA X CI 273	-50.444**	12.111	0.289	-0.156	0.333	-0.911	-0.068	-1.622	-1.067
CI 889 X CR 54 A3	-36.467**	-32.689	-1.956**	-0.644*	-0.533	-2.356*	-0.033	-2.333	-1.422
CI 889X IMS2-5	20.800	68.911**	0.111	0.689*	0.200	0.044	0.074**	-0.933	-0.289
CI 889X CI 273	15.667	-36.222	1.844**	-0.044	0.333	2.311*	-0.041**	3.267	1.711
9S 75 X CR 54 A3	-27.689*	4.089	0.267	0.133	0.133	-0.133	-0.019	-0.889	-0.200
9S 75X IMS2-5	-55.089**	-31.978	-1.333	-0.200	-0.133	0.933	0.015	0.178	0.267
9S 75X CI 273	82.778**	27.889	1.067	0.067	0.001	-0.800	0.003	0.711	-0.067
CD (5%)	**26.36**	**45.13**	**1.37**	**0.60**	**0.59**	**2.09**	**0.04**	**3.29**	**1.85**

*Significant at 5% ** Significant at 1%

Table21 : (continued)

CROSSES	Weight of foliage (kg/plant)	Tuber yield (kg/plant)	Harvest Index	Dry matter content (%)	Starch (%)	Sugar (%)	Cooking quality	CMD
S. JAYA X CR 54 A3	0.449**	1.047**	0.323	-3.017**	-1.393*	-0.087*	0.378**	-0.133
S. JAYA X IMS2-5	0.169*	-1.500**	-2.967**	-0.920	1.827**	0.002	-0.622**	0.600
S. JAYA X CI 273	-0.618**	0.453*	2.644**	3.937**	-0.433	0.085*	0.244*	-0.467
S. VIJAYA X CR 54 A3	-0.229**	-0.064	-0.802*	3.194**	0.029	-0.052	-0.289**	1.200**
S. VIJAYA X IMS2-5	-0.309**	0.356	3.324**	1.024	-0.451	0.104**	0.711**	-0.400
S. VIJAYA X CI 273	0.538**	-0.291	-2.522**	-4.219**	0.422	-0.053	-0.422**	-0.800*
V. HRASWA X CR 54 A3	0.060	0.680**	1.091**	1.817**	0.973	0.155**	-0.289**	-0.356
V. HRASWA X IMS2-5	-0.087	0.500**	0.801	-1.820**	1.293*	0.024	0.711**	0.044
V. HRASWA X CI 273	0.027	-1.180**	-1.892**	0.003	-2.267**	-0.180**	-0.422**	0.311
CI 889 X CR 54 A3	-0.151	-0.531**	0.353	-0.339	0.596	0.004	-0.178	-1.022**
CI 889X IMS2-5	0.036	0.956**	0.540	3.891**	-1.384*	0.003	-0.178	0.044
CI 889X CI 273	0.116	-0.424*	-0.893*	-3.552**	0.789	-0.007	0.356**	0.978**
9S 75 X CR 54 A3	-0.129	-1.131**	-0.965*	-1.656*	-0.204	-0.020	0.378**	0.311
9S 75X IMS2-5	0.191**	-0.311	-1.698**	-2.176**	-1.284*	-0.134**	-0.622**	-0.289
9S 75X CI 273	-0.062	1.442**	2.663**	3.831**	1.489*	0.155**	0.244*	-0.022
CD (5%)	0.16	0.37	0.80	1.33	1.26	0.07	0.20	0.63

*Significant at 5% ** Significant at 1%

Table 22.Genetic components of variance and gene action

Sl. No.	GENETIC COMPONENTS	GCA/SCA RATIO	GENE ACTION
1	Height at first branching (cm)	0.3062	DOMINANCE
2	Plant Height (cm)	0.2954	DOMINANCE
3	Stem Girth (cm)	0.2991	DOMINANCE
4	Leaf Retention(1-5 Scale)	0.2192	DOMINANCE
5	Internodal distance (cm)	0.4975	DOMINANCE
6	No. of tubers/plant	0.5100	DOMINANCE
7	Mean weight of tuber(kg)	0.5294	DOMINANCE
8	Length of tuber(cm)	0.7069	DOMINANCE
9	Girth of tuber(cm)	0.5537	DOMINANCE
10	Weight of foliage (kg/plant)	0.3757	DOMINANCE
11	Tuber Yield(kg/plant)	0.3528	DOMINANCE
12	Harvest index	0.2087	DOMINANCE
13	Dry matter content (%)	0.5375	DOMINANCE
14	Starch (%)	0.4540	DOMINANCE
15	Sugar (%)	0.6174	DOMINANCE
16	Cooking Quality	0.4212	DOMINANCE
17	CMD (1-5 Scale)	0.4221	DOMINANCE

Table 23. Proportional contribution (%) of lines, testers and Line x Tester interaction to total variance in hybrids

Sl. No.	Characters	Lines	Testers	Line x Tester
1	Height at first branching (cm)	39.38	5.03	55.58
2	Plant Height (cm)	22.31	15.96	61.72
3	Stem Girth (cm)	25.34	13.95	60.69
4	Leaf Retention(1-5 Scale)	14.29	17.71	68.00
5	Internodal Distance (cm)	51.69	5.79	42.51
6	No. of tubers/plant	51.48	8.52	40.01
7	Mean weight of tuber(kg)	67.41	0.50	32.20
8	Length of tuber(cm)	74.05	2.95	22.99
9	Girth of tuber(cm)	65.15	1.68	33.18
10	Weight of foliage (kg/plant)	51.77	1.84	46.39
11	Tuber Yield(kg/plant)	42.92	6.22	50.87
12	Harvest Index	27.66	4.47	67.87
13	Dry matter content (%)	66.17	2.59	31.24
14	Starch (%)	21.19	67.89	50.16
15	Sugar (%)	69.09	5.08	25.85
16	Cooking Quality	57.89	0.84	41.26
17	CMD (1-5 Scale)	41.22	12.02	46.77

4.3.3.2.1 Height at First Branching (cm)

Out of 5 female parents, L1 (62.29) had significant positive gca effect, while other parents, L2 (-28.15) L3 (-11.49) and L5 (-15.71) had significant negative *gca* effect. In the remaining parent (L4) exhibited negative gca effects. Among the males T2 (12.09) showed significant positive gca effects, while T1 (-15.31) had significant negative gca effect.

In all 15 crosses, three had significant positive and five significant negative sca effects. In seven crosses sca effects were non-significant. The sca effect ranges from -55.089 (L5 x T2) to 82.778 (L5 x T3).

4.3.3.2.2 Plant Height (cm)

Significant positive gca effects in L5 (34.58) and significant negative gca effects L2 (-19.75) were noticed in female parents. In males, T3 (-4.11) with negative and T1 (-17.64) with negative and significant and T2 (21.76) exhibited significant positive gca effect. Only one cross displayed significant sca effects with positive and seven negative effects respectively. The estimates of specific combining ability effects ranged from 68.911 (L4 x T2) to -40.089 (L3 x T2).

4.3.3.2.3 Stem Girth (cm)

Significant positive gca effects for this trait in L4 (0.96) and significant negative gca effects in L2 and L5 (-0.93 each) were noticed in female parents. In males, T3 (-0.62) with negative and significant, T1 (-0.16) with negative and T2 (0.78) exhibited significant positive gca effect. Only two crosses each displayed significant sca effects with positive and negative effects respectively. The estimates of specific combining ability effects ranged from -2.6 (L1 x T3) to 1.93 (L1 x T1).

4.3.3.2.4 Leaf Retention (1-5 scale)

Two out of five females exhibited significant gca effects, one each of them exhibited significant negative and positive gca effect. Among males, only one parent T1 (-0.24) exhibited significant negative gca effects, T2 (0.09) and T3 (0.16) registered positive gca effects.

In all crosses, one had negative significant and one with positive significant sca effect, respectively. The maximum and minimum sca effect was noticed in crosses L4 x T2 (0.689) and L4 x T3 (-0.044) respectively. Remaining 13 crosses had non-significant sca effects.

4.3.3.2.5 Internodal Distance (cm)

The highest positive gca effects for this trait in L1 (0.31) and significant negative gca effects in L2 (-0.58) were noticed in female parents. In males, T3 (0.02), T2 (0.13) with positive and T1 (-0.13) exhibited negative gca effect.

None of the crosses displayed either significant sca effects with positive and negative effects. The estimates of specific combining ability effects ranged from -0.089 (L2 x T1) to 0.356 (L1 x T1).

4.3.3.2.6 Number of Tubers per Plant

Significant positive gca effects for this trait in L1 (2.42) and significant negative gca effects in L2 (-1.36) and L4 (-1.58) were noticed in female parents.

In males, T1 (0.80) with positive, T2 (-0.60) and T3 (-0.20) with gca effect.

Only one of the cross displayed significant positive sca effect and two with negatively significant effects. The estimates of sca effects ranged from -2.356 (L4 x T1) to 2.311 (L4 x T3).

4.3.3.2.7 Mean Weight of Tuber (kg)

Out of 5 female parents, L1 (0.08) and L2 (0.08) had significant positive gca effect, while among other parents, L4 (-0.05) and L5 (-0.09) had significant negative gca effect. In the remaining parent (L3) exhibited negative gca effects. Among the males T2 and T3 (-0.01 each) showed negative gca effects, while T1 (0.01) had positive gca effect.

In all 15 crosses, three had significant positive and two significant negative sca effects. In nine crosses sca effects were non-significant. The sca effect ranges from -0.098 (L1 x T2) to 0.07 (L1 x T3 and L4 x T2).

4.3.3.2.8 Length of Tuber (cm)

All the females were noticed with significant gca effects, among which L1 (5.98) had highest positive significant value and highly significant negative gca effects in L4 (-4.41). In males, T1 (0.89) with positive and significant, T2 (-0.84) with negative and significant and T3 (-0.04) with negative gca effect were noticed.

Only one of the cross displayed significant sca effects with negative. The estimates of specific combining ability effects ranged from -3.844 (L1 x T3) to 3.267 (L4 x T3).

4.3.3.2.9 Girth of Tuber (cm)

Out of 5 female parents, L1 (1.33) and L2 (1.44) had significant positive gca effect, while among other parents L5 (-3.33) had significant negative gca effect. In the remaining parents (L3 and L4) exhibited negative gca effects. Among the males T1 (0.09) and T2 (0.29) showed positive gca effects, while T3 (-0.38) had negative gca effect.

In all 15 crosses, only L1 x T1 (2.80) had significant positive sca effects. In 14 crosses sca effects were non-significant. The sca effect ranges from -1.733 (L1 x T2) to 2.8 (L1 x T1).

4.3.3.2.10 Weight of Foliage (kg per plant)

Significant positive gca effects for this trait in L1 (0.55) and highly significant negative gca effects in L4 (-0.28) were noticed in female parents. In males, T1 (0.08) with positive and significant, T2 (-0.02) and T3 (-0.06) with negative gca effect.

Four of the crosses displayed significant sca effect with positive and three with significantly negative effects. The estimates of sca effects ranged from -0.618 (L1 x T3) to 0.538 (L2 x T3).

4.3.3.2.11 Tuber Yield (kg per plant)

All five females exhibited significant gca effects, two of them exhibited significant positive gca effect. Among males, T2 (-0.41) exhibited significant negative gca effects, T3 (0.26) positive significant and T1 (0.14) registered positive gca effects.

In all crosses, four had negative significant and six with positive significant sca effect. The maximum and minimum sca effect was noticed in crosses L5 x T3 (1.42) and L1 x T2 (-1.50) respectively. Remaining 4 crosses had non-significant sca effects.

4.3.3.2.12 Harvest Index

Significant positive gca effects for this trait in L1 (1.97) and highly significant negative gca effects in L4 (-1.58) were noticed in female parents. In males, T1 (-0.62) with negative and significant, T2 (0.07) with positive and T3 (0.53) with significant and positive gca effect.

Seven of the crosses displayed significant sca effects with negative and four with significantly positive effects. The estimates of sca effects ranged from -2.967 (L1 x T2) to 3.324 (L2 x T3).

4.3.3.2.13 Dry Matter Content (%)

The highest positive gca effects for this trait in L2 (6.64) and significant negative gca effects in L3 (-5.08) were noticed in female parents. In males, T3 (0.39) with positive, T1 (-1.09) with significant negative and T2 (0.71) exhibited positive and significant gca effect.

Six of the crosses displayed significant sca effects with negative and five with significantly positive effects. The estimates of sca effects ranged from -4.219 (L2 x T3) to 3.937 (L1 x T3).

4.3.3.2.14 Starch (%)

The highest significant positive gca effects for this trait in L4 (0.89) and significant negative gca effects in L1 (-1.29) were noticed in female parents. In males, T1 (1.17) with significant positive, T2 (-0.22) with negative and T3 (-0.96) exhibited negative and significant gca effect.

Four of the crosses displayed significant sca effects with negative and three with significantly positive effects. The estimates of sca effects ranged from -2.267 (L3 x T3) to 1.827 (L1 x T2).

4.3.3.2.15 Sugar (%)

The highest significant positive gca effects for this trait in L5 (0.24) and significant negative gca effects in L1 (-0.16) were noticed in female parents. In males, T1 (0.06) with significant positive, T2 (-0.02) with negative and T3 (-0.04) exhibited negative and significant gca effect.

Three of the crosses displayed significant sca effects with negative effects and four with significantly positive effects. The estimates of sca effects ranged from -0.180 (L3 x T3) to 0.155 (L3 x T1 and L5 x T3).

4.3.3.2.16 Cooking Quality

The highest significant positive gca effects for this trait in L5 (0.55) and significant negative gca effects in L2 (-0.78) were noticed in female parents. In males, T3 (0.09) with significant positive, T1 and T2 (-0.04) with negative and T2 gca effect.

Six of the crosses showed significant sca effects with negative and seven with noteworthy constructive outcomes. The estimates of sca effects ranged from -0.622 (L1 x T2 and L5 x T2) to 0.711 (L2 x T2 and L3 x T2).

