ASSESSMENT AND INDUCTION OF VARIABILITY THROUGH *IN VITRO* MUTAGENESIS IN CASSAVA (*Manihot esculenta* Crantz)

By
HILÁRIO ERNESTO MAGAIA
(2011-21-118)

Department of Plant Breeding and Genetics
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA
2015

ASSESSMENT AND INDUCTION OF VARIABILITY THROUGH *IN VITRO* MUTAGENESIS IN CASSAVA (*Manihot esculenta*, Crantz)

By
HILÁRIO ERNESTO MAGAIA
(2011-21-118)

THESIS

Submitted in partial fulfillment of the requirement for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture
Kerala Agricultural University

Department of Plant Breeding and Genetics
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA
2015

DECLARATION

I hereby declare that the thesis entitled "**Assessment and induction of variability through *in vitro* mutagenesis in cassava (*Manihot esculenta* Crantz)**" is a bonafide record of research done by me during the course of study and the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other university or society.

Vellanikkara
08-04-2015

Hilário Ernesto Magaia
(2011-21-118)

CERTIFICATE

Certified that this thesis entitled "**Assessment and induction of variability through *in vitro* mutagenesis in cassava (*Manihot esculenta* Crantz)**" is a record of research work done independently by **Hilário Ernesto Magaia** (2011-21-118) 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.

Vellanikkara
08-04-2015

Dr. Jiji Joseph
Associate Professor and
Chairperson (Advisory Committee)
Department of Plant Breeding and Genetics
College of Horticulture
Vellanikkara

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Mr. Hilário Ernesto Magaia** (2011-21-118), a candidate for the degree of **Doctor of Philosophy in Agriculture** with major in **Plant Breeding and Genetics**, agree that this thesis entitled "**Assessment and induction of variability through *in vitro* mutagenesis in cassava (*Manihot esculenta* Crantz)**" may be submitted by him in partial fulfillment of the requirement for the degree.

Dr. Jiji Joseph
(Chairperson, Advisory Committee)
Associate Professor
Dept. of Plant Breeding and Genetics
College of Horticulture
Vellanikkara, Thrissur

Prof. C. R. Elsy
(Member, Advisory Committee)
Professor and Head
Dept. of Plant Breeding and Genetics
College of Horticulture
Vellanikkara, Thrissur

Dr. Dijee Bastian
(Member, Advisory Committee)
Associate Professor
Seed Technology Unit
College of Horticulture
Vellanikkara, Thrissur

Dr. Rose Mary Francies
(Member, Advisory Committee)
Associate Professor
Seed Technology Unit
College of Horticulture
Vellanikkara, Thrissur

Dr. C. Beena
(Member, Advisory Committee)
Associate Professor
AICRP on MAP&B
College of Horticulture
Vellanikkara, Thrissur

Dr. R. Sujatha
(Member, Advisory Committee)
Associate Professor and Head
Department of Plant Biotechnology
College of Agriculture
Padannakkad

(EXTERNAL EXAMINER)

Acknowledgement

"Love the LORD your GOD with all your heart and with all your soul and with your entire mind. Love your neighbor as yourself."

First and foremost, I kneel down before God Almighty and give my thanks for enlightening and blessing me with confidence, will power and courage to get through the difficult circumstances faced by me to complete this study.

My words cannot express the deep sense of immeasurable gratitude and undoubtful indebtedness to ***Dr. Jiji Joseph****, Associate Professor, Department of Plant Breeding and Genetics, College of Horticulture and chairperson of my advisory committee. With great respect and devotion, I wish to place my heartfelt thanks to her for the inspiring guidance, untiring interest, unstinted co-operation, esteemed advice, constructive criticism, valuable suggestion and immense help rendered by her during the investigation and preparation of the thesis. I am genuinely indebted to her for the constant encouragement and affectionate advice rendered throughout my academic programme.*

I take this opportunity to place on record my sincere gratitude and thanks to ***Dr. C.R. Elsy****, Professor and Head, Department of Plant Breeding and Genetics, College of Horticulture and member of my advisory committee for her critical suggestions and encouragement throughout the investigation for the successful completion of this programme and for the impartial scrutiny of the manuscript.*

I am deeply obliged to ***Dr. Rose Mary Francies****, Associate Professor, Seed Technology Unit, College of Horticulture and member of my advisory committee for her unfailing support and help during the investigation and especially for the manuscript enhancement.*

I am grateful to ***Dr. Dijee Bastian****, Associate Professor, Seed Technology Unit, College of Horticulture and member of my advisory committee for her*

valuable help, guidance rendering through the period of the research work. I thank her for all the help and co-operation she has extended to me.

I am thankful to ***Dr. R. Sujatha****, Associate Professor and Head, Department of Plant Biotechnology, College of Agriculture, Padannakkad, member of my advisory committee for her technical advice, valuable suggestions, guidance rendered and support throughout the period of this research work.*

I am ever grateful to ***Dr. C. Beena****, Associate Professor, AICRP on Medicinal and Aromatic Plants and Betel vine, College of Horticulture and member of my advisory committee for treasured technical support, sustained interest, kind concern and ever willing help rendered for the successful completion of my research work especially biochemical analysis.*

I am thankful to ***Dr. V.V. Radhakrishnan****, Professor (retired), Department of Plant Breeding and Genetics, College of Horticulture, for his valuable co-operation during the initial stage of investigation.*

I express my sincere gratitude to ***Dr. K.T. Presana Kumari****, Professor, Department of Plant Breeding and Genetics, College of Horticulture for her valuable support.*

I am thankful to ***Dr. Biju. S.****, Associate Professor, Department of Plant Breeding and Genetics, College of Horticulture, for his support especially in the preparation of the thesis.*

With deep reverence, I express my heartfelt thanks to ***Dr. Santhosh Kumar A.V.****, Associate Professor of College of Forestry, Thrissur, for his crucial support and the facilities provided for undertaking the laboratory studies and the whole hearted co-operation throughout the period of investigation.*

I would like to specially thank ***Dr. K. Ajith Kumar****, Associate Professor, Department of Pomology and Floriculture, for his timely help during the*

hardening and acclimatization stage of my study and also for his suggestions and unconditional support for the successful completion of my research work.