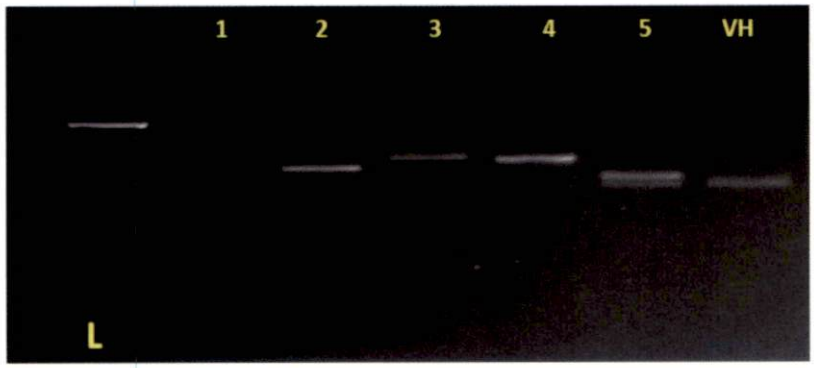
4.3.3.2.17 CMD Disease Score (1-5 scale)

The highest significant positive gca effects for this trait in L4 (0.69) and significant negative gca effects in L1 (-0.87) were noticed in female parents. In males, T3 (0.14) with positive, T1 (-0.38) with significant negative and T2 (0.36) exhibited positive and significant gca effect.

Two of the crosses showed critical sca effects with negative and two with noteworthy constructive outcomes. The appraisals of sca effects ran from - 1.022 (L4 x T1) to 1.200 (L2 x T1).

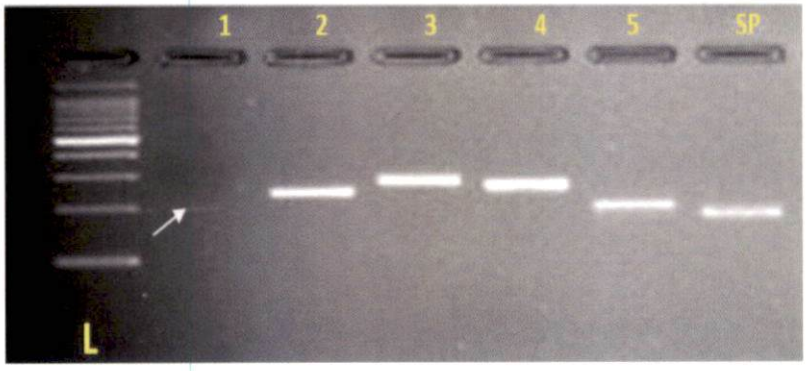
4.4 EXPERIMENT IV: BULK SEGREGANT ANALYSIS

DNA from the early bulking clones was isolated and pooled. Similarly DNA from the late bulking clones was isolated and pooled. Using molecular marker analysis pooled DNA from the early and late bulking segregants was compared.



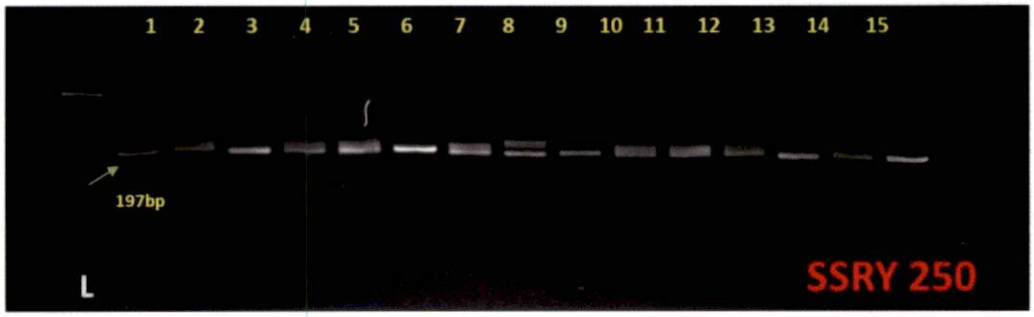
1- SSRY 250, 2- (ESTs) SSRY 47, 3- SSRY 63, 4- (ESTs) SSRY 292,
5- SSRY 239, L- Ladder (100bp), VH- Vellayani Hraswa

Plate 11. Gel Image of Early Bulking DNA Pool

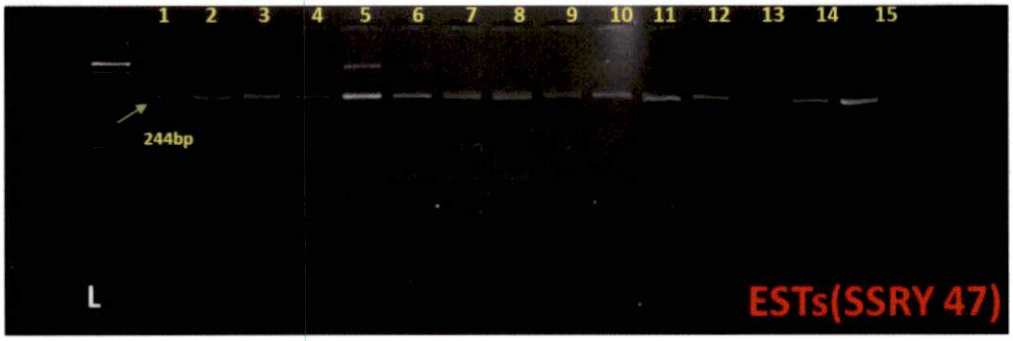


1- SSRY 250, 2- (ESTs) SSRY 47, 3- SSRY 63, 4- (ESTs) SSRY 292,
5- SSRY 239, L- Ladder (100bp), SP- Sree Padmanabha

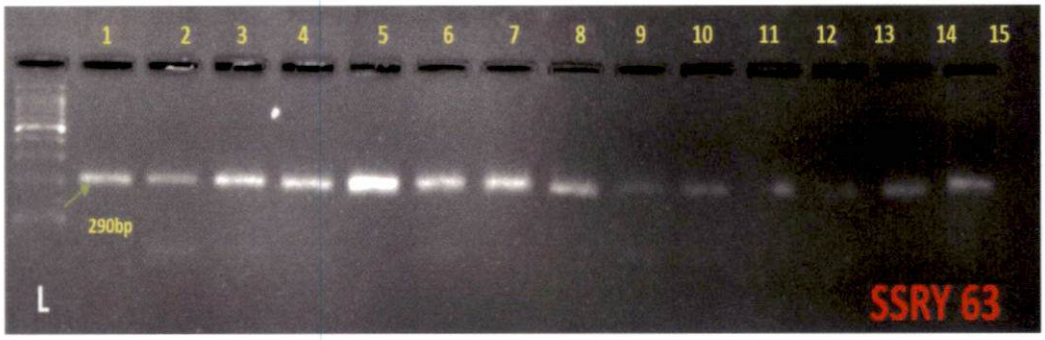
Plate 12. Gel Image of Late Bulking DNA Pool



(a)

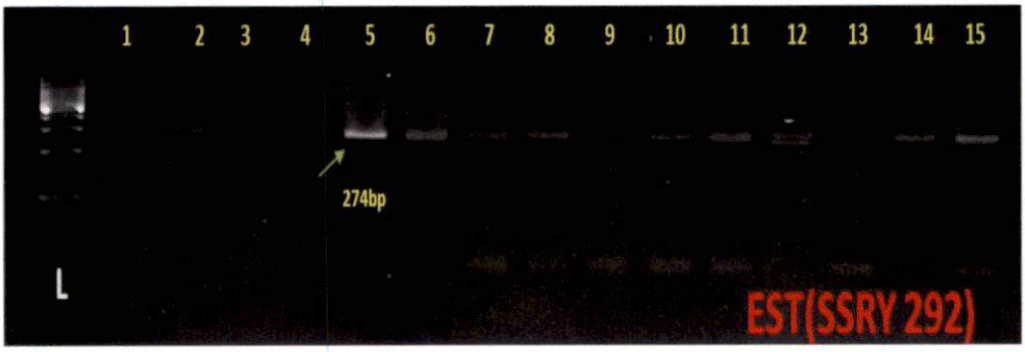


(b)

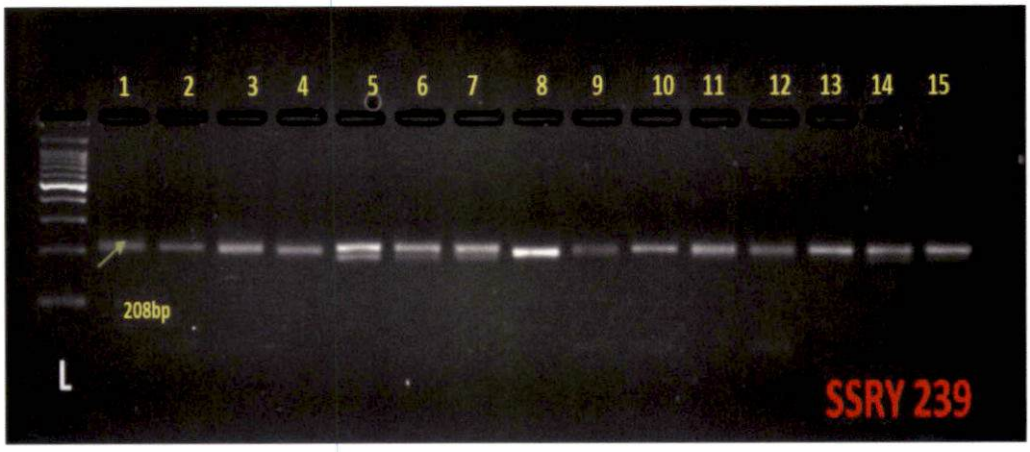


(c)

1-Sree Jaya X CR 54 A3, **2**- Sree Jaya X IMS2-5, **3**- Sree Jaya X CI 273, **4**- Sree Vijaya X CR 54 A3
5- Sree Vijaya X IMS2-5, **6**- Sree Vijaya X CI 273, **7**- V. Hraswa X CR 54 A3, **8**- V. Hraswa X IMS2-5
9- V. Hraswa X CI 273, **10**- CI 889 X CR 54 A3, **11**- CI 889 X IMS2-5, **12**- CI 889 X CI 273,
13- 9S 75 X CR 54 A3, **14**- 9S 75X IMS2-5, **15**- 9S 75X CI 273, **L**- Ladder 100bp



(d)



(e)

1-Sree Jaya X CR 54 A3, **2**- Sree Jaya X IMS2-5, **3**- Sree Jaya X CI 273, **4**- Sree Vijaya X CR 54 A3 **5**- Sree Vijaya X IMS2-5, **6**- Sree Vijaya X CI 273, **7**- V. Hraswa X CR 54 A3, **8**- V. Hraswa X IMS2-5 **9**- V. Hraswa X CI 273, **10**- CI 889 X CR 54 A3, **11**- CI 889 X IMS2-5, **12**- CI 889 X CI 273, **13**- 9S 75 X CR 54 A3, **14**- 9S 75X IMS2-5, **15**- 9S 75X CI 273, **L**- Ladder 100bp

Plate 13. Gel Images of Early Bulking SSR Markers

(a) **Image of SSRY 250 Marker**
(b) **Image of (ESTs)SSRY 47Marker**
(c) **Image of SSRY 63Marker**
(d) **Image of (ESTs)SSRY 44Marker**
(e) **Image of SSRY 239Marker**

Table 24.Early bulking SSR markers profile for parents and crosses

	SSRY 250	ESTs (SSRY) 47	SSRY 63	ESTs (SSRY) 292	SSRY 239
PARENTS					
SREE JAYA	0	1	0	1	1
SREE VIJAYA	1	0	0	1	0
VELLAYANI HRASWA	0	1	1	1	1
CI 889	0	0	0	0	1
9S 75	0	0	0	0	0
CR 54A3	0	0	0	1	0
IMS2-5	0	0	0	1	0
CI 273	0	0	0	0	0
CROSSES					
S. JAYA X CR 54 A3	0	1	1	0	1
S. JAYA X IMS2-5	1	0	0	1	1
S. JAYA X CI 273	1	1	0	1	1
S. VIJAYA X CR 54 A3	1	1	0	0	1
S. VIJAYA X IMS2-5	1	1	0	0	0
S. VIJAYA X CI 273	1	1	0	0	0
V. HRASWA X CR 54 A3	0	1	0	0	0
V. HRASWA X IMS2-5	0	0	0	0	0
V. HRASWA X CI 273	0	0	0	0	0
CI 889 X CR 54 A3	0	0	0	0	1
CI 889X IMS2-5	0	0	0	0	1
CI 889X CI 273	0	0	0	0	0
9S 75 X CR 54 A3	0	0	0	0	0
9S 75X IMS2-5	0	0	0	0	0
9S 75X CI 273	0	0	0	0	0

Table 25. Early bulking SSR primers gel scoring

	SSRY 250		ESTs (SSRY) 47		SSRY 63	ESTs (SSRY) 292		SSRY 239	
	1	2	1	2	1	1	2	1	2
S. JAYA X CR 54 A3	0	1	0	1	1	0	0	1	0
S. JAYA X IMS2-5	1	1	0	1	1	1	0	1	0
S. JAYA X CI 273	0	1	0	1	1	0	0	1	0
S. VIJAYA X CR 54 A3	1	1	0	1	1	1	0	1	0
S. VIJAYA X IMS2-5	1	1	1	1	1	1	1	1	1
S. VIJAYA X CI 273	0	1	0	1	1	1	1	1	1
V. HRASWA X CR 54 A3	1	1	0	1	1	1	0	1	1
V. HRASWA X IMS2-5	1	1	0	1	1	1	0	1	1
V. HRASWA X CI 273	0	1	0	1	0	1	0	1	0
CI 889 X CR 54 A3	1	1	0	1	1	1	0	1	0
CI 889X IMS2-5	1	1	0	1	0	1	1	1	0
CI 889X CI 273	1	1	0	1	0	1	1	1	0
9S 75 X CR 54 A3	0	1	0	1	1	0	0	1	0
9S 75X IMS2-5	1	1	0	1	1	1	0	1	1
9S 75X CI 273	0	1	1	1	1	1	0	1	1

Table 26.Jacccard similarity matrix for 5 early bulking SSR primer gel scoring

Crosses	L1xT1	L1xT2	L1xT3	L2xT1	L2xT2	L2xT3	L3xT1	L3xT2	L3xT3	L4xT1	L4xT2	L4xT3	L5xT1	L5xT2	L5xT3
L1×T1	1.00	.	.	.	.	.	.	.	.	.	.	.	.	.	.
L1×T2	0.67	1.00	.	.	.	.	.	.	.	.	.	.	.	.	.
L1×T3	1.00	0.67	1.00	.	.	.	.	.	.	.	.	.	.	.	.
L2×T1	0.67	1.00	0.67	1.00	.	.	.	.	.	.	.	.	.	.	.
L2×T2	0.44	0.67	0.44	0.67	1.00	.	.	.	.	.	.	.	.	.	.
L2×T3	0.57	0.63	0.57	0.63	0.78	1.00	.	.	.	.	.	.	.	.	.
L3×T1	0.57	0.86	0.57	0.86	0.78	0.75	1.00	.	.	.	.	.	.	.	.
L3×T2	0.57	0.86	0.57	0.86	0.78	0.75	1.00	1.00	.	.	.	.	.	.	.
L3×T3	0.60	0.67	0.60	0.67	0.44	0.57	0.57	0.57	1.00	.	.	.	.	.	.
L4×T1	0.67	1.00	0.67	1.00	0.67	0.63	0.86	0.86	0.67	1.00	.	.	.	.	.
L4×T2	0.43	0.71	0.43	0.71	0.67	0.63	0.63	0.63	0.67	0.71	1.00	.	.	.	.
L4×T3	0.43	0.71	0.43	0.71	0.67	0.63	0.63	0.63	0.67	0.71	1.00	1.00	.	.	.
L5×T1	1.00	0.67	1.00	0.67	0.44	0.57	0.57	0.57	0.60	0.67	0.43	0.43	1.00	.	.
L5×T2	0.57	0.86	0.57	0.86	0.78	0.75	1.00	1.00	0.57	0.86	0.63	0.63	0.57	1.00	.
L5×T3	0.57	0.63	0.57	0.63	0.78	0.75	0.75	0.75	0.57	0.63	0.44	0.44	0.57	0.75	1.00

Where, L1- SREE JAYA, L2- SREE VIJAYA, L3- VELLAYANI HRASWA, L4- CI 889, L5- 9S 75
T1- CR 54A3, T2- IMS2-5, T3- CI 273

Total of 9 primers were selected (Olasanmi, 2014), out of which only 5 got amplified (Table 8) and used for the analysis.

Early bulking DNA pool along with V. Hraswa as a control (Plate 11) and late bulking DNA pool along with Sree Padmanabha (Plate 12) as a control were framed and analysed for the selected 5 early bulking SSR markers.

Early bulking SSR maker SSRY 250 resulted in 13 amplifying bands (Plate 13. A) ranging from 170bp to 214bp and found to be polymorphic. One allele (194bp) found to be present in early bulking parental check (V. Hraswa) and absent in late bulking parental check (Sree Padmanabha) used for analysis. Clones from crosses *viz.* S. Jaya x CR 54A3, S. Jaya x CI 273, S. Vijaya x CR 54A3, S. Vijaya x IMS2-5 and S. Vijaya x CI 273 also found to amplify this band (194bp) and are identified as genetically early bulking clones.

Early bulking SSR maker (ESTs) SSRY 47 resulted in 10 amplifying bands (Plate 13. B) ranging from 200bp to 251bp. One allele (236bp) found to be present in early bulking parental check (V. Hraswa) and absent in late bulking parental check (Sree Padmanabha) used for analysis. Clones from crosses *viz.* S. Jaya x CR 54A3, S. Jaya x CI 273, S. Vijaya x CR 54A3, S. Vijaya x IMS2-5, S. Vijaya x CI 273, V. Hraswa x CR54A3 also found to amplify this band (236bp) and are identified as genetically early bulking clones.

Early bulking SSR maker SSRY 63 resulted in 11 amplifying bands (Plate 13. C) ranging from 270bp to 311bp and none exhibited polymorphism. One allele (290bp) found to be present in early bulking parental check (V. Hraswa) and absent in late bulking parental check (Sree Padmanabha) used for analysis. Only one cross S. Jaya x CR54A3 found to amplify this band (290bp) and are identified as genetically early bulking clones

Early bulking SSR maker (ESTs) SSRY 292 resulted in 6 amplifying bands (Plate 13. D) ranging from 226bp to 297bp and all found to be polymorphic in nature. One allele (294bp) found to be present in early bulking parental check (V. Hraswa) and absent in late bulking parental check (Sree Padmanabha) used for analysis. Clones from crosses *viz.* S. Jaya x CR 54A3 and S. Jaya x CI 273 found to amplify this band (294bp) and are identified as genetically early bulking clones.

Among early bulking SSR maker SSRY 239 resulted in 10 amplifying bands (Plate 13. E) ranging from 181bp to 221bp and all found to be polymorphic in nature. One allele (208bp) found to be present in early bulking parental check (V. Hraswa) and absent in late bulking parental check (Sree Padmanabha) used for analysis. Clones from crosses *viz.* S. Jaya x CR 54A3, S. Jaya x IMS2-5, S. Jaya x CI 273, V. Hraswa x CR 54A3 and V. Hraswa x IMS2- 5 found to amplify this band (208bp) and are identified as genetically early bulking clones.

Early bulking SSR markers profile for parents and crosses were tabulated in Table 24. Among the cross combinations S. Jaya x CR54A3, S. Jaya x IMS2-5 and S. Vijaya x CR 54A3, three of five markers revealed alleles associated with early bulking nature.

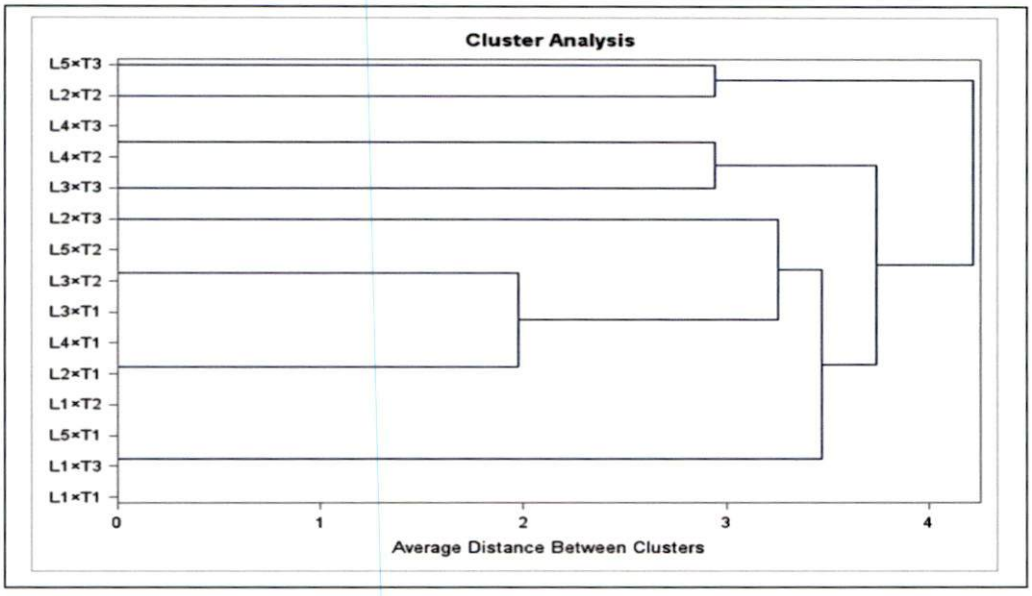


Figure 3. Cluster diagram for early bulking SSR primer gel scoring data

Sree Jaya x CR 54A3 (L1 x T1)

Sree Jaya x CI 273 (L1 x T3)

9S 75 x CR 54A3 (L5 x T1)

Sree Jaya x CR 54A3 (L1 x T1)

Plate 14. Selected Crosses with CMD resistance and Early Bulking Nature

DISCUSSION

5. DISCUSSION

Cassava, a native to South America is one of the important tuber crops of world. For an organized plant breeding, it is important to identify the plant parents, as well as crosses which might be exploited to bring further genetic improvement in economic traits. The objectives in crop improvement programme continue to be those which are of crucial importance in negating the world food crisis. The pressure of population and the consequent increase in demand for food on one hand and the depleting resources on the other hand has led to a ceiling in the crop yield improvement. Several parameters of selection have been developed in plant breeding research to achieve desirable genetic improvement for yield and other desirable quality components.

It is a harvest with one of kind favorable circumstances of delivering satisfactory yields on fruitless soils in the midst of unpredictable precipitation when most different products would fall flat. Disregarding cassava's strength, its yield potential is only sometimes acknowledged due to various constraining components including: soil barrenness, dry seasons, bugs, sicknesses, utilization of sub-par and low yielding cultivars, and absence of enhanced early bulking cultivars. Regardless, there are various open doors for hereditary change of cassava, particularly as far as protection from CMD and early bulking. Change of these attributes, especially early bulking, and stands into extraordinarily advantage numerous subsistence agriculturists and their families who depend on cassava and its items for their day by day life. Early bulking cultivars are urgent underway zones where there is developing weight on rural land compelling agriculturists to strengthen generation, and in drier territories where early bulking cultivars can be reaped after just a single cycle of rain. Ordinary cassava rearing through hybridization is, in any case, influenced by various cassava natural components, including abnormal amounts of hereditary heterozygosity, variable blossoming designs, low seed set and germination. By and

large, gives a comprehension of the difficulties, openings, and advance in cassava reproducing.

The experiment with an objective of introgression of cassava mosaic disease resistance to short duration varieties of cassava through marker assisted selection and to study the inheritance of early bulking nature, the results obtained were discussed in this chapter.

5.1 EXPERIMENT I: RAISING OF POLLINATION BLOCK AND HYBRID SEED PRODUCTION

The experimental materials comprising of Five early bulking high yielding lines *viz,* S. Jaya, S. Vijaya, V. Hraswa, CI 889 & 9S 75 and three testers *viz,* CR 54A3, IMS2-5 and CI 273 with resistance to cassava mosaic disease were crossed to produce fifteen F_1 combinations, from which reliable amount of seeds and seedlings were obtained.

5.2 EXPERIMENT II: SCREENING OF SEEDLINGS FOR RESISTANCE AND EARLY BULKING NATURE

The F_1 seedlings obtained were subjected to detailed study on variation among them and correlation of morphological characters with tuber yield and CMD.

5.2.1 Mean Performance of F_1 Seedlings

In the current experimental data, results from ANOVA shown highly significant difference among the genotypes for all the quantitative traits studied, thereby justifying the selection of parents for the study. The mean sum of squares due to crosses was highly significant, indicating the diverse performance of different cross combinations. The mean sum of square values due to plant parents v/s crosses was greatly significant for all characters illuminating the presence of heterosis due to

the significant difference in the mean performance of hybrids and parents. Significant differences were also detected for all characters studied in lines and testers, indicating the preponderance of non-additive gene action. The hybrids exhibited highly significant variation for all the characters studied. The growth, yield and yield attributing traits like plant height, stem girth, no. of tubers plant^{-1}, mean weight of tuber, length of tuber, girth of tuber and tuber yield have shown remarkable variation among the parents studied. Such variances for these characters were in accordance with the earlier reports in cassava by Mandal (1993); Brown and Caligari (2009).

Among the traits plant height and stem girth exhibited significant and positive correlation for all the characters except tuber yield which holds same as earlier record by Rajendran *et al.*(1985), Naskar *et al.* (1991).

No. of tubers per plant, mean weight of tuber, tuber length and tuber girth exhibited significant and positive correlation for all the characters which holds same as earlier recorded by Fukuda (1979) and Balashanmugham (1980). The trait CMD exhibited significant and positive correlation for all the characters studied and also highest negative significance with tuber yield, indicating yield and disease correlation for the hybrid seedlings studied. Such correlations for these traits were in accordance with earlier reports in cassava by Manga (1994), Akinwale *et al.* (2010), Adeniji *et al.* (2011), Mulualem and Ayenew (2012), Babu Rao *et al.* (2015); Patrick *et al.* (2016).

5.2.2 Marker Aided Selection of CMD Resistant Clones

Multiplex PCR, a tool used for simultaneous finding of cassava with CMD (Alabi *et al.*, 2008). Through multiplex PCR, hybrid seedlings of Sree Jaya x IMS2-5, Vellayani Hraswa x IMS2-5 and CI 889 x CI 273 confirmed presence of SLCMV in causing cassava mosaic disease in the most of hybrid seedlings studied among crosses. The viral load scale in RT PCR recorded CI 889 and 9S 75 to be highly resistant. S. Jaya, S. Vijaya and V. Hraswa grouped to be field tolerant among parents. Among crosses S. Jaya x CR 54A3, S. Jaya x IMS2-5, S. Jaya x CI 273, 9S

75 x CR 54A3, 9S 75 x IMS2-5 and 9S 75 x CI 273 grouped to be highly resistant seedlings. Among the crosses V. Hraswa x CI 273 found to record with highest viral load.

Among F_1 clones only the line 9S 75 grouped to be highly resistant, CI 889 as field tolerant and rest of the lines, S. Jaya, S. Vijaya and V. Hraswa confined to be susceptible to CMD virus. Viral load scale among crosses, S. Jaya x CR 54A3, S. Jaya x CI 273 and 9S 75 x CR 54A3 grouped as highly tolerant. Crosses, 9S 75 x IMS2-5 and 9S 75 x CI 273 found to be field tolerant. Among the crosses, S. Vijaya x IMS2-5 had a highest viral load and highly susceptible to CMD. A Standard curve with No. of cycles v/s Florescence has indicated S. Jaya x CR 54A3 with zero viral load based on the amplifying cycles. The results obtained were evident of the earlier works done by Deepthi (2017)

5.2.3 Molecular Characterisation to the Resistance of Selected Clones by using SSR Markers Linked to CMD Resistant

The different SSR primers selected based on review of literature were screened by Bulk Segregant Analysis. Out of SSR markers screened, five markers (SSRY 28, SSRY 40, SSRY 44, SSRY 106 and SSRY 235) showed association with cassava mosaic disease resistance and were selected for MAS to identify CMD resistance among the clones.