I am grateful to ***Dr. P. Sureshkumar****, Professor and Head, Radio Tracer Laboratory, College of Horticulture and* ***Dr. Tankaraj****, Professor, Centre of Plant Breeding and Genetics, Tamil Nadu Agricultural University for their timely help on the irradiation of research material for mutation study.*

I extend my thanks to the all the successive ***Associate Deans*** *of the College of Horticulture, to* ***Smt. V. S. Swapna*** *and* ***Dr. A.T. Francis****, Librarians, to* ***Mrs. Shakhila****, Academic Section Officer, to* ***Mr. Godwin A.G.****, Cashier, to* ***Mr. Aravind K.S.*** *of Students' Computer Club and to* ***Mrs. Anitha****, Photostat Section of the College of Horticulture for rendering necessary help and support whenever needed.*

I extend my sincere thanks to ***Mr. Presanth****,* ***Ms. Anu*** *and* ***Ms. Divya*** *from College of Forestry for their prompt help and cooperation on chemical analysis.*

I would like to acknowledge the help extended by each of non teaching staff of the Department of Plant Breeding and Genetics for all their remarkable support, especially ***Dr. Gayathri G.****,* ***Mrs. Smitha P.S.****,* ***Ms. Geethu A.R.****,* ***Ms. Lalitha M.V.****,* ***Ms. Daya Lakshmi****,* ***Ms. Mittu Thomas****,* ***Mr. Prince P.J.****,* ***Mrs. Bhavani****,* ***Mr. Krishnakuty K.K.****,* ***Ms. Saritha O.S.****,* ***Ms. Jincy Joseph****,* ***Ms. Asha K.G.****,* ***Ms. Sanitha M.V.****,* ***Ms. Revathy V.S.****,* ***Mrs. Shymol P.M.*** *and* ***Ms. Susan George.***

More personally I am thankful evermore to all my caring friends ***Saini Pawan****,* ***Manjunath Forico****,* ***Dhanesh N.J.****,* ***Jyothi Lekshmi****,* ***Fahida****,* ***Vikhe Parimal****,* ***Bansode Ravindra****,* ***Rajesh T.****,* ***Subhashini Shinde****,* ***Agatha Shiny****,* ***Tess Joseph****,* ***Amaranatha Reddy****,* ***Vilas K.****,* ***Sarath P.S.****,* ***Tincy Tom****,* ***Jeevan S.****,* ***Preethy T.T.****,* ***Rubhena****,* ***Asna A.C.****,* ***Suma A.*** *and* ***Asish I. Edakkalathur****.*

I am sincerely thankful to my companions in the International Students Hostel, ***Abdu Ibrahim****,* ***Baraket nugus****,* ***Sahle Yeibyo*** *and* ***Msgna Sium*** *(Eritrea),* ***Shifferaw M.*** *(Ethiopia),* ***Aston*** *and* ***Charles*** *(Malawi),* ***Auxilio*** *(Indonesia),* ***Sarvar****,* ***Abdul Razaq Shinwari****,* ***Ali Fekrat*** *and* ***Sherzad Ahjaz*** *(Afghanistan),* ***Emmanuel Zivenge*** *(Zimbabwe),* ***Yaha Nakaande*** *(Namibia),* ***Milly Sedi*** *(Kenya),* ***Ramnarace Sukhna*** *(West Indies),* ***Clement*** *and* ***Joan*** *(Rwanda) and* ***Kalilo Donzo*** *(Liberia),* ***Shyju*** *(Caretaker),* ***Kamrodin****,* ***Mohan****,* ***Unny*** *and* ***Rachandran*** *(Chief cooks) and all security staff.*

I am deeply grateful to the Twelve Apostles Church in Maputo, similarly to the bible study group and the Assembly of God Church in Thrissur, especially Senior Pastor ***Samuel****, Pastor* ***Santhosh*** *and Evangelist* ***Rajesh*** *for all their sustaining prayers. I am also grateful to my closely friends, Portuguese language speakers,* ***Dr. Davees C.J.*** *(St. Thomas College, Thrissur) and* ***Mr. Anoop Lazar*** *for their memorable and precious friendship.*

The award of Indo-Africa Agriculture Scholarship by African Union and Government of India (through ICAR) is thankfully acknowledged.

To all those, not mentioned here, but contributed directly or indirectly to the completion of the present study, my deep sense of gratitude.

Above all, with gratitude and affection, I recall the moral support, boundless love, constant encouragement, blessings and motivation of my family without which this endeavour would never have become a reality.

Hilário Ernesto Magaia

Dedicated to
My Dearest Family:
Victória,
Samira, Dércia,
Léria, Hizerílio
and all
Magaia's

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

2,4-D	-	2,4-Dichlorophenoxyacetic acid
BA	-	Benzyl Amino purine
CMD	-	Cassava Mosaic Disease
cm	-	Centimeter
CTCRI	-	Central Tuber Crops Research Institute
CIAT	-	Centro Internacional de Agricultura Tropical
CLS	-	Cercospora Leaf Spot
Co 60	-	Cobalt 60
CRD	-	Completely randomized design
EMS	-	Ethyl Methane Sulphonate
FRY	-	Fresh root yield
FEC	-	Friable embryogenic callus
Gy	-	Gray
HI	-	Harvest Index
ha	-	Hectare
HCl	-	Hydrochloric acid
HCN	-	Hydrogen cyanide
IBA	-	Indole-3-Butyric Acid
IITA	-	International Institute of Tropical Agriculture
kg	-	Kilogram
LAF	-	Laminar Air Flow
LD50	-	Lethal Dose 50
MSL	-	Mean Sea Level
µM	-	Micromolar
mg	-	Milligram
ml	-	Milliliter
mM	-	Millimolar
MS3 media	-	Murashige and Skoog media with 3.0 per cent sucrose
MS2, MS1, MS0	-	MS media with 2.0, 1.0 and nil per cent sucrose
nm	-	Nanometer
NAA	-	Naphthalene acetic acid

N	-	Normality
No.	-	Number
KCN	-	Potassium cyanide
RBD	-	Randomized block design
SI	-	Seletion index
NaOH	-	Sodium hydroxide
SE	-	Somatic embryo
i.e.	-	That is
TDZ	-	Thidiazuron
t ha^{-1}	-	Tonne per hectare
TG	-	Tuber girth
UV	-	Ultra Violet
WAC	-	Weeks after culturing

Introduction

1 INTRODUCTION

Cassava (*Manihot esculenta*) is a perennial woody shrub domesticated around 7000 BC in the Amazonian region of Brazil, South America. It was introduced in Africa and Asia in 16th century and more recently in Australia during 20th century (Lebot, 2009). Cassava is believed to have reached India in the 16th century through Portuguese travelers who embarked on the southern part of the country (Nair and Unnikrishnan, 2007).

Cassava with a worldwide annual production of 250 million metric tonnes (t) is the third most important source of carbohydrate, after rice and maize, in the tropics and subtropics. Africa is the leading producer of tapioca, producing fifty-three per cent of world's total production while India is credited with the highest productivity, *i.e.*, 35 t ha^{-1} (FAO STAT, 2013). Kerala is the major cassava producer state in India, followed by Tamil Nadu and Andhra Pradesh (Vanitha *et al.*, 2013).

According to Onwueme and Sinha (1991), cassava tuber is rich in starch and contain small amount of free sugars (non-starch), but weak in protein and vitamin A. However, Westby (2002), reported existence of yellow varieties containing nearly one per cent concentration of β-carotene. Cassava starch is an important component of both human diet and animal feed. Cassava starch is also exploited as raw material in textile, paper, adhesives, glucose, soap, detergent, laundry, ethanol, cosmetic powders, pharmaceuticals and insecticide, and food processing industries (Benesi *et al.*, 2004). Hence, cassava breeding mainly targets increasing tuber yield as well as improving its quality. Breeding efforts aims for higher dry matter and starch content, good cooking quality, low cyanide content, high vitamin A and protein content. Longer shelf life and shorter duration are also important considerations in cassava crop improvement.

As a naturally out-crossing and clonally propagated crop, genetic improvement in cassava is limited by poor knowledge of genetic diversity within the species. Many years of farmers' selection could have resulted in diverse

landraces with genes required for adaptation to biotic and abiotic stresses in higher frequencies. Many of the cassava types under cultivation in Kerala and other states of India are either chance seedling or bud mutations selected for desirable characteristics and maintained by vegetative propagation. Cultivars best suited to the local environments are generally adopted and gained popularity in the growing location.

Majority of cassava cultivars mature in ten months and thus occupy land for a longer period. In Kerala, cassava is usually cultivated as a monocrop in the uplands. However, presently, it is being cultivated more and more in low lying areas in rotation with short duration rice varieties (Abraham *et al.*, 2002). According to Nair *et al.* (1996) and Nair and Unnikrishnan (2007), attention is to be given to develop early maturing varieties which can be harvested in six months with stress resistance and good cooking quality so that they can be effectively be utilized in cassava – rice double cropping systems practiced in Kerala.

Being a cross-pollinated crop, inbreeding depression is common in cassava. Large number of cultivars has been obtained through intra and interspecific crosses by conventional breeding approaches. Nevertheless, the heterozygous nature of cassava weakens the selection procedure when conventional plant breeding approaches are practiced. The tangled additive and heterotic effects of gene action makes the estimation of parental breeding values difficult. Thus, the useful recessive traits go undetected and the backcross method is not feasible (Ceballos *et al.*, 2004).

Taking into consideration of the genetic nature of cassava, the limitations of conventional approaches of breeding and the need for further genetic improvement of the crop, the use of innovative breeding approaches such as mutagenesis is inevitable. *In vitro* mutagenesis has been identified as a potential tool to create genetic variability for cassava improvement because it gives faster results compared to true seed breeding approach. It can generate large genetic variability with greater chance of successful selection for new cassava clones, as

cassava is a vegetatively propagated crop with strong outcrossing characteristics (Taylor *et al.,* 1996). In cassava, mutation breeding approach has been practiced in India by Central Tuber Crops Research Institute (CTCRI) in Trivandrum, for development of varieties with cassava mosaic disease resistance and low level of HCN in the root (Abraham, 2002, Abraham *et. al.*, 2002).

In vitro mutagenesis in cassava requires an efficient regeneration system, appropriate treatment with mutagens and proper selection as the fundamentals. Tissue culture is essential for *in vitro* mutagenesis because it shortens the cloning process through expansion of the multiplication rate, supports genetic manipulation through totipotency and overcomes *in vivo* intra/interspecific barriers improving mutagenic efficiency. According to Raemakers *et al.* (1997), there are four *in vitro* regeneration methods in cassava, namely primary and secondary somatic embryogenesis (SSE), adventitious shoot formation (ASF), friable embryogenic callus (FEC) and protoplast culture (PC). One of these regeneration systems can be combined with *in vitro* physical or chemical mutagens, like Gamma-radiation (γ-rays) or Ethyl-Methane-Sulphonate (EMS), respectively, for successful induction of genetic variability.

Considering the importance of *in vitro* mutagenesis in cassava in inducing genetic variability, the present investigation entitled 'Assessment and induction of variability through *in vitro* mutagenesis in cassava (*Manihot esculenta* Crantz)' was initiated with the following objectives:

- Assessment of variability in short duration cassava genotypes
- Standardization of *in vitro* propagation method in cassava
- Induction of variability through *in vitro* mutagenesis in selected genotypes
- Evaluation of mutated population for quantitative and qualitative traits

Review of Literature

2 REVIEW OF LITERATURE

2.1 Introduction

The scientific study of cassava (*Manihot esculenta*, Crantz) has been neglected for a long time in the past, and considered as poor man's crop by the western based research. Now being one of the most important crops for hunger alleviation around the world, it has been targeted for more research attention (Blench, 1997). Genetic improvement of a tuber crop like cassava contributes to food security, especially in periods of nutritional stress in the major tropical regions, therefore justifying more public sector attention (FAO, 2013).

Studies on variability in cassava contribute to genetic improvement of the crop by providing information on the raw material for selection of high quality clones with better productivity.

2.1.1 Origin, expansion and taxonomy

Cassava may possibly have been cultivated in Colombia and Venezuela about 7,000 years ago (Purseglove, 1982). Nowadays the crop is grown practically in all tropical and subtropical countries (Onwueme and Sinha, 1991). Portuguese introduced cassava to Asia, around 17th century and to India around 1840-60 (Nair and Unnikrishnan, 2006; Namitha, 2010).

The genus *Manihot* belong to the family Euphorbiaceae and it is a large family of flowering plants with 300 genera and around 8,000 species of which cassava (*Manihot esculenta* Crantz) is most important economically (Rahman and Akter, 2013).

2.1.2 Production statistics and importance

In Kerala, cassava used to be the crop of food security for the entire state until 19th century. In Kerala, cassava root is a secondary staple food that is consumed at breakfast, lunch, dinner or as snack. The history of introduction of cassava in Kerala can be traced back to 1880`s when Vishakham Thirunal Rama Varma Maharaja (1880-85) of Travancore imported some planting material from

Brazil and planted in a fenced area. A royal proclamation was made stating that the root of the plant had great qualities and was only for palace use, and any one interfering with the plants would be severely punished. According to the story, all the plants were stolen overnight. Thus the Maharaja achieved his objective of spreading the cultivation of cassava.