The marker SSRY 28 resulted in four bands and is found to be polymorphic. Few clones from crosses *viz.* S. Jaya x CR 54A3, S. Vijaya x CR 54A3, CI 889 x CR 54A3, CI 889 x CI 273 and 9S 75 x CR 54A3 also found to amplify resistant band (190bp) and are identified as genetically resistant clones. The marker SSRY106 found to be linked to CMD resistant among crosses, S. Jaya x CR 54A3, S. Vijaya x CR 54A3, V. Hraswa x CI 273 and found to be genetically susceptible. In marker SSRY 235 the unique resistant allele occurred in the crosses *viz.,* S. Jaya x IMS2-5,

CI 889 x IM2-5 and S. Vijaya x IMS2-5 and found to be genetically resistant clones. The SSRY44 amplified six alleles and were found to be polymorphic. The resistant allele was found to be present in parent CI 273 and cross S. Jaya x CR 54A3. In SSRY40 rare alleles was present only in parent CR 54A3 and among crosses S. Jaya x CR 54A3, S. Jaya x IMS2-5, S. Jaya x CI 273 and S. Vijaya x IMS2-5 and were identified as genetically resistant clones to CMD.

Among the SSR markers studied, the maximum polymorphism was elucidated by SSRY 28, SSRY 44 and followed by SSRY 235. These polymorphic markers are found to be linked to CMD and hence can be used in marker assisted selection in cassava. These results were found to associate with the earlier reports by Fragene (2000) and Mohan *et al.* (2013) on marker association with CMD.

SSR markers profile for CMD in parents and crosses along with disease scoring revealed that the cross combination S. Jaya x CR 54A3, S. Vijaya x IMS2-5, V. Hraswa x CR 54A3 and V. Hraswa x CI 273 three of five SSR markers found associated with CMD 2 gene. These reports holds as good to the earlier reports of Akano *et al.* (2002), Fragene *et al.* (2000), Okogbeni *et al.* (2012) and Mohan *et al.* (2013).

5.3 EXPERIMENT III: EVALUATION OF EARLY BULKING CLONES WITH RESISTANCE TO CMD

5.3.1 Line x Tester Analysis

5.3.1.1 Analysis of Variance

It has shown highly significant differences among genotypes for all the traits. This indicated that great amount of genetic variation is present in the materials for all the characters studied. The significant mean squares attributable to parents as well as hybrids depicted presence of adequate variability in them for all the characters. The

higher magnitude of mean squares of parents as compared to hybrids indicated that the parents are more variable as compared to hybrids. Comparison of mean squares due to parents v/s hybrids was found to be significant for all the traits. This specified that average performance of hybrids (Lines x Testers) may significantly differ for these traits depending upon the genetic makeup of the constituent parents.

5.3.1.2 Mean Performance of Parents and Hybrids for all the characters.

The higher mean values among parent (Lines) varies as, Sree Jaya recorded highest values only for the character of stem girth, Sree Vijaya for height at first branch and no. of tubers plant^{-1}, Vellayani Hraswa for dry matter content and sugar content, CI 889 recorded to be high in plant height, length of tuber and weight of foliage; 9S 75 was found to be high in many of the characters *viz*, leaf retention, inter-nodal distance, mean weight of tuber, girth of tuber, tuber yield, harvest index, starch per cent and recorded with CMD scale yielding to no symptoms.

Mean values of higher end among testers, CR54 A3 for stem girth and length of tuber, IMS2-5 for height at first branch, plant height, leaf retention, inter-nodal distance, girth of tuber and high sugar per cent. Tester CI 273, high mean value for stem girth, inter-nodal distance, no. of tuber plant^{-1}, mean weight of tuber, girth of tuber, weight of foliage, tuber yield, harvest index, dry mater content and starch per cent. All the selected testers were CMD resistant parents

The mean height at first branching was found to be highest in S. Vijaya and IMS2-5 among parents. Among crosses S. Jaya x CR54A3 found to be recorded with the maximum height at first branch.

5.3.2 Heterosis

Plant breeding can be divided into three stages; creation of a gene pool of variable germplasm, selection of superior individuals from the gene pool and

utilization of the selected individuals directly for commercial cultivation or in hybridization to create a superior variety. The improvement in yield, which is considered as a final product in almost all the crop plants is usually obtained by screening and selecting the suitable genes from a huge collection of germplasm and accumulating them in a productive genotype for commercial cultivation.

The genetic yield potential of varieties and hybrids can be improved by using suitable parents in hybridization. The information regarding extent of heterosis and combining ability for various characters is of great value in handling the breeding materials. Development of hybrids necessitates the incorporation of good parents in their genetic makeup. Sometimes high yielding parents may not produce superior hybrids. Thus, the identification of specific parental combination capable to produce the desired level of heterotic effect by their F_1 is also important in improvement of yield potential. The knowledge of combining ability provides a useful clue for selection of desirable parents for the development of better hybrids. Information regarding gene action is also very essential for developing superior genotype.

In the present investigation heterosis and combining ability effects were studied for twenty traits to identify and develop early bulking and CMD resistant hybrids. The mean performance of lines, testers and LxT crosses obtained for different characters were compared with resultant mid-parent (MP), better parent (BP) and V. Hraswa, a standard variety for estimation of heterosis.

5.3.2.1 Growth Parameters

Heterosis for growth parameters is an indication of heterosis for yield because growth and yield parameters are strongly associated. The ideal plant type depends on height at first branching, plant height, inter-nodal distance and weight of foliage. These are the major parameter which acts as source trait to support yield and its component traits.

For these traits hybrids showed high mean value over standard check. The data on heterosis also showed that the hybrids in general were best performer. Out of fifteen crosses, three showed significant positive standard heterosis for height at first branching. This suggested importance of dominant gene action in the study.

The cross S. Jaya x CR54A3 showed highest standard heterosis for this character. Out of fifteen crosses, two showed significantly positive standard heterosis for height of plant. The cross V. Hraswa x IMS2-5 showed highest standard heterosis for this trait. Out of fifteen crosses, none of crosses showed significantly positive standard heterosis for the trait of intermodal distance. Out of fifteen crosses, eight showed significantly positive standard heterosis for weight of foliage. The cross S. Jaya x CR54A3 showed highest standard heterosis for this trait. Similar findings have also been reported by Okogbenin and Fregene (2002) to examine crop traits associated with early bulking. In consequence, they put forward that those crop traits were key factors in triggering early root yield as a complex trait along with other crop growth traits. These results were found to be similar as reported by Hershey (2012) Tumuhimbise (2013) and Okogbenin *et al.* (2013)

5.3.2.2 Yield (and early bulking) and its Components

Presently trend towards emerging early bulking cassava cultivars in response to increasing demand by farmers is increasing.

Yield components greatly influence the yield and expression of heterosis for stem girth, no. of tubers plant^{-1}, mean weight of tuber, length of tuber, girth of tuber, tuber yield and harvest index can greatly contribute for total tuber yield per plant and early bulking of tubers. For all these traits, positive heterosis is desirable.

Out of fifteen crosses, five showed significantly positive standard heterosis for stem girth. This suggested the importance of dominant gene action. The cross S. Jaya x CR54A3 showed highest standard heterosis for this character, followed by CI 889 x CI 273.

Out of 15 crosses, 8 showed significantly positive standard heterosis for no. of tubers plant[1]. This suggested the importance of dominant gene action. The cross S. Jaya x CR54A3 showed highest standard heterosis for this character.

The cross S. Jaya x CI 273 followed by S. Vijaya x CI 273 and S. Jaya x CR54A3 showed highest standard heterosis for mean weight of tuber. Out of 15 crosses, 6 showed significantly positive standard heterosis.

Out of fifteen crosses, only one showed significantly positive standard heterosis for length of tuber. This suggested the importance of dominant gene action. The cross S. Jaya x CR54A3 showed highest standard heterosis for this character.

None of the cross out of 15 exhibited positive significance through girth of tuber. Highest negative significant value was with cross 9S 75 x CI 273.

Out of fifteen crosses, nine showed significantly positive standard heterosis for tuber yield. This suggested the importance of dominant gene action. The cross S. Jaya x CR54A3 showed highest standard heterosis for this character, followed by S. Jaya x CI 273.

The cross S. Jaya x CI 273 followed by S. Jaya x CR54A3 showed highest standard heterosis for character harvest index. Out of fifteen crosses, 5 showed significantly positive standard heterosis.

These outcomes were in adaptation with contemplates by Okogbenin and Fregene (2002) and Ceballos *et al.,* (2004) which demonstrated that size of a tuber, weight of foliage and Harvest Index (HI) were the most essential variables for root bulking. This was likewise affirmed by Hershey (2012) Tumuhimbise (2013) and Okogbenin *et al.* (2013) who showed that productivity of roots at 12 MAP can be utilized as criteria to screen for early bulking in light of the fact that early fresh storage root yielders (FSRY) are the high yielders at later phases of FSRY assessment.

For the biochemical traits like dry matter content, the expression of significant heterosis values over mid parent values in desired positive direction was revealed in 5 crosses. Per cent heterosis over standard check ranged high in cross S. Vijaya x

CR54 A3. Out of fifteen crosses, 3 showed significantly positive standard heterosis for this trait.

The starch (%) content holds higher positive standard heterosis value with cross CI 889 x CR54 A3 and only one cross showed positive value out of 15 crosses.

The sugar (%) content holds higher negative standard heterosis value with cross CI 889 x CI 273 and only none of crosses showed positive value out of 15 crosses. Cooking quality among the crosses implies, none of crosses showed positive value. All these biochemical traits were in conformation with studies by, Easwari Amma *et al.,* (1995), Easwari Amma and Sheela (1995), Ceballos *et al.,* (2004) and Tumuhimbise (2013).

CMD Disease Scoring (1-5 scale), negative heterosis is desirable. The degree of heterosis over better parent ran high in cross 9S 75 x CR54A3. Among 15 crosses, only 2 revealed significant negative heterosis over better parent for CMD disease scoring. Correspondingly, high magnitude of economic heterosis was observed in crosses CI 889 x CI 273 (200 %) and followed by S. Jaya x CR54 A3 and S. Jaya x CI 273 (-25.00 each) for lowest magnitude heterosis over commercial check. Similar types of results were also obtained by Tumuhimbise (2013).

Thus, Standard heterosis was positive and significant in the combinations Sree Jaya x CR 54A3 (L1xT1) and Sree Jaya x CI 273 (L1xT3) for all the yield contributing traits. The crosses Sree Jaya x CR54 A3 (L1x T1) and Sree Jaya x CI 273 (L1xT3) exhibited negative standard heterosis for CMD.

5.3.3 Combining Ability Analysis

5.3.3.1 ANOVA for Combining Ability and Gene Action

The examination of ANOVA for combining ability demonstrated that mean squares due to gca and sca were significant.

Nature of gene action as measured by GCA and SCA variances is particularly useful in deciding the inheritance of character and thereby selection of a suitable breeding programme. Greater GCA variance for a character indicates predominance of additive gene action and if SCA variance is greater, non-additive gene action plays an important role in controlling that trait. Simple selection is enough for a character controlled by additive gene action as it as fixable, but if non-additive gene action is predominant for a character, which is non-fixable, heterosis breeding may be rewarding or selection has to be postponed to later generations.

In the present study, for all traits sca variance was higher than gca variance as evidenced by the ratio being less than one, suggesting significant part of non-additive gene action like dominance, epistasis besides other interaction effects in expression of these characters. When non-additive genes govern the characters this suggest that there is an opportunity of improvement of these traits by using selection methods as well as to go for hybrid breeding programme in exploitation of heterosis. These outcomes are like the prior reports by Calle *et al.,* (2005), Jaramillo *et al.,* (2005), Perez *et al.,* (2005), Cach *et al.,* (2006), Sleper and Poehlman, (2006), Zacarias and Labuschagne, (2010) and Kulembeka *et al.,* (2012)

5.3.3.2. Estimation of Combining Ability Effects.

General and specific combining ability effects and variances got from a set of F_1's would empower a plant breeder to choose attractive parents and crosses for every one of the quantitative attributes. General combining ability impacts of parents and specific combining ability impacts of crosses were exceptionally critical for the characters considered. From the present investigation, it was evident that gca or sca effects in parents or crosses were in desirable direction for some characters and in undesirable direction for some other traits. Therefore it is important to ascertain the status of parent or hybrid with respect to combining ability effects over a number of component characters (Arunachalam and Bandopadhay, 1979).

Parents with great GCA for particular characters might be helpful in a hybridization program for development of those required attributes (Parkes *et al.*, 2013). Among lines, Sree Jaya (L1) was seen to be a good combiner for yield and yield contributing attributes. Sree Vijaya (L2) was observed as good general combiner for mean weight of tuber, girth of tuber and dry matter content. Vellayani Hraswa (L3) is a poor general combiner in this study. CI 889 (L4) is a good general combiner for stem girth and starch content 9S 75 (L5) is a decent general combiner for plant height, sugar (%) and cooking quality.

Among testers, CR54 A3 (T1) is a good general combiner for length of tuber, weight of foliage, starch and sugar content. IMS2-5 (T2) is a good general combiner for CMD disease resistance breeding. CI 273 (T3) is a good general combiner for harvest index and cooking quality. They could be considered as the best combining parents of the present study in yield attributes.

It was evident from these results that high gca effects for mean weight of tuber and tuber yield in the genotypes L1 (S. Jaya) was mainly due to important yield contributing characters mentioned above. Therefore, it would be worthwhile to use the above parental line in the hybridization programme for improvement of yield in cassava. The potentiality of a crop parent in hybridization programme may be assessed by its *per se* enactment and gca effects. The results revealed that most of the characters had comparatively high degree of correspondence between *per se* enactment and gca effects. This could be ascribed to the predominant role of additive and additive x additive type of gene action for the inheritance of these traits. Significant sca effects of those combinations involving good x good combiners showed the major role of additive type of gene effects, which is fixable.

The estimates of sca effects revealed the positive effect with cross combination 9S 75 x CI 273 and significant positive gca effects for height at first branch. Cross combination CI 889 x IMS2-5 exhibited highest significant positive

sca effects for plant height, S. Jaya x CR 54A3 for stem girth, CI 889 x IMS2-5 for leaf retention, CI 889 x CI 273 for no. of tubers plant $^{-1}$, S. Jaya x CI 273 for mean weight of tubers and length of tuber, S. Jaya x CR 54A3 for girth of tuber, S. Vijaya x CI 273 for weigth of foliage, 9S 75 x CI 273 for tuber yield, S. Vijaya x IMS2-5 for HI, S. Jaya x CI 273 for dry matter content, S. Jaya x CR54 A3 for starch per cent, V. Hraswa x CR54 A3 for sugar per cent, S. Vijaya x IMS2-5 and V. Hraswa x IMS2-5 for cooking quality and S. Vijaya x CR54 A3 for CMD disease score exhibited highest significant positive sca effects.