However, the crop is now cultivated in an area of about one lakh ha producing 24 lakh tonnes with an average productivity of 33.30 t ha^{-1}. Until 1970s cassava was mainly cultivated as an upland crop, then started occupying low land areas replacing paddy. It has been observed that the area under cassava in upland is declining compared to its earlier scenario. This is due to competition from more remunerative plantation crops like rubber and coconut (Abraham *et al*., 2002). Also cultivation of cassava as a monocrop in the uplands started declining (Vanitha *et al*., 2013). Now the crop is progressively being cultivated in low lying areas after main crop of rice and for this reason short duration varieties are needed (Edison *et al*., 2006).

Cassava with its tuberous roots provides a major food source for more than 800 million people in tropics (Lebot, 2009). Its leaves are also used for human consumption as vegetable in some parts of Africa (Purseglove, 1982; Chavez *et al*., 2000) and as mineral supplement (after dried and milled) for babies in Brazil (Henry *et al*. 1998).

2.1.3 Genetic population structure and propagation

The population structure of cassava is highly heterozygous and is propagated vegetatively. Thus, its true seed is only confined to plant breeding work (Allem, 2002). Inbreeding depression occur when selfing is practiced. The conventional breeding method used for cassava genetic improvement is clonal selection which impedes genetic recombination (Ceballos *et al*., 2004). As a result, for further genetic improvement of the crop, especially addressing fresh root traits, duration and stress resistance, innovative breeding approaches are required.

2.2 Studies on variability

For genetic improvement of any crop, a comprehensive knowledge of genetic variability of various quantitative characters and their contribution to yield is a fundamental requirement. To understand the genetic variability, three main aspects should be considered by a breeder, they are (i) the reproductive system of the species, (ii) the genetic control of the trait under study, and (iii) the selection methods to be used to come out with improved genotypes. Therefore, studies on variability constitute the starting point for planning any breeding programme and the success of genetic improvement of any trait depends on the nature of variability present for the trait (Falconer and Mackay, 1996).

Phenotypic variation of any trait is a combination of genetic variation, environmental variation and variation due to the interaction between the genetic and the environmental factors. The extent of variability is measured by genotypic and phenotypic coefficient of variance (GCV and PCV) which provides information about relative amount of variation in different characters and heritability provides a quantitative measure of this proportion (Acquaah, 2009). Since heritability is also influenced by environment, the information on heritability alone may not help in pin pointing characters enforcing selection (Brown and Caligari, 2008). The heritability estimates along with the predicted genetic advance will be more reliable and helpful in formulating suitable selection procedure (Roychowdhury and Tah, 2013).

2.2.1 Biometrical traits

The genetic improvement programme needs extensive knowledge of genetic variations in the available in the germplasm. Variability studies in cassava by Mahangu *et al*. (1983) reported moderate coefficient of variation (CV) for total number of branches and low CV for stem girth, canopy width and plant height at harvest. Stem girth, canopy width and plant height at harvest showed moderate to low heritability values associated with low expected genetic gain.

Naskar *et al*. (1991), obtained high heritability estimates and genetic advance for plant height and stem girth. According to Suthanthirapandian *et al*. (1994), highest genetic advance was noticed for the number of leaves. The height of plant showed maximum phenotypic variation and genotypic coefficient of variations were high for all plant characteristics except stem girth (Rai *et al*., 1986; Naskar *et al*. 1991; Suthanthirapandian *et al*., 1994).

Durasaimy *et al*. (2011) carried out the factor analysis for 28 morphological traits to identify the combination of traits contributing for maximum variability. Among the variables, petiole colour was found to be of maximum variation (60.8per cent) and neck length of the tuber had the least variation (2.1per cent), whereas bud colour and number of tubers were contributing to variation of 57 per cent and 49.5 per cent respectively.

Heritability, as well as expected genetic gain for root traits was commonly high in cassava. The root yield as well as the number of roots showed moderately high heritability and high response to selection. The PCV and GCV were high for root yield and number of roots per plant. Relatively high heritability estimates were obtained for dry matter content, but its expected gain was only 29 per cent (Mahangu *et al*. 1983).

Naskar *et al*. (1991) obtained high heritability and genetic advance for number of tuber and tuber yield in cassava. PCV and GCV for different root traits in cassava did not vary much revealing that they were not influenced by environment and selection can be done based on phenotypic values (Rai *et al*., 1986; Naskar *et al*. 1991; Suthanthirapandian *et al*., 1994).

According to Kawuki *et al*., 2011, the elite genotypes were having relatively higher dry matter content than local genotypes. Durasaimy *et al*., 2011 observed considerable variations to tuber length followed by number of tubers and tuber girth. The CV was found to be the maximum for number of tubers (67.3 per cent).

It has been reported that there was enormous genetic variation for harvest index (HI) in seedling populations of cassava. By virtue of its high heritability, HI

could be effectively used as an indicator for seedling selection. In cassava, the heritability of harvest index was much higher than that of root yield and total plant weight under both very high yielding and low yielding environments (Sinclair, 1998). This suggests that harvest index is a highly stable character over a wide range of environment whereas yield and total plant weight are not as stable. The coefficient of variation for HI was found to be moderate in cassava. The highest average HI observed was 0.60 and lowest 0.32 among African cassava germplasm. Relatively high heritability estimates also were obtained for harvest index with a genetic gain of 50 per cent (Mahangu *et al*., 1983; Kawuki *et al*., 2011).

2.2.2 Qualitative traits

As farmers identify and select their best genotypes of cassava based on qualitative traits of the shoot portion, proper description of the genotypes using standardized morphological descriptors is important. Kawuku *et al*., (2011) studied 29 qualitative traits in cassava and grouped the germplasm in to eight major groupings. Some of these groupings comprised cassava genotypes from the different countries, but some consisted primarily of germplasm from a single or two countries. Viera *et al*., (2011) evaluated 33 qualitative traits and observed that six qualitative characters do not have any variability. Studies conducted by Mezette *et al*., (2013) observed a higher proportion of white or cream root cortex colour (71 per cent), while, flesh colours were predominantly white (49 per cent) and cream (42 per cent).

2.2.3 Biochemical traits

Cassava is a staple food in Indonesia and Southern India. Cassava tubers and leaves are used for human consumption in Africa. It is a source of carbohydrates, protein and minerals providing about 200 to 700 calories per day per person (Cock, 1985). Cassava tuber for human consumption is categorized as table or sweet cassava and bitter cassava is used in animal feed and industrial purposes (FAO and IFAD, 2006).

Typical fresh cassava roots consist of 62 per cent water, 21 per cent carbohydrate, 1.0 per cent protein and 0.5 per cent fibre (Onwueme & Sinha, 1991). Pillai *et al*. (2006) screened twelve accessions for biochemical traits and highest amylose content of 30 per cent was observed in accession No. E-108 and accession No. E-109 showed the highest amylopectin to amylose ratio of four.

The safety limit for cyanogens for cassava products was set at 10 mg kg^{-1} dry weight (FAO/WHO, 1991). On average, unprocessed fresh cassava roots contain about 15 – 440 mg kg^{-1} cyanide equivalents, while high cyanide cultivars can contain up to 1,500 mg kg^{-1} (O'Brien *et al*., 1992). King and Bradbury (1995) concluded that many compounds contributed to the taste of cassava tubers and the bitterness of cassava is not always correlated positively with the cyanide potential.

High cyanogenic potential of 12.86 mg HCN equivalent per 100 g fresh tuber weight was found by Raji *et al*., 2007 in some improved cultivars, while Nigerian cassava landraces had lower cyanogenic potential of 1 – 5 mg HCN equivalent per 100 g fresh tuber weight, which is considered as non-toxic. Bakayoko *et al*. (2009) found a range of 9.0 - 13.3 mg HCN per kg of fresh root in improved varieties which were significantly low compared to 32.2 mg per kg HCN of local varieties. They also observed that the starch content was about 87 per cent and the fibre 2.0 per cent on dry weight basis.

2.2.4 Organoleptic traits

Safo-Katanka *et al*. (1997) investigated cooking quality of cassava in terms of mealiness of the cooked roots, elasticity and freedom from lumpiness of the pounded paste. Microscopic study of the cells of raw and cooked roots showed that the cooking quality was related to the size of the starch granules, and the difference between varieties could be explained on the basis of cell disorganization. Dry matter and starch content were related to the differences in mealiness, and amylose content of the starch to the elasticity and smoothness of the pounded paste. Fibre content was negatively correlated with cooking quality. The loss in cooking quality during rainy season was more due to the reduction of

dry matter than starch content. Changes in the gelatinization properties of the starch were also related to the loss in cooking quality.

Raji *et al.* (2007) observed that improved cultivars were having low mealiness and the landraces were having high mealiness of boiled tubers. Although, the landraces gave lesser yields than the elite cultivars, they carried genes for adaptation to local conditions, and have preferred tuber quality attributes that can be introgressed into elite germplasm. The quality traits for cassava with respect to consumers' preference before cooking are fresh tuber colour, easiness for peeling and cooking time and after cooking are taste, texture and fibre content (Gonzalez & Johnson, 2009). The sweetness of fresh tubers on chewing can be an indication to categorize tuber into sweet or bitter cassava, based on low and high HCN content (Frank, *et al*., 2011).

2.2.5 Biotic stress

Hahn *et al*. (1980) estimated the heritability for resistance to Cassava Mosaic Disease (CMD) as 50 – 70 per cent and the trait was assumed to be polygenic. Prabakar and Raguchander (2001) observed that white fly population was positively correlated with mosaic incidence. Varieties having resistance to white fly infestation may be able to evade (Pillai *et al*., 2006). A survey conducted by Jose *et al*., (2011) on the incidence of CMD in Kerala, showed that the disease occurred throughout the state at low to high incidences (44.5 – 96.75 per cent). Manu-Aduening *et al*., (2013) observed significant genotypic differences amongst the progenies and the parents in susceptibility to CMD. *Cercospora* Leaf Spot disease (CLS) is the most important fungal disease of cassava and is found wherever the crop is grown. Severe incidences of the disease occurred particularly under wet conditions, causing great loss in leaf and tuber yield (Hillocks and Wydra, 2002).

2.3 Correlation studies

One of the most important objectives in a breeding programme is to incorporate the genetic potential for high yield in a variety. Yield being a complex

character, it is worthwhile to estimate the influence of the association existing between the variable characters and yield. The knowledge of its magnitude and sign helps in understanding how the improvement in one character will cause simultaneous change in other characters. A comparison of phenotypic and genotypic correlations would give an indication on the effect of environment on the genetic performance of individuals of a population.

Sreekumari and Abraham (1985) reported that girth of stem and tuber showed significant positive correlation with tuber yield under shade condition. Correlation studies in cassava showed that tuber yield was positively and significantly correlated with all characters except petiole length (Naskar *et al*., 1991). The correlation between bitterness in cassava and cyanogenic potential was found to be high, but a cause-effect relationship is still questionable (Bokanga, 1994). Asante and Dixon (2002), found that the correlation between economically important traits, *viz*., root number, root weight and root fresh yield was highly significant and positive.

Aina *et al*. (2007) reported correlation coefficient of 0.95 for number of medium-sized roots with yield. Number of roots (r = 0.91) and small-sized roots (r = 0.77) were also correlated with root yield. According to Padma *et al*. (2009), tuber yield was positively correlated with plant height, tuber weight and tuber width. Fresh root yield in cassava was positively correlated with canopy mass, number of storage roots per plant, plant height and HI. It was suggested that harvest index should be used with caution in the selection process because plants with high harvest indices and little canopy yield, were undesirable because they produced little propagation material (Aina *et al*., 2007; Ntawuruhunga and Dixon, 2010).

Manu-Aduening *et al*. (2013) found negative correlation between incidence and other traits. Mulualem and Dagne (2013), observed that the genotypic correlation between fresh root yield and number of vertical stem per plant, root girth, root diameter, weight of above ground plant parts and root dry weight was

highly significant, while positive and significant phenotypic correlation was observed between root fresh yield per plot, root girth, root length and diameter.

2.4 Path Coefficient Analysis

Path coefficient analysis devised by Wright (1921) measures the direct influence of one variable upon another and permits the separation of correlation coefficients into components of direct and indirect effects. Rekha *et al*. (1991) reported that single tuber weight, girth of tuber and length of tuber were found to be the three factors exerting considerable influence directly and indirectly on tuber yield in cassava.

Path analysis by Aina *et al*. (2007) revealed that number of roots had the largest direct effect on root yield, followed by number of medium-sized roots. Small-sized roots had a negative direct effect on root yield but a positive indirect effect via number of roots. According to Padma *et al*. (2009) tuber weight followed by plant height had maximum positive direct effect on tuber yield.