Assurance of legacy of morphological characters in cassava plant utilizing consolidating capacity has picked up noticeable quality, the outcomes holds great with the greater part of the work done by CIAT and the national research establishments where cassava is a staple sustenance edit by Perez *et al.*, 2005; Jaramillo *et al.*, 2005; Calle *et al.*, 2005; Cach *et al.*, 2006; Kamau, 2006; Munga, 2008; Owolade *et al.*, 2009; Mtunda, 2009; Zacarias and Labuschagne, 2010; Kulembeka *et al.*, 2012 and Parkes *et al.*, 2013.

5.4 EXPERIMENT IV: BULK SEGREGANT ANALYSIS

DNA from the early bulking clones was isolated and pooled. Similarly DNA from the late bulking clones was isolated and pooled. Using molecular marker analysis pooled DNA from the early and late bulking segregants was compared.

Early bulking DNA pool along with V. Hraswa as a positive control because of its early maturity for harvest and late bulking DNA pool along with Sree Padmanabha, a late maturing as a control were framed and analysed for the selected 5 early bulking SSR markers.

Among the entire early bulking SSR maker studied, particular allele of interest for early bulking nature was noticed in the positive control being used. Based on the allele of interest studied for identification of genetically proved early bulking

clones, marker SSRY 250 resulted in selection of clones from crosses *viz.* S. Jaya x CR 54A3, S. Jaya x CI 273, S. Vijaya x CR 54A3, S. Vijaya x IMS2-5 and S. Vijaya x CI 273 as genetically early bulking clones.

SSR maker (ESTs) SSRY 47 resulted from crosses *viz.* S. Jaya x CR 54A3, S. Jaya x CI 273, S. Vijaya x CR 54A3, S. Vijaya x IMS2-5, S. Vijaya x CI 273, V. Hraswa x CR54A3. SSR maker SSRY 63 resulted in non-polymorphic bands. Only one cross S. Jaya x CR54A3 found as genetically early bulking clone. Early bulking SSR maker (ESTs) SSRY 292 resulted in clones from crosses *viz.* S. Jaya x CR 54A3 and S. Jaya x CI 273 as genetically early bulking clones. SSR maker SSRY 239 resulted in clones from crosses *viz.* S. Jaya x CR 54A3, S. Jaya x IMS2-5, S. Jaya x CI 273, V. Hraswa x CR 54A3 and V. Hraswa x IMS2- 5 as genetically early bulking clones. These molecular markers results for early bulking through BSA implies the earlier report by Olasanmi *et al.,* (2014)

Early bulking SSR markers profile for parents and crosses revealed cross combinations S. Jaya x CR54A3, S. Jaya x IMS2-5 and S. Vijaya x CR 54A3, three of five markers revealed alleles associated with early bulking nature.

SUMMARY

6. SUMMARY

The experiment entitled "Introgression of mosaic disease resistance in popular short duration cassava varieties of Kerala through marker assisted selection" was carried out at Division of Crop Improvement, ICAR-CTCRI, Sreekariyam, Thiruvananthapuram during 2014-17. In the first experiment, Five early bulking high yielding lines *viz,* S. Jaya, S. Vijaya, V. Hraswa, CI 889 and 9S 75 and three testers *viz,* CR 54A3, IMS2-5 and CI 273 with resistance to cassava mosaic disease were crossed to produce fifteen F_1 combinations, from which reliable amount of seeds and seedlings were obtained.

In the second experiment, the F_1 seedlings obtained were subjected to detailed study on variation among them and correlation of morphological characters with tuber yield and CMD. ANOVA expressed highly significant difference among the genotypes for all the quantitative traits; it justifies the selection of parental material for the study. From the analysis, mean sum of square due to crosses showed highly significant, indicating towards diverse performance of all cross combinations. Significant differences were also observed for all traits studied in lines and testers indicating the predominance of non-additive gene action. Correlation study revealed association of tuber yield and CMD with other traits among seedlings.

In continuation of second experiment, marker aided selection of CMD resistant clones was carried out. The seedlings without CMD visual symptoms were subjected to multiplex PCR and real time quantitative estimation to study the presence of virus in field tolerant plants. The resistance of the selected clones was further verified by using SSR markers linked to CMD resistance. Through multiplex PCR Sree Jaya was confirmed to be affected with SLCMV and V. Hraswa was confirmed to be affected with both ICMV and SLCMV. Among crosses, Sree Jaya x IMS2-5, Vellayani Hraswa x IMS2-5 and CI 889 x CI 273 were affected by SLCMV.

The viral load scale in RT PCR recorded CI 889 and 9S 75 to be highly resistant among parents. Among crosses S. Jaya x CR 54A3, S. Jaya x IMS2-5, S. Jaya x CI 273, 9S 75 x CR 54A3, 9S 75 x IMS2-5 and 9S 75 x CI 273 grouped to be highly resistant seedlings. Among the crosses V. Hraswa x CI 273 found to record with highest viral load. Among F1 clones only the line 9S 75 grouped to be highly resistant, among crosses, S. Jaya x CR 54A3, S. Jaya x CI 273 and 9S 75 x CR 54A3 grouped as highly tolerant to CMD.

The different CMD linked SSR primers were selected for continuation part of the second experiment, Out of SSR markers screened, five markers (SSRY 28, SSRY 40, SSRY 44, SSRY 106 and SSRY 235) showed association with cassava mosaic disease resistance were selected for MAS for the identification of CMD resistance among clones. The maximum polymorphism was elucidated by SSRY 28, SSRY 44 and followed by SSRY 235. SSR markers profile for CMD in parents and crosses along with disease scoring revealed that the cross combination S. Jaya x CR 54A3, S. Vijaya x IMS2-5, V. Hraswa x CR 54A3 and V. Hraswa x CI 273 three of five SSR markers found associated with CMD 2 gene.

In the third experiment, CMD resistant clones obtained through L x T crosses with short duration were multiplied through miniset technology and evaluated along with parents for the confirmation of early bulking nature. Harvesting was done at six months after planting. The results of ANOVA expressed highly significant difference among the genotypes for all quantitative traits studied. Among lines 9S 75 found to be recorded with highest mean values for maximum of traits, *viz.* leaf retention, intermodal distance, mean weight of tuber, girth of tuber, tuber yield, harvest index, starch per cent and recorded no CMD symptom. Among testers CI 273 found with maximum higher mean values compare to others. Among the crosses, Sree Jaya x CR54 A3 recorded the highest mean values for many of the yield attributing traits *viz.*

height at first branch, stem girth, intermodal distance, number of tubers per plant, tuber length, tuber girth, weight of foliage, tuber yield, HI and no symptoms of CMD.

The data on different types of heterosis were calculated and used Vellayani Hraswa as a standard check; this revealed superiority of some outstanding cross combinations. The maximum standard heterosis for tuber yield was observed in the cross S. Jaya x CR54 A3 followed by S. Jaya x CI 273. The maximum standard heterosis was revealed by S. Jaya x CR54 A3 for height at first branch, stem girth, no. of tubers plant^{-1}, stem girth, length of tuber and weight of foliage.

Standard heterosis was positive and significant in the combinations Sree Jaya x CR 54A3 (L1xT1) and Sree Jaya x CI 273 (L1xT3) for all the yield contributing traits. The crosses Sree Jaya x CR54 A3 (L1x T1) and Sree Jaya x CI 273 (L1xT3) exhibited negative standard heterosis for CMD.

The *gca* values revealed that, among lines, Sree Jaya (L1) was found to be a good general combiner for crop yield and yield contributing characters. Sree Vijaya (L2) was observed as good general combiner for mean weight of tuber, girth of tuber and dry matter content. Vellayani Hraswa (L3) is a poor general combiner in this study. CI 889 (L4) is a good general combiner for stem girth and starch content 9S 75 (L5) is a decent general combiner for plant height, sugar (%) and cooking quality.

Among testers, CR54 A3 (T1) is a good general combiner for length of tuber, weight of foliage, starch and sugar content. IMS2-5 (T2) is a good general combiner for CMD disease resistance breeding. CI 273 (T3) is a good general combiner for harvest index and cooking quality. They could be considered as the best combining parents of the present study in yield attributes. Among the cross combination S. Jaya x CR 54A3 had highly positive significant sca effect for girth of tuber, Earliness is an important trait in cassava. Earliness of bulking is required in such crops for realizing the potential tuber yield at less duration of crop maturity.

In the last experiment, bulk segregant analysis was carried out; DNA from the early bulking clones was isolated and pooled. Similarly DNA from the late bulking clones was isolated and pooled. Using early bulking SSR marker analysis pooled DNA from the early and late bulking segregants was compared and analysed. Among the cross combinations (S. Jaya x CR54 A3, S. Jaya x IMS2-5, S. Vijaya x CR54 A3) three of five markers revealed alleles associated with early bulking nature.

This study has identified two SSR markers (SSRY 28 and SSRY 106) associated with resistance to CMD. One SSR marker, ESTs (SSRY) 292 associated to early bulking nature and among the crosses, clones from S. Jaya x CR54 A3, S. Jaya x CI 273 and 9S 75 x CR54 A3 are being confirmed with CMD resistance as well as early bulking nature.

Future Line of Work

1. The present study identified SSR markers that can be used in marker assisted selection in cassava. However these markers need further validation on larger population.
2. Also there is scope for identifying more molecular markers showing strong association with cassava mosaic disease resistance and early bulking using bioinformatics tools
3. Despite the fact, efficiency of these molecular markers in MAS still need to be examined, their identification will lead for further studies in developing more molecular markers with high link to CMD and early bulking.
4. Furthermore validation of Early bulking markers need to done with large population of selected clones
5. Selected clones from S. Jaya x CR54 A3, S. Jaya x CI 273 and 9S 75 x CR54 A3 are needed to multiply for further evaluation.

REFERENCES

7. REFERENCES

Abraham, A. 1956. Tapioca cultivation in India. *Farm bulliten.* 17 (ICAR).

Acquaah, G. 2009. *Principles of plant genetics and breeding* (2nd Ed.). Blackwell publishing, Oxford, UK, 584p.

Adeniji, O. T., Odo, P. E., and Ibrahim, B. 2011. Genetic relationships and selection indices for cassava root yield in Adamawa State, Nigeria. *Afr. J. Agric. Res.* 6 (13): 2931-2934.

Adjebeng Danquah, Vernon Edward Gracen, Samuel Kwame Offei, Isaac Kwadwo Asante and Joseph Manu-Aduening. 2016. Genetic variability in storage root bulking of cassava genotypes under irrigation and no irrigation. *Agric. and Food Security.* 5:9.

Aina, O. O., Dixon, A. G. O., and Akinrinde, E. A. 2007. Genetic variability in cassava as it influences storage root yield in Nigeria. *J. Biol. Sci.* 7: 765-770.

Aina, O. O., Dixon, A. G. O., and Akinrinde, E. A. 2007. Trait association and path analysis for cassava genotypes in four agro ecological zones of Nigeria. *J. Biol. Sci.* 7 (5): 759-764.

Aina, O. O., Dixon, A. G. O., Ilon, P. and Akinrinde, E. A. 2010. G x E interaction effects on yield and yield components of landraces and improved cassava genotypes in the savanna regions of Nigeria. *World J. Agric. Sci.* 6(1): 67-78.

Akano, A.O., Dixon, A., Mba, C. Barrera, E., Fregene, M. 2002. Genetic mapping of a dominant gene conferring resistance to cassava mosaic disease. *Theoretical and Applied Genetics* 105: 521-525.

Akinwale M. G., Akinyele, B. O., Dixon, A. G. O. and Odiyi, A. C. 2010. Genetic variability among forty-three cassava genotypes in three agro-ecological zones of Nigeria. *J. Pl. Breed. Crop Sci.* 2(5): 104-109.

Alabi. O. J., Lava Kumar, Naidu R. 2008. Multiplex PCR for the detection of African cassava mosaic virus and East African cassava mosaic Cameroon virus in cassava. *J. Virological Methods.* 154(1-2):111-20.

Allard, R. W. 1960. *Principles of plant breeding.* John Wiley & Sons, New York.

Allen, A. C. 2002. The origins and taxonomy of cassava. In: Hillocks, R. J., Thresh, J. M. and Bellotti, A. C. (eds), *Cassava biology, production and utilization.* CABI Publishing, Wallingford, UK, pp. 1- 16.

Almeida, J. M. R. 1995. *Manual de mandioca*. Cultivar, Porto, Portugal.

Alves, A. A. C. 2002. Cassava botany and physiology. In: Alves, A. A., Hillocks, R. J., Thresh, J. M. and Bellotti, A. C. (eds), *Cassava: Biology, production and utilization*. CABI, Wallingford, UK, pp. 67-89.

Amenorpe, G., Amoatey, H. M. A., Darkwa, A., Banini, G.K., and Elloh, V.W. 2007. Peak root and starch weights of ten early bulking cultivars of cassava (*Manihot esculenta* Crantz) in Ghana. *J. Ghana Sci. Assoc.* 9: 54-60.

Ariyo O.A., Dixon. A.G.O., Atiri. G.I. 2005. Whitefly Bemisia tabaci infestation on cassava genotypes grown at different ecozones in Nigeria. *J. Econ. Entomol.* 98(2):611-617.

Arunachalam, V. and Bandopadhyay, A. (1979). Are "multilple cross- multiple pollen hybrids" an answer for productive populations in Brassica copestries variety brown serson?. *Theor. Appl. Genet*ics, 54: 203-207.

Asare, P. A., Galyuon, I. K. A., Sarfo, J. K., and Tetteh, J. P. 2011. Morphological and molecular based diversity studies of some cassava (*Manihot esculenta* Crantz) germplasm in Ghana. *Afr. J. Biotechnol.* 10(63): 13900-13908.

Asiedu, R.; Hanh, S. K.; Bai, K. V. and Dixon, A. G. O. 1989. Inter-specific hybridization in the genus progress and prospects. Act. Hort. 380:110-113.

Babu Rao, B, Ashok, P, Ramanandam, G. and Sasikala, K. 2015. Assessment of genetic diversity in cassava (*manihot Esculenta* crantz.) Genotypes. *The Bioscan.* 10(4): 1935-1938.