Study by Mulualem and Ayenew (2012), showed that diameter of roots exhibited highest direct effect of (1.98) towards root fresh weight; while minimum was indicated by plant height (-1.83). Mulualem and Dagne (2013) observed that, root diameter had the highest direct effect towards root fresh weight; while minimum was indicated by number of branches per plant. Number of vertical stem per plant, stem girth, weight of shoot and root dry weight also contributed to the root fresh weight.

2.5 Cluster analysis

Selection of parents based on the extent of genetic divergence had been successfully utilized in different crop plants by Moll *et al*. (1969), Miller and Marani (1963), Murthy and Anand (1966) and Bhatt (1970). Pillai (2002) working with 73 exotic collections of cassava germplasm, evaluated 10 yield and quality characters, and eight out of ten characters showed high variability. Following non-hierarchical Euclidean cluster analysis all the 73 genotypes were grouped into six clusters with variable number of genotypes. Accessions of heterogenous origin or

place of release often grouped together in the same cluster, suggesting some degree of ancestral relationship between the genotypes. Chioma (2009) using agglomerative hierarchical cluster analysis performed on squared Euclidean distance matrix for 43 Nigerian resistant genotypes identified six groups at 0.97 coefficients of similarity. This indicated likeness among the genotypes, suggesting that the selection can be done from each cluster depending on the requirement.

2.6 *In vitro* propagation in cassava

Cassava multiplication through micropropagation, besides being a faster and convenient system, can be used as a tool to improve the genetic make-up of cassava through genetic manipulation. In addition, *in vitro* propagation facilitates the storage and international exchange of cassava germplasm (Smith *et al*., 1986; Onuoch and Onwubiku, 2007). Properly optimized, micropropagation is a cheap and simplified laboratory based technique for cassava propagation, not affected by environmental conditions that limit the time of planting, nor by phytosanitary conditions of propagation based on stem cuttings (Santana *et al*. 2009).

2.6.1 Explant collection and sterilization

Explants from indoor samples have low contamination compared to outdoor fields. Also samples collected from outdoor field at midday are easier to sterilize than those collected in the morning and at nightfall (Ruimei *et al.*, 2009). About four to ten cassava stakes per clone can provide sufficient propagule, one month after planting (Cacai *et al*., 2013). Contrary, a period of seven months is needed for excision of axillary explants from the young branches for the same purpose (Chishimba and Lungumbwanga, 1997; HaiWang *et al*., 2010).

Disinfection with 0.1 per cent solution of mercuric chloride on the axillary explants of cassava for 6-10 minutes showed survival rate of explants ranging from 32.3 per cent to 37.9 per cent (HaiWang *et al*., 2010). Many commercial detergents or surfactants can be used for pre-sterilization, such as sodium hypochlorite at 5 – 10 per cent concentration for 10 – 20 minutes with gentle shaking, followed by 3 – 5 times rinse with distilled water. The absolute

sterilization is performed under Laminar Air Flow at two stages using 70 per cent ethanol followed by 0.05 - 0.10 per cent Mercuric Chloride ($HgCl_2$), for 1 – 5 minutes each, and then followed by 3 – 5 times rinsing with distilled water (Ruimei *et al.,* 2009; Bheranu, 2011; Cacai *et al*., 2013).

2.6.2 Establishment of *in vitro* regeneration system

In vitro regeneration system in cassava comprises three different types, *i.e.*, embryogenesis, organogenesis and germination. It can be caused by direct and indirect organogenesis or embryogenesis. Essentially, somatic embryos (SE) constitute the common indirect source for all regeneration types. Nodes, axillary buds and meristems forms the explants for organogenesis, and biological seed for germination. Direct embryogenesis produces hard callus and or friable embryogenic callus (FEC), whereas, indirect embryogenesis produces FEC but via secondary embryogenesis (Konan *et al*., 1994, 1997; Raemakers *et al*., 1997; Mussio *et al*., 1998; Li *et al*., 1998; Fregene *et al*., 1999; Zhang *et al*., 2001).

2.6.3 Plant growth regulators and *in vitr*o culture media

Auxins and cytokinins constitute the main classes of plant growth regulators used for *in vitro* regeneration systems, besides gibberellins and abscisic acid. The commonly utilized auxins in cassava regeneration are 2,4-D (2,4-dichlorophenoxy acetic acid), NAA (naphtalene acetic acid) and picloram (4-amino-3,5,6-trichloropicolinic acid). Callus formation or rhizogenesis induction is achieved using high or lower auxins concentrations, respectively. On the other hand, BA (6-benzyl adenine), kinetin and TDZ (Thidiazuron) are the commonly used cytokinins. Their application at low concentrations when combined with auxins induces cell enlongation and rhizogenesis, whereas their solely use at high concentration promotes organogenesis rather than rhizogenesis (Chawla, 2010).

Rapid *in vitro* propagation method of cassava can be achieved using Murashige and Skoog (MS) solid media. In which nodal explants are induced to grow as multiple-shoots in the MS medium containing 1.0 μM BA, supplemented with 0.25 μM NAA as growth regulators. On every three weeks of growth, nodes

were removed from the shoots and subcultured on fresh culture medium. Each growth cycle can produce an average of 7.0 nodes (Smith *et al*., 1986). Bhagwat *et al*. (1996) reported higher efficiency of regeneration yielding an average of 31.5 shoots per nodal explant after 10 weeks of culture. It consisted one week exposure to 0.11–0.22 µM TDZ in liquid MS medium followed by culture on solidified MS medium supplemented with 2.2 µM BA and 1.6 µM GA3.

Konan *et al*., 1997 could produce 25 shoots per culture in MS medium with 10 mg l^{-1} BA within 4-6 weeks. Half-strength MS medium without plant growth regulators showed consistently superior results when compared to Linsmaier and Skoog (LS) medium supplemented with 0, 1, 2, or 3 µM BA (Chishimba *et al*., 1997), similar result were obtained by IAEA (2006) in ½ MS + 20 g Sugar + 5.8 pH + 1.8 g Gelrite.

In order to promote root initiation on the developing plantlets, the nodal explants were transferred to a MS medium containing 2.5 µM IBA (Smith *et al*., 1986). MS medium alone is able to initiate *in vitro* rooting in plantlets that can be successfully transferred to soil conditions, and once established in the field they show no abnormalities in growth, form and tuber quality (Chishimba *et al*., 1997).

Multiplication of *in vitro* propagules initiated from nodal explants on a MS + 20 g l^{-1} sucrose + 0.5 mg l^{-1} thiamine + 0.04 mg l^{-1} BA + 0.02 mg l^{-1} NAA + 0.05 mg l^{-1} GA3 + 7.5 g l^{-1} agar was effective for axillary propagation without noticeable callusing (de Melo, 2002). HaiWang *et al*. (2010) obtained multiple shoots in MS+NAA 0.3 mg l^{-1} media but with occasional callusing. Formation of callus is one of the critical handicaps for *in vitro* multiplication of some cassava varieties (IAEA, 2006).

The full MS supplemented with 0.01 mg l^{-1} NAA and 0.05 mg l^{-1} BA gave 100per cent plantlets survival with the highest mean plant height of 1.49 cm and mean root number of 0.82 (Mapayi *et al*., 2013). Roca *et al*. (1984), Konan *et al*. (1997), Groll *et al*. (2002) and Danso and Ford-Lloyd (2002) gave detailed protocols for *in vitro* regeneration using nodal explants on different media

composition however, they were later proven less effective than ½ MS2 (IAEA, 2006).

2.6.3.1 Induction media for friable embryogenic callus

Friable embryogenic callus (FEC) is an important stage for indirect organogenesis of cassava. Gresshoff and Doy (GD) basal medium supplemented with picloram or MS media + 50 µM picloram gave the best results for producton of FEC (Taylor *et al*., 1996; Sheela *et al*., 2007). Based on this system high quality embryogenic suspension cultures can be established routinely. The system is valuable for different biotechnological purposes such as cassava genetic transformation (Taylor *et al*., 2001) or mutation breeding.

According to Groll *et al*. (1997), nodal explants gave the highest frequency of embryogenic callus formation especially when nodes are excised from the uppermost nodal position on the parent plant. Basal media containing BA, 2iP, Kinetin, or TDZ at levels of 10 µM when supplemented with IAA at 0.1 µM, induced embryogenic callus after 12 days of initial culturing. Direct root organogenesis 5 days after culturing was also observed, but shoot organogenesis was never observed.

Callus formation was reported by de Melo (2002) in young leaf segments cultured *in vitro* using MS + 5-20 mg l^{-1} 2,4-D medium. This resulted in the formation of both FEC and compact calli after two weeks of culturing. Ogburia, 2002 investigated the recalcitrant property of roots, hypocotyl, epicotyl, petiole, leaf and ovary cassava explants on their ability for callus formation. Roots and leaves were the most recalcitrant explants. Callogenesis frequency of 46.7 per cent was observed in the MS + 8 mg l^{-1} 2,4-D + 1.0 mg l^{-1} BA.

2.6.3.2 Induction of somatic embryos

De Melo (2002) obtained somatic embryos (SE) by exposing FEC on MS + 0.2 mg l^{-1} GA3 +0.2 mg l^{-1} BA, which were immediately germinated into plants on multiplication media. Embryogenic property can be maintained for prolonged period of time by repeated cycles of secondary embryogenesis (Raemakers *et al*.,

1995). High genotypic dependence is the major problem with this system. The development of an efficient system for production of SE by inducing primary SE from leaf explant and cycling SE through Secondary somatic embryogenesis was described by Raemakers *et al*. (1997), Li *et al*. (1998), Saelim *et al*. (2006) and Ibrahim *et al*. (2008).

Somatic embryogenesis in cassava has also been induced from floral tissue (Woodward and Puonti-Kaerlas, 2001), young leaf lobes (Groll *et al*., 2001, Joseph *et al*., 2001), immature leaves (Ma and Xu, 2002), leaf segments of *in vitro* plantlets (Takahashi *et al*., 2000; de Melo, 2002), zygotic embryos (Raemakers, 1993; Stamp and Henshaw, 1982; Szabados *et al*., 1987), friable embryogenic callus, cell suspension cultures (Taylor *et al*. 1996), and thin cell layers from petiole and internodes (Groll *et al*., 2001).

Direct somatic embryogenesis can be achieved from cassava immature leaves through MS media + 2,4-D or NAA, as reported by Ma and Xu (2002), or on MS + 1.0 mg l^{-1} GA3 (de Melo, 2002). Some genotypes fail to produce somatic embryos (Rossin and Rey, 2011). The embryogenic cells mainly are induced within 4–12 days. From FEC auxins enhanced the formation of somatic embryos, while BA stimulated development of adventitious shoots (Ma and Xu, 2002).

2.7 *In vitro* mutagenesis

Mutagenesis is the process of exposing propagules to mutagens in order to generate change in genotypes. According to Toffino *et al*. (2011), seeds, cuttings, pollen grain, as well as *in vitro* plantlets, nodes, meristems and calli can be exposed to physical and chemical mutagens at effective intensities either *in vivo* or *in vitro*. As vegetatively propagated crops do not produce seed and often the size of the propagule is too big to treat with mutagen *in vivo* mutagenesis is difficult. However, in *vitro* techniques allow mutagenic treatments of large numbers and multiplication of the selected genotypes in a small space and duration under disease free conditions.

In vitro culture techniques also allow selection of the desirable variants from large population of cells. Even though, the occurrence of desired mutation is empirical and random, the combination *in vitro* and mutation techniques can speed up the breeding of vegetatively propagated plants like cassava (Ahloowalia, 1995).

2.7.1 *In vitro* mutagenesis *via* gamma radiation

Physical mutagen such as gamma-rays, X-rays and neutrons are known to be more effective in vegetatively propagated crops because they reach the target easily and can be applied precisely as compared to chemical mutagen (Konzak, 1984). Irradiation has been more useful in producing new genotype variants in asexually propagated crops (Nybom and Koch, 1965).

This may be due to insufficient penetration of the chemicals and generally poor chromosome breaking ability in seed crops. For crops like cassava with severe constraints posed on its genetic improvement by inherent heterozygosity, the adoption of this methodology is necessary. However, there is scant information on protocols for inducing mutations in cassava (Owoseni *et al*., 2006).

Ahiabu *et al*. (1997) irradiated *in vitro* cassava shoot meristem culture, where a lethal dose (LD50) was fixed as 40Gy. Lee *et al*. (1997) irradiated *in vitro* cultures of cassava from young leaves with doses between 4.0 to 3.80 Gy. Compact and FEC were observed after 10 – 15 days of culture in dark condition, then SE were produced through maturation media, which displayed morphological aberrations. The LD50 was fixed as 20 Gy for leaf explant and 10 Gy for SE.