Babu Rao, B, Ashok, P, Ramanandam, G. and Sasikala, K. 2016. Studies on genetic variability, heritability and genetic advance for quantitative traits in cassava (*Manihot esculenta* Crantz). *The Bioscan.* 11(3): 1991-1994

Baguma, Y. 2004. Regulation of starch synthesis in cassava. PhD thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden, 44p.

Balashanmugam, P. V. 1980. Genetic variability and correlation in cassava. *Proceedings of National Seminar on cassava production Technology.* Coimbatore. 18-22.

Bassey, E.E., and I.H. Gamaliel. 2013. Screening cassava (*Manihot esculenta* Crantz) genotypes for tuber bulking, early maturity and optimum harvesting time in Uyo, South Eastern Nigeria. Peak Journal of Agricultural Sciences 1:83-88.

Beeching, J.R., Marmey, P., Gavalda, M.C., Noirot, M., Haysom, H.R., Hughes, M.A., Charrier, A. 1993. An assessment of genetic diversity within a collection of cassava (*Manihot esculenta* Crantz) germplasm using molecular markers. *Annals of Botany* 72: 515-520.

Benesi, I. R. M. 2005. Characterization of Malawian cassava germplasm for diversity, starch extraction and its native and modified properties. PhD thesis, University of the Free State, Bloemfontein, South Africa, 217p.

Bhagath, N. R., Jadye, A. T., Mhaskar, N. V., Mehendale, S. K., and Jadhav, B. B. 2011. H-119 A High Yielding Short duration Cassava Variety for Konkan conditions. *N S C F T ABSTRACTS.*

Biradar, R. S., Rajendran P. G., and Hrishi. 1978. Genetic variability and correlation in cassava (*Manihot esculenta* Crantz). *J. Root Crops.* 4(1): 7-10.

Bonierbale, M., Guevara. C., Dixon., Ng, N.O., Asiedu, R. and Ng, S.Y.C. 1997. Chapter 1: Cassava, In: Fuccillo, D. Sears, L., and Stapleton, P. (eds) Biodiversity in Trust. Cambridge University Press, Cambridge. Pp. 1-20.

Bradbury, J. H. and W. D. Holloway. 1988. Chemistry of tropical root crops: significance for nutrition and agriculture in the pacific. ACIAR Monograph No. 6, Australian Centre for International Agricultural Research, Canberra.

Buitrago, J. A. 1990. La yucca em alimentacion animal. Publicacion 85, CIAT, Cali, Colombia

Burns, A., Gleadow, R., Cliff, J., Zacarias, A., and Cavagnaro, T. 2011. Cassava, the drought, war and famine crop in a changing world. *Sustainability* 2: 3572-3607.

Busogoro, J. P., Masquellier, L., Kummert, J., Dutrecq, O., Lepoivre, P., Jijaki, H. 2008. Application of simplified molecular protocol to reveal mixed infection with begomovirus in cassava. *J. Phytopathology.* 156:452-457.

Cach, N.T., Lenis, J. I., Perez, J. C., Morante, N., Calle, F., and Ceballos, H. 2006. Inheritance of useful traits in cassava grown in sub-humid conditions. *Pl. Breed.* 125: 177-182.

Caetano-Anolles, G., Bassam, B.J. and Gressholf, P.M. 1991. DNA amplification, fingerprinting using very short arbitrary oligonucleotide primers. *Biotechnol.* 9:553-557.

Calle, F., Perez, J. C., Gaitan, W., Morante, N., Ceballos, H., Llano, G., and Alvarez, E. 2005. Diallel inheritance of traits in cassava (*Manihot esculenta* Crantz) adapted to acid soil savannas. *Euphytica* 144: 177-186.

Ceballos, H., Iglesias, C. A., Pérez, J. C., and Dixon, A. G. O. 2004. Cassava breeding: opportunities and challenges. *Pl. Mol. Biol.* 56: 503-516.

Ceballos, H., P. Kulakow, and C. Hershey. 2012. Cassava breeding: Current status, bottlenecks and the potential of biotechnology tools. *Tropical Plant Biology* 5:73-87.

Chang-Ho, P., K. Kim, H. Aswidinnoor, and F. Rumawas. 2005. Varietal differences of drymatter accumulation and related characters in cassava (*Manihot esculenta* Crantz). *Korean J. Crop Science* 50: 45-54.

Chavarriaga-Aguirre, P., and M. Halsey. 2005. Cassava (*Manihot esculenta* Crantz): Reproductive biology and practices for confinement of experimental field trials. Report prepared for the programme for biosafety systems, Washington, USA. p. 1-20.

Chavez, A. L., Sanchez, T., Jaramillo, G., Bedoya, J. M., Echeverry, J., Bolanos, E. A., Ceballos, H., and Iglesias, C. A. 2005. Variation for quality traits in cassava roots evaluated in landraces and improved clones. *Euphytica* 143: 125-133.

Chikoti, P. C. 2011. Development of cassava (*Manihot esculenta* Crantz) cultivars for resistance to cassava mosaic disease in Zambia. PhD thesis, University of KwaZulu-Natal, South Africa.

Chitiyo, M. and Kasele, I. 2004. Evaluation of cassava varieties for yield and adaptability in Zimbabwe. *Afr. Crop Sci. J.* 12(3): 197-200.

CIAT .1972. Investigacion, produccion y utilizacion de yuca. Documento de trabajo n 50.

CIAT. 2002. Improved cassava for the developing world. Annual report for 2001.

Costa, I. R. S. and Silva, S. O. 1992. Coleta de germoplasma de mandioca no Nordeste do Brasil. Revista Brasileira de mandioca, 11 (1), Bahia, Brasil, pp. 19-27.

Dabholkar, A. R. 1992. *Elements of biometrical genetics*. South Asia Books Publications, New Delhi, 431p.

Dahniya, M. T. 1994. An overview of cassava in Africa. *Crop Sci. J.* 2: 337-343.

De Carvalho, R. D. and Guerra, M. 2002. Cytogenetics of *Manihot esculenta* Crantz (cassava) and eight related species. *Hereditas* 136: 159-168.

Deepthi, D. C. 2017. Molecular characterization of symptom recovery in cassava genotypes during cassava mosaic disease infection. Ph. D. thesis. pp 212.

DeVries, J., and Toenniessen, G. 2001. Securing the harvest: biotechnology, breeding and seed systems for African crops. Chapter 13: Cassava. CABI Publishing, Oxon, UK and New York, USA. p. 147-156.

Dixon, A.G.O., M.O. Akoroda, R.U. Okechukwu, F. Ogbe, P. Ilona, L.O. Sanni, C. Ezedinma, J. Lemchi, G. Ssemakula, M.O. Yomeni, E. Okoro, and G. Tarawali. 2008. Fast track participatory approach to release of elite cassava genotypes for various uses in Nigeria's cassava economy. *Euphytica* 160:1-13.

Djirabaye Nadjiam, Sarr, P.S., Naïtormbaïdé, M., Mbaïguinam, J.M.M. and Guisse, A. 2016. Agro-Morphological Characterization of Cassava (*Manihot esculenta* Crantz) Cultivars from Chad. *Agricultural Sciences*, 7, 479-492. http://dx.doi.org/10.4236/as.2016.77049

Doku, E.V. 1969. Cassava in Ghana. Accra, Ghana Universities Press. p.44.

Edison, S. 2002. Plant protection problems in cassava in India. In: Proceedings of the 7th Regional Cassava Workshop. 28 October – 1 November, 2002. Bangkok, Thailand, pp. 264-270.

Ekanayake I.J., D.S.O. Osiru, and M.C.M. Porto. 1998. Physiology of cassava. IITA Research guide no. 55. Third edition. IITA, Ibadan, Nigeria. p. 32.

El-Sharkawy, M. A. 2003. Cassava biology and physiology. *Pl. Mol. Biol.* 56: 481-501.

El-Sharkawy, M. A. 2007. Physiological characteristics of cassava tolerance to prolonged drought in the tropics: Implications for breeding cultivars adapted to seasonally dry and semiarid environments. *Brazilian J. Pl. Physiol.* 19: 257-286.

El-Sharkawy, M. A. 2012. Stress-tolerant cassava: the role of integrative ecophysiology-breeding research in crop improvement. *Open J. of Soil Sci.* 2: 162-186.

Eswari Amma, C. S and Sheela, M. N., 1995. Combining ability, heterosis and gene action for three major quality traits in cassava. *J. Root Crops* 21(1): 24–29.

Eswari Amma, C. S and Sheela, M. N., 1998. Genetic analysis in a diallel cross of inbred lines of cassava. *Madras Agric. J.* 85(5, 6): 264–268.

Eswari Amma, C. S and Sheela, M. N., and Pillai, T. P. K. 1995. Combining ability analysis in cassava. *J. Root Crops* 21(2): 65–71.

Eswari Amma, C. S. and Sheela, M. N. 1993. Heterosis in cassava: Nature and magnitude. In: Proceedings from the Symposium on Tropical Tuber Crops "Problems, prospects and future strategies". ISRC, Trivandrum, India, 6–9 November 1993, pp. 88–94.

Falconer, D. S and Mackay, T. F. C. 1996. Introduction to quantitative genetics. Fourth edition. Longman Scientific and technical Co., Exssex, England.

Falconer, D. S. 1989. *Introduction to Quantitative Genetics*, Longman Group Limited, England, pp. 48-263.

FAOSTAT [Food and Agricultural Organisation Statistical Database]. 2015 [on-line]. Available at http://apps.fao.org/faostsat. [03 Aug. 2017].

Fauquet, C. and Fargette, D. 1990. African cassava mosaic-virus-aetiology, epidemiology and control. *Pl. Dis.* 74: 404-411.

Fokunang, C.N., C.N. Akem, T. Ikotun, A.G.O. Dixon and E.A. Tembe. 2000. Role of the insectvector *Pseudotheraptus devastans* in cassava anthracnose disease development. *European J.Plant Pathology* 106: 319-327.

Food and Agriculture Organization (FAO). 2012. Food and Agriculture Organisation statistical database for agriculture. Crops and products domain. http://faostat.fao.org..

Food and Agriculture Organization (FAO). 2013. Food and Agriculture Organisation statistical database for agriculture. Crops and products domain. http://faostat.fao.org.

Franche. J. C., Jorge, M. A. B. and Zacarias, A. M. 1991. A cultura da mandioca em Moçambique: estado actual e suas potencialidades. *Revista Brasileira de Mandioca*, 11 (2), Bahia, Brasil, 156-163.

Fregene, M., Angel, F., Gomez, R., Rodriquez, F., Chavarriaga, P., Roca, W., Tohme, J. and Bonierbale, M. 1997. A molecular genetic map of cassava (*Manihot esculenta* Crantz). *Theor. Appl. Genet.* 95: 431-441.

Fregene, M., Bernal, A., Doque, M., Dixon, A., and Tohme, J. 2000. AFLP analysis of African cassava (*Manihot esculenta* Crantz) germplasm resistant to cassava mosaic disease (CMD). *Theor. Appl. Genet.* 100: 678-685.

Fregene, M., Okogbenin, E., Mba C., Angel F., Suarez, M.C., Guitierez, J., Chavarriaga, E., Roca, W., Bonierbale, M., and Tohme J. 2001. Genome mapping in cassava improvement: challenges, achievements and opportunities. *Euphytica*. 120:159-165.

Fukuda, N. M. G. 1979. Production and preliminary selection of new cassava hybrids. In *Congresso Brasilerio de Mandioca,* 10, Cruz das almas-B A, Brazil, 535-545 (Port).

Fukuda, W.M. G., S. de Oliveira, Silva, and C. Iglesias. 2002. Cassava breeding. *Crop Breeding and Applied Biotechnol.* 2:617-638.

Hahn S. K., Terry E. R., and Leuschner, I. 1980. Breeding cassava for resistance to cassava mosaic disease. *Euphytica* 29: 673-683.

Hallauer, A. R. and Miranda, J. B. 1982. *Quantitative Genetics in Maize Breeding*. IOWA State University Press, Ames, IOWA.

Hamada, H., Petrino, M., Kalunaga, T. 1982. A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. *Proceedings of National Academy of Sciences* 79: 6465-6469.

Henry, G., and Hershey, C. 2002. Cassava in South America and the Caribbean. In: Hillocks, R. J. and Thresh, J. M. (eds), *Cassava: Biology, production and utilization*. CABI, Wallingford, UK, pp. 17-40.

Hershey, C. H. 1993. *Manihot genetic diversity*. Report of the international network for cassava genetic resources. CIAT, Cali, 179p.

Hershey, C., 2012. Cassava genetic improvement: theory and practice. FAO Publishing, Rome, Italy. In press.

Hu, J., Quiros, C.F. 1991. Identification of broccoli and cauliflwer cultivars with RAPD markers. *Plant Cell Report* 10: 505-511.

Huiping Bi, Mahender Aileni and Peng Zhang. 2010. Evaluation of cassava varieties for cassava mosaic disease resistance jointly by agro-inoculation screening and molecular markers. *African J. Plant Sci.* 4(9):330-338.

Iglesias, C., Mayer, J., Chavez, A. L., and Calle, F. 1997. Genetic potential and stability of carotene content in cassava roots. *Euphytica* 94: 367–373.

IITA (International Institute of Tropical Agriculture), 1990. Cassava in tropical Africa. Reference Manual, IITA, Ibadan, Nigeria

IITA, 2001: Cassava. IITA/Crops. http://www.iita.org .

Indira, P. and Sinha, S. K. 1970. Calorimetric method for determination of HCN in tubers and leaves of cassava. *Ind. J. Agric. Sci.* 39(11): 1021-1023.

Izumi, Y., E. Yuliadi, Sunyoto, and M. Iijima. 1999. Root system development including root branching in cuttings of cassava with reference to shoot growth and tuber bulking. *Plant Production Science* 2:267-272.

Jansson, C., Westerbergh, A., Zhang, J., Hu, X., and Sun, C. 2009. Cassava, a potential biofuel crop in (the) People's Republic of China. *Appl. Energy* 86: 95-99.

Jaramillo, G., Morante, N., Perez, J. C., Calle, F., Ceballos, H., Arias, B., and Bellotti, A. C. 2005. Diallel analysis of cassava adapted to the mid-altitude valleys environment. *Crop Sci.* 45: 1058-1063.

Jatasra, D. S. and Paroda, R. S. 1978. Genetic divergence in wheat under different environmental conditions. *Cereals Res. Commun.* 6: 307-317.

Jennings, D. L. 1976. Cassava, *Manihot esculenta* (Euphorbiaceae). In: Simmonds, N. (ed.), *Evolution of crop plants*. Longman, London. pp. 81-51.

Jennings, D. L. and Iglesias, C. A. 2002. Breeding for crop improvement. In: Hillocks, R. J., Thresh, J. M., and Bellotti, A.C. (eds), *Cassava: Biology, Production and Utilization.* CABI Publishing. Oxon, UK and New York, USA, pp. 149-166.

Jones, W. O. 1959. Manioc in Africa. Stanford University Press, Starford.

Jose, A., Makeshkumar, T. and Edison, S. 2011. Survey of cassava mosaic disease in Kerala. *J. Root Crops*. 37(1): 41-47.

Kamau, J. W. 2006. Participatory based development of early bulking cassava varieties for the semi-arid areas of Eastern Kenya. PhD thesis, University of KwaZulu-Natal, South Africa.

Karp, A., Seberg, O., Buiatti, Golan-Goldhirsh. 1996. Polymoephism and DNA markers of asparagus cultivars identified by dando amplified polymorphic DNA. *Euphytica* 87: 39-44.

Kawano, K. 1990. Harvest index and evolution of major food drop cultivars in the tropics. *Euphytica* 46:195-202.

Kawano, K. 2003. Thirty years of cassava breeding for productivity- biological and social factors for success. *Crop Sci.* 43: 1325-1335.

Kawano, K., Goncalvez Fukuda, W.M., Cenpukdee, U. 1987. Genetic and environmental effects on dry matter content of cassava root. *Crop Science* 27: 69-74.

Kawano, K., Narintaraporn, K., Narintaraporn, P., Sarakarn, S., Limsila, A., Limsila, J., Superhan, D., Sarawat, V., and Watananonta, W. 1998. Yield improvement in multistage breeding program for cassava. *Crop Sci.* 32(2): 325-332.

Kawuki, R.S., M. Ferguson, M.T. Labuschagne, L. Herselman, J. Orone, I. Ralimanana, M. Bidiaka, S. Lukombo, M.C. Kanyange, G. Gashaka, G. Mkamilo, J. Gethi, H. Obiero. 2009. Variation in qualitative and quantitative traits of cassava germplasm from selected national breeding programmes in sub-Saharan Africa. Field Crops Research 122:151-156.

Keutgen, M., F. Kubota, and K. Saitou. 2001. Effects of exogenous injection of sucrose solution to plant on the carbon distribution to tuberous root and tuberous root production in sweet potato (*Ipomoea batatas* Lam.). *Japan J. Crop Science* 70:575-579.

Kizito, E.B., Bua, A., Fregene, M., Egwang, T., Gullberg, U., and Westerbergh, A. 2007. The effect of cassava mosaic disease on the genetic diversity of cassava in Uganda. *Euphytica.* 146: 45-54.

Kulembeka, H. P., Ferguson, M., Herselman, L., Kanju, E., Mkamilo, G., Masumba, E., Fregene, M., and Labuschagne, M. T. 2012. Di-allele analysis of field

resistance to brown streak disease in cassava (*Manihot esculenta* Crantz) landraces from Tanzania. *Euphytica* 187: 277-288.

Kumar, S., Tamura, K., Jakobsen, I., Nei, M. 2000. *MEGA, Molecular Evolutionary Genetics Analysis*, version 2.1. Institute of Molecular Evolutionary Genetics, The Pennsylvania State University, University Park, PA.

Kundy. A., Mkamilo, R. and misangu. 2015. Genetic variability among six traits in twelve cassava genotypes in Southern Tanzania. *J. Natural Sci. Research.* 5: 12.

Kunkeaw, S., Tangphatsornruang, S., Smith, D. R., and Triwitayakorn, K. 2009. Genetic linkage map of cassava based on AFLP and SSR markers. Plant Breeding. 129(1).

Lava Kumar, P., S.A. Akinbade, A.G.O. Dixon, N.M. Mahungu, M.P. Mutunda , D. Kiala L. andLonda and J.P. Legg. 2009. First report of the occurrence of *East African cassava mosaic virus*-Uganda (EACMV-UG) in Angola. Plant Pathology 58: 402.

Lee, M. 1995. DNA markers and plant breeding programs. *Advanced Agron.* 55: 265-344

Lefevre, F., Charrier, A. 1993. Heredity of seventeen isozyme loci in cassava (*Manihot esculenta* Crantz). *Euphytica* 66: 171-178.

Leitão, A. B. 1970. A mandioca no sul do Save. Comunicações, INIA, Lourenço Marques, Moçambique.

Lenis, J. I., Calle, F., Jaramillo, G., Perez, J. C., Ceballos, H., and Cock, J. H. 2006. Leaf retention and cassava productivity. *Field Crops Res.* 95: 126–134.

Lokko, Y., Danquah, E.Y, Offei, S.K, Dixon, A.G.O, Gedil, M.A. 2005. Molecular markers associated with a new source of resistance to the cassava mosaic disease. *African Journal of Biotechnology* 4: 873-881.

Lush, J. L. 1940. Intra-sire correlation on regression off-spring on dams as a method of estimating heritability of characters. *Proc. Am. Soc. Anim. Prod.* 33: 292-301.

Magoon, M. L., Krishnan, R., and Lakshmi, K. 1972. Association of plant and tuber characters with yield of cassava. *Tropical root and tuber crops newsletter* no. 5: 29-30.

Mamba – Mbayi, G., Tshilenge, P., Nkongolo, Mbuyi, A.K. 2014. Characterisation of Congolese strains of *Xanthomonas axonopodis pv. manihotis*. Associated with cassava bacterial blight. *American J. Plant Sci.* 5: 1191-1201.

Mandal, M. A. A. 2003. Improvement of potato (*Solanum tuberosum* L.) through hybridization and *in-vitro* culture technique. Ph.D. Thesis. Rasshahi University, Rasshahi, Bangladesh.

Manga, G. A. 1994. Sprouting and establishment of cassava. M.Sc. Thesis. University of Ibadan, Ibadan, Nigeria, 112p.

Mba, R.E.C., Stephenson, P., Edwards, K., Melzer, S., Nkumbira, J., Gullberg, U., Apel, K., Gale, M., Tohme, J. and Fregene, M. (2001). Simple sequence repeat (SSR) marker survey of the cassava (*Manihot esculenta* Crantz) genome: towards an SSR-based molecular genetic map of cassava. *Theor. Appl. Genet.* 102: 21-31.

Melaku Gedil, Slabaugh, Berry, S., Johnson, R., Michelmore, R, Miller, J. Gulya, T., Knappa, S.J. 2010. Candidate disease resistance genes in Cassava to CMD. Genetics. 127:1113-1120.

Mignouna, H.D., and Dixon, A.G.O. (1997). Genetic relationships among cassava clones with varying levels of resistance to the African mosaic disease using RAPD markers. *Afr. J. Root and Tuber Crops*: 2(1and2): 28-32.

Mkumbira, J., Chiwona-Karltun, L., Lagercrantz, U., Mahungu, N.M., Saka, J., Mhone, A., Bokanga, M., Brimer, L., Gullberg, U., Rosling, H. 2001. Classification of Cassava into "Bitter" and "Cool" in Malawi: The Farmers' Method. In: Fauquet, C.M. and N.J. Taylor (Eds.). *Cassava: An ancient crop for modern times. Proceedings of the fifth International Meeting of the CBN.*

Mkumbira, J., Largercrantz, U., Mahungu, N.M., Chiwona, K.L., Saka, J., Mhone, A., Bokanga, M., Brimer, I., Gullberg, U. and Roslin, H. 2002. *Euphytica* (In press).

Mohan, C., Ponnusamy Shanmugasundaram, Marappan Maheswaran, Natesan Senthil, Duraisamy Raghu and Mullath Unnikrishnan. 2013. Mapping New Genetic Markers Associated with CMD Resistance in Cassava (*Manihot esculenta* Crantz) Using Simple Sequence Repeat Markers. *J. Agril Sci.* 5(5).

Mtunda, K. J. 2009. Breeding, evaluation and selection of cassava for high starch content and yield in Tanzania. PhD thesis, University of KwaZulu-Natal, South Africa,

Mulualem, T. and Ayenew, B. 2012. Correlation and path coefficient analysis of Cassava (*Manihot esculenta* Crantz) at Jimma, Southwestern, Ethiopia. *J. Nat. Sci. Res.* 2(9): 1-7.

Munga, T. L. 2008. Breeding for cassava brown streak resistance in coastal Kenya. PhD Thesis, University of KwaZulu-Natal, South Africa,

Murthy, B. R. and Arunachalam, V. 1965. The nature of divergence in relation to breeding system in some crop plants. *Indian J. Genet. Pl. Breed.* 30: 47-58.

Muthukrishnan, C., Thambujaraj, R., and Shanmugam, A. 1973. Tapioca needs less water and care. *Farm Factory* 7(9): 29-30.

Nageswari, K. and Palaniswamy, V. 2011. Correlation and Genetic variability studies in cassava (*Manihot esculenta* Crantz). *NSCFT, CTCRI proceedings*. 219 - 222.

Nageswari, K. and Palaniswamy, V. 2011. Correlation and Genetic variability studies in cassava (*Manihot esculenta* Crantz). *NSCFT, CTCRI proceedings*. 219 - 222.

Naskar, S. K., Singh, D. P., and Lakshmi R. K. 1991. Variability and correlations in F_1 population of cassava. *J. Root Crops* 17(2): 139-141.

Nassar, N. M. A., and Ortiz, R. 2006. Cassava improvement: challenges and impacts. *J. Agric. Sci.* 145: 163-171.

Nassar, N. M. A., and Ortiz, R. 2008. Cassava genetic resources: manipulation for crop improvement. *Pl. Breed. Rev.* 31: 247–275.

Ntawuruhunga, P. and Dixon, A. 2010. Quantitative variation and interrelationship between factors influencing cassava yield. *J. Appl. Biosci.* 26: 1594-1602.

Ntui, V. O., Uyoh, E. A., Affangideh, U., Udensi, U., and Egbonyi, J. P. 2006. Correlation and genetic variability in cassava (*Manihot esculenta* Crantz). *J. Food Agric Env.* 4 (3/4): 147-150.

Nweke, F. I. 1994. Cassava distribuition in Africa. Study of cassava in Africa. IITA, Ibadan, Nigeria, work paper no 12.

Obonyo, R. , Owor, B., Legg, J.P. Okao-okuja, G., , Kyamanywa, S., Ogenga-Latigo, M.W. 2007. Interaction between cassava mosaic geminiviruses and the

African pandemic of cassava mosaic disease. *Integrated Pest Management Conference Proceedings*. Kampala, Uganda: Makerere University: 355- 361.

Odedina, S. A., Odedina, J. N., Ogunkoya, M. O., and Ojeniyi. S. O. 2009. Agronomic evaluation of new cassava varieties introduced to farmers in Nigeria. *Afr. Crops Sci. Conf. Proc.* 9: 77 - 80.

Ojulong, H., Labuschagne, M. T., and Fregene, M. 2007. A cassava clonal evaluation trial based on a new cassava breeding scheme. *Euphytica* 160: 119-129.

Ojulong, H.F., M.T. Labuschagne, L. Herselman, and M. Fregene. 2010. Yield traits as selection indices in seedling populations of cassava. Crop Breeding and Applied Biotechnology 10:191-196.

Okogbenin, E., and M. Fregene. 2002. Genetic and QTL mapping of early root bulking in an F_1 mapping population of non-inbred parents in cassava (*Manihot esculenta* Crantz). Theoretical and Applied Genetics 106:58-66.

Okogbenin, E., M.C.M. Porto, C. Egesi, C. Mba, E. Ospinosa, G. L. Santos, C. Ospina, J. Marin, E.Barera, J. Gutierrez, I. Ekanayake, C. Iglesias and M. Fregene. 2007. Marker aidedintrogression of CMD resistance in Latin American germplasm for genetic improvement ofcassava in Africa. *Crop Sci.* 47: 1895-1904.

Okogbenin, E., Marin, J., and Fregene, M. 2006. An SSR-based molecular genetic map of cassava. *Euphytica* 147: 433-440.

Okogbenin, E., Setter T.L. and M. Fregene. 2002. Genetic and QTL mapping of early root bulking in an F_1 mapping population of non-inbred parents in cassava (*Manihot esculenta* Crantz). *Theoretical and Applied Genetics* 106:58-66.

Okogbenin, E., T.L. Setter, M. Ferguson, R. Mutegi, H. Ceballos, B. Olasanmi, and M. Fregene. 2013. Phenotypic approaches to drought in cassava: review. *Frontiers in Physiology* 4:1-15.

Onwueme, I. C. 1978. *The tropical tuber crops: yams, cassava, sweet potato, and cocoyam's.* Wiley, New York, USA, 36p.

Onwueme, I. C. 1982. The Tropical Tuber Crops. John Willey and sons. Chichester.

Osiru, D. S., Porto, M. C. M., and Ekanayake, I. J. 1996. Morphology of Cassava. In: Ekanayake, I. J., Osiru, D. S., and Porto, M. C. M. (eds), *IITA Research Guide.* Ibadan, Nigeria, 32p.

Owolade, O. F., Dixon, A. G. O., Adeoti, A. Y. A. 2009. Screening of Cassava Genotypes to Anthracnose Disease. J. Agric. Sci 12: 9-14.

Owolade, O. F., Dixon, A. G. O., and Adeoti, A.Y.A. 2006. Diallel analysis of cassava genotypes to anthracnose disease. World J. Agric. Sci. 2: 98-104.

Padma, S. S. V., Kameswari, P. L., and Ramana, K. T. V. 2009. Correlation studies and path analysis in edible cassava germplasm in the tribal zone of Andhra Pradesh. *Indian J. Hort.* 66(3): 319 - 322.

Panse, V. G. 1957. Genetics of quantitative characters in relation to plant breeding. *Indian J. Genet. Pl. Breed.* 17 (2): 318-328.

Panse, V. G. and Sukhatme, P. V. 1985. *Statistical methods for agricultural workers.* Indian Council of Agricultural Research, New Delhi, 125p.

Paramguru, P., Sundararajan, S., Venkatachalam, R., and Seemanthi, R. 1990. Evaluation of cassava varieties for earliness, yield and quality. *J. Root Crops* (ISRC National Symposium, Special): 12-18.

Parkes, E. Y., Fregene, M., Dixon, A., Boakye-Peprah, B., and Labuschagne, M. T. 2013. Combining ability of cassava genotypes for cassava mosaic disease and cassava bacterial blight, yield and its related components in two ecological zones in Ghana. *Euphytica* 194: 13-24.

Patrick, C.C., Paul Shanahan and Rob Melis. 2016. Evaluation of cassava genotypes for resistance to cassava mosaic disease and agronomic traits. *American J. Plant Sci.* 7: 1122-1128.

Perez, J. C., Ceballos, H., Celle, F., Morante, N., Gaitan, W., Llano, G., and Alvarez E. 2005. Within family genetic variation and epistasis in cassava (*Manihot esculenta* Crantz) adapted to the acid soil environment. *Euphytica* 145: 77-85.

Peroni, N. Emmy and N. Hanazaki. 2007. Current and lost diversity of cultivated varieties, especially cassava, under swidden cultivation systems in the Brazilian Atlantic Forest. Agriculture, Ecosystems and Environment 92: 171-183.

Pillai, S. V., Nair, P. G. K., and Nair R. R. 2000. Identification of Genetic stock from cassava germplasm. Abstract: *International symposium on tropical tuber and root crops,* January 19th to 22nd, Thiruvananthapuram: 9-10.

Radhakrishnan, V. V. and Gopakumar, K. 1984. Correlation for yield and its component in Tapioca. *Indian J. Agric. Sci.* 54: 975-978.

Raghu, D., Senthil, N., Saraswathi, T., Raveendran, M., Gnanam, R., Venkatachalam, R., Shanmugasundaram, P., and Mohan, C. 2007. Morphological and Simple sequence repeats (SSR) based finger printings of south Indian cassava germplasm. *Int. J. Integrative Biol.* 1(2): 141-148.

Rajendran, P. G., Lakshmi, K. R., and Unnikrishnan, M. 1985. Genetic and path co efficient analysis in cassava. *National Symposium on Tropical tuber crops.* 1-5.

Ramanujam, T. and Biradar, R. S. 1987. Growth analysis in cassava. *Indian J. Pl. Physiol.* 30: 144-153.

Ravi, V., S.K. Naskar, T. Makeshkumar, B. Babu, and B. S. Prakash-Krishnan. 2009. Molecular physiology of storage root formation and development in sweet potato (*Ipomoea batatas* (L.) Lam.). *J. Root Crops* 35:1-27.

Rekha, V. R., Manikantan, N. P., Sreekumar, S. G. Balakrishnan, A. R., and Pillai, M. R. C. 1991. Path analysis of yield components in a few cassava cultivars. *J. Root Crops.* 17(1): 35-38.

Richordson, K. V. A. 2011. Evaluation of three cassava varieties for tuber quality and yield. *Gladstone Road Agricultural Centre Crop Research Report NO. 4*: 1-12.

Rogers, D. J. and Appan, S. G. 1973. Manihot and manihotoides (Euphorbiaceae): a computer assisted study. Organization for Flora Neotropica. Monograph 13. Hafner, New York. pp 278.

Rogers, D.J., Appan, S.G. 1973. Manihot and manihotoides (Euphorbiaceae): a computer assisted study. Organization for Flora Neotropica. Monograph 13. Hafner, New York. pp 278.

Rubaihayo, P.R., Whyte, J.B.A., Dixson, A.G.O. and Osiru, D.S.O. 2001. Inter-relationship among traits and path analysis for yield components of cassava. A research for storage root yield indicator. *African Journal of Crop Science.* 9 (4): 599-606.

Saiki, R.K., Scharf, S., Faloona, F., Mullis, K., Horn, G.T., Erlich, H.A., and Arnheim, N. 1988. Enzymatic amplification of b-globin genomic sequences and restriction site analysis of diagnosis of sickle cell anaemia. *Science*. 230: 1350-1354.

Sankaran, M., Singh, N. P., Datt, C., Santhosh, B., Nedunchezhiyan, M., Nasker, S. K., and Ngachan, S. V. 2008. Evaluation of high yielding cassava varieties under upland conditions of Tripura. *J. Tuber Crops*. 34(1): 73 - 76.

Second, G., Allem, A.C., Mendes, R.A., Carvalho, L.J.C.B., Emperaire, L., Ingram, C., Colombo, C. 1997. Molecular marker (AFLP)-based *Manihot* and cassava numerical taxonomy and genetic structure analysis in progress: Implications for their dynamic conservation and genetic mapping. *African Journal of Root and Tuber Crops* 2: 140-147.

Sheela, M. N., Radhika, K. N., Susan, J. K., and Abraham, K. 2008. Variation in crude protein, dry matter and starch in inbred and backcross lines of cassava. *J. Root Crops*. 34(2): 115-119.

Silva, J. R. D. 1977. Some indexes of cassava productivity. *Empressca Brasileria de Pesquisas Agropecuarias*, 6 (Port).

Sleper, D.A., and J.M. Poehlman. 2006. Breeding field crops. Fifth edition. Blackwell Publishing. Iowa, USA.

Sreekumari, M. T. and Abraham, K. 1991. Correlation studies in shade grown cassava. *J. Root Crops* 17 (1): 56-59.

Stegemann, H. 1984. Retrospect on 25 years of cultivar identification by protein patterns and prospects for the future. *Seed Science and Technology* 15: 20-31.

Suja, G., John, K. S., Sreekumar, J., and Srinivas, T. 2009. Short-duration cassava genotypes for crop diversification in the humid tropics: growth dynamics, biomass, yield and quality. *J. Sci. Food Agri.* 90: 188-198.

Surya kumari, S. and Anuradha, T. 2000. Genetic variability in edible lines of cassava under rainfed conditions of Andhra Pradesh. *J. Root Crops. 26(1): 8-9.*

Swarup, B. and Chaughale, D. S. 1962. Studies on genetic variability in sorghum phenotypic variation and its heritability components in important characters towards yield. *Indian J. Genet. Pl. Breed.* 22: 31-35.

Tautz, D., Renz, M. 1984. Simple sequences are ubiquitous repetitive components of eukaryote genomes. *Nucleic Acids Research* 12: 4127-4138.

Thottappilly, G., Mignouna, H.D., Omitogun, O.G. 2000. The use of DNA markers for rapid improvement of crops in Africa. *African Crop Science Journal* 8: 99-108.

Tonukari, N. J. 2004. Cassava and the future of starch. Electronic Journal of Biotechnology7.http://www.ejbiotechnology.info/content/vol7/issue1/ISSN:

Tumuhimbise, Melis, R. R., Shanahan, P., and Kawuki, R. 2012. Farmers' perceptions on early storage root bulking in cassava (*Manihot esculenta* Crantz) in east and central Uganda and their implication for cassava breeding. *World J. Agri. Sci.* 8: 403-408.

Tumuhimbise, R. 2013. Breeding and evaluation of cassava for high storage root yield and early bulking in Uganda. PhD thesis, University of KwaZulu-Natal, South Africa, 194p.

Venkatachalam, R., Saraladevi, D., Yassin, M. M., and Seemanthini, R. 1990. Evaluation of some short duration of cassava under Coimbatore conditions. *J. Root Crops* (ISRC-National Symposium, Special): 19-22.

Vieira, E.A., Carvalho, F.I.F., Bertran, I., Kopp, M.M., Zimmer, P.D., Benin, G., Silva, J.A., Hartwig, I., Malone, G., Oliveira., A.C. 2007. Association between genetic distance in wheat (*Triticum aestivum* L.) as estimated by AFLP and morphologic markers. *Genetic and Molecular Biology* 30: 392-399.

Vijaya, B. K. and Jos, J. S. 1981. Distribution of stomata and trichomes in cassava leaves. *J. Root Crops* 7(1): 11-14.

Wang, C., Lentini, Z., Tabares, E., Quintero, M., Ceballos, H., Dedicova, B., Sautter, C., Olaya, C., and Peng, Z. 2011. Microsporogenesis and pollen formation in cassava (*Manihot esculenta* Crantz). *Biologia Plantarum* 55: 469-478.

Welsh, J., McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* 18: 7213–7218.

Westby, A. 2002: Cassava utilization, storage and small-scale processing. In: R.J. Hillocks et al. (eds), Cassava: Biology, production and utilization. CABI, Wallingford, UK. p. 281-300.

Westwood, N. N. 1990. Maintenance and storage: clonal germplasm. *Pt. Breed. Rev.* 7: 111–128.

Wholey, D.W., and J.H. Cock. 1974. Onset and rate of root bulking in cassava. *Experimental Agriculture*. 10:193-198.

Williams, J.G., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18: 6531–6535.

Zacarias, A. M., and Labuschagne, M. T. 2010. Diallele analysis of cassava brown streak disease, yield and yield related characteristics in Mozambique. *Euphytica* 176: 309-320.

INTROGRESSION OF MOSAIC RESISTANCE IN POPULAR SHORT DURATION CASSAVA VARIETIES OF KERALA THROUGH MARKER ASSISTED SELECTION

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ABSTRACT

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ABSTRACT

The present study entitled "Introgression of mosaic disease resistance in to popular short duration cassava varieties of Kerala through marker assisted breeding"was conducted in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Kerala Agricultural University and Division of Crop Improvement, ICAR- Central Tuber Crops research Institute, Sreekariyam, Thiruvananthapuram, Kerala during the period 2014 - 2017 with the core objective of introgression of cassava mosaic disease (CMD) resistance to short duration varieties of cassava through marker assisted selection (MAS) and to study the inheritance of early bulking nature. The research work was carried out as four experiments.

In the first experiment, Five early bulking high yielding lines *viz,* Sree Jaya, Sree Vijaya, Vellayani Hraswa, CI 889 and 9S 75 and three testers *viz*, CR 54A3, IMS2-5 and CI 273 with resistance to cassava mosaic disease were selected and planted in a pollination block and crossed in Line x Tester (LxT) design to produce hybrid seeds of 15 F_1 combinations. Experiment II was conducted in two parts. Screening of F_1 seedlings for CMD resistance and early bulking nature was carried out in the first part of experiment II, where hybrids along with the parents were evaluated. Analysis of variance revealed significant differences among the genotypes for all the traits studied. All the agronomic traits were recorded and inheritance of early bulking and its correlation with other traits were studied. The CMD incidence expressed significant and negative correlation with tuber yield per plant where as significant and positive correlation for all other traits with tuber yield per plant was observed among the F_1's.

As a part of experiment II (b), seedlings without the CMD visual symptoms were subjected to multiplex PCR and the results revealed that among the parents Sree Jaya, Sree Vijaya, Vellayani Hraswa expressed presence of Srilankan Cassava mosaic

Virus (SLCMV) and Vellayani Hraswa expressed the presence of both SLCMV and Indian Cassava mosaic Virus (ICMV). Among the crosses, Sree Jaya x CR54 A3 (L1x T1), Sree Jaya x IMS2-5 (L1xT2), Vellayani Hraswa x IMS2-5 (L3xT2), CI 889 x CI 273 (L4xT3) expressed the presence of SLCMV. Real time PCR (qPCR) assay for seedlings identified CI 889 (L4), 9S 75(L5), CR 54A3 (T1), IMS2-5 (T2) and CI 273(T3) among the parents and Sree Jaya x CR54 A3 (L1x T1), Sree Jaya x IMS2-5 (L1xT2), Sree Jaya x CI 273(L1x T3) and 9S 75 x CR54 A3 (L5x T1), 9S 75 x IMS2-5 (L5xT2) and 9S 75 x CI 273 (L5x T3) among the crosses as highly resistant, based on viral load present in the DNA sample.

Based on the previous report ten CMD resistance linked markers were screened through BSA and five of which SSRY 28, SSRY 44 SSRY 40, SSRY 106 and SSRY 235 were selected. Among the CMD linked SSR markers studied, the maximum polymorphism was elucidated by SSRY 28, SSRY 44 and followed by SSRY 235. SSRY 28 is a strongly linked marker to *CMD2* which is a dominant gene conferring resistance among the clones of combinations (L1xT1, L2xT2, L3xT1 and L3xT3) three of five markers revealed alleles associated with *CMD2* gene

In the third experiment to evaluate the early bulking clones, field was laid out in randomized block design (RBD) with three replications consisting of CMD resistant clones along with parental clones using miniset technique.

Analysis of variance revealed significant differences among the genotypes for all the traits. Measurement of heterosis was carried out considering parent Vellayani Hraswa (L3) as check and results revealed that standard heterosis was positive and significant in the combinations Sree Jaya x CR 54A3 (L1xT1) and Sree Jaya x CI 273 (L1xT3) for all the yield contributing traits. The crosses Sree Jaya x CR54 A3 (L1x T1) and Sree Jaya x CI 273 (L1xT3) exhibited negative standard heterosis for CMD.

Combining ability analysis showed significant gca, sca variances and gca, sca effects for all the traits. Moreover gca/sca variance ratio indicated preponderance of dominance / non-additive gene action for the inheritance of all traits. Among the lines, Sree Jaya (L1) exhibited positive and significant *gca*effect for tuber yield and yield contributing traits. Among the testers, IMS2-5 (T2) exhibited negative and significant *gca* effect for CMD. Among the crosses Sree Jaya x CR54 A3 (L1x T1) exhibited positive and significant *sca*effect for girth of tuber and stem girth, 9S 75 x CI 273 (L5xT3) exhibited positive and significant *sca*effect for tuber yield per plant, CI 889 x CR 54A3 (L4xT1) exhibited negative and significant *sca*effect for CMD.

In the last experiment, through bulk segregants analysis using 5 SSR markers linked to early bulking in cassava were selected out of 9 SSR markers selected. Among 5 SSR markers of CMD and early bulking nature two SSR markers (SSRY 28 and SSRY 106) associated with resistance to CMD and One SSR marker, ESTs (SSRY) 292 associated to early bulking nature has been identified. Among the crosses, clones from Sree Jaya x CR54 A3 (L1xT1), Sree Jaya x CI 273 (L1x T3) and 9S 75 x CR 54A3 (L5xT1) are being confirmed with CMD resistance as well as early bulking nature.