The most commonly used physical mutagen is gamma radiation (Gamma rays), using cobalt (Co 60), at 200 grays (Gy) exposure for seeds and between 10-60 Gy for cuttings and pollen grain. In case of *in vitro* propagules the exposure varied from 12 to 50 Gy (Toffino *et al*., 2011).

A cyclic somatic embryogenic system can be used to induce mutations in cassava, through irradiation of selected globular-stage SE. The optimal dose for inducing mutations was determined to be 50Gy. Evaluation of mutants showed that more than 50 per cent of the regenerated plants varied morphologically from original type plants. Thus, this approach could be applied to induce genetic variability for production of novel cassava cultivars (Joseph *et al*., 2004).

2.7.2 *In vitro* mutagenesis *via* Ethyl Methane Sulfonate

Ethyl methanesulfonate (EMS) is an alkylating agent and the most commonly used chemical mutagen, especially for *in vitro* mutagenesis as a strategy to mitigate the confounding effects of chimeras in a crop such as cassava. This is achieved through regeneration of plants from one or a few cells that have been induced to mutate. The same procedure of *in vitro* culture establishment, either based on nodal explant or somatic embryo followed by mutagen treatment, and quantifying the observed damage on regenerated plants for gamma irradiation are the same when chemical mutagens are used (Escobar-Perez *et al.*, 2008; Mba *et al*., 2010).

The explants are transferred into the homogeneous EMS solution and kept for hours standing on a gyratory shaker. After this treatment the explants are washed passing through sterile sieves three times with sterile distilled water, and transferred into conical flasks containing liquid growth media. Afterwards, the flasks are incubated on a horizontal gyratory shaker under continuous light at 26ºC, replacing the media every 24 hours at least two times. Lastly, the explants are transferred into sterile whatman filter paper to remove the excess of liquid media and then transferred to semisolid MS basal growth media (Escobar-Perez *et al.*, 2008; Mba *et al*., 2010).

According to Toffino *et al*., 2011, EMS can be used at concentrations of 0.5 to 1.1 and 0.25 to 1.0 per cent for in vivo and *in vitro* treatments, respectively. Similar protocols for induction of mutation in nodal explants, using EMS in *in vitro* suspension culture systems can be successfully applied for mutagenesis in

somatic embryos. This has been reported in cassava (Escobar-Perez *et al.*, 2008) and soybean at concentration of 1 - 30 mM for 4 hours (Hofman *et al*., 2004; KyuJung *et al*., 2008), sweet potato at 0.5per cent for 3 hours (Luan-YuShi *et al*., 2007) and in grape vine at 10 – 20 mM (Acanda *et al*., 2014). EMS can also be incorporated into MS media at different concentrations, avoiding the need for suspension cultures and rotary shakers.

Tejaswini *et al*. (2006) incorporated EMS in tissue culture media at three different concentrations (0.01 per cent, 0.05 per cent and 0.1 per cent) and compared with EMS treatment in suspension culture solution at 0.1, 0.5 and 1.0 per cent for ¼, ½ and 1 hour each in *in vitro* shootlets of carnation. It was shown that lower concentration of EMS is sufficient for incorporation, whereas comparatively a higher concentration need to be used in case where explants are dipped in the mutagen.

2.8 Hardening of *in vitro* plantlets

Hardening is gradual strengthening of the delicate, soft and fragile tissue of in vitro plants, in order to achieve maximum survival when transferred to *ex vitro* conditions. It is commonly performed under lab conditions by reducing the media strength and sugars and avoiding plant growth regulators (Ziv, 1986). Whereas, acclimatization refers to the similar process but performed under green house conditions by gradually reducing the high relative humidity, as well as, increasing the temperature and light to natural/ambient *ex vitro* levels.

Significant loss of cassava *in vitro* plantlets occur during hardening in semi-arid climatic zones (George, 1996) as well as in culture rooms where the environment is less critical than greenhouses (Jorge, 2002). IITA and CIAT guidelines guarantees only 35 – 50 per cent plant survival rates in Mozambique and Zimbabwe (Jorge, 1996; Jorge *et al*., 2000) with similar rates reported from Brazil and Costa Rica (da Silva *et al*., 1995; Saborio *et al*., 1998). This situation hampers the adoption of tissue culture as a tool for cassava mass propagation and genetic improvement by developing countries around the world.

Studies conducted by Jorge *et al*. (2000) revealed that the increase in light intensity resulted in increased fresh and dry mass and increase in stem thickness of *in vitro* plants. But it also increases the leaf senescence. Consequently, during acclimatization those plants suffer 30 – 70 per cent reduction in survival. They also found that root regeneration to be critical, revealing the importance of well established callus free rooting system strongly connected to the mother plant. In the green house high humidity and shade conditions are essential in the first week, thus, it is advisable to perform acclimatization during rainy and cool season if performed in (sub) tropical areas.

During hardening transplanting must be done immediately after extracting the *in vitro* plants, irrigated with the correct quantity of water and nutrient solution at field capacity, and placed in humidity chamber. If the high relative humidity created by the environment within the chamber is guaranteed, the plantlets will not need further irrigation. Each plantlet can be irrigated with 10 cc of a nutrient solution consisting of a mixture of two gram of fertilizer rich in phosphorus to promote root formation per liter of deionized water. After 70 to 90 days of acclimatization in the mesh house or greenhouse, the plants can be transplanted to the field. During the transporting the plantlets must be protected from strong air currents to prevent abrasion or dehydration (Ospina *et al.*, 2007).

In vitro plants survive better under both natural and low levels of light and can tolerate high temperatures and the development of *ex vitro* functional roots is critical to guarantee the plant survival during the first days after transplant, the standard recommendations for a successful survival in four weeks are: (i) covering the plants with a humidity tent and a shade cover for two weeks, (ii) partial removal of humidity tent but the shade had to be left for two more weeks, to allow a gradual adaptation to the high light natural conditions (Jorge, 2002; Ospina *et al*., 2007).

The availability of microsprays in a greenhouse suspended either over the tables or from the roof and installed along the floor to control the temperature and

relative humidity, especially in the first days of the hardening process is crucially essential. Maximum temperature should be 33 – 38 ºC, and the minimum 18 – 22 ºC. The sterilized substrate in which the plantlets will be grown can be one part of black milled and sieved soil mixed with three parts of washed and sieved coarse sand. The installations should be disinfected before transplanting (Ospina *et al*., 2007). For temporary planting of the plantlets, the wood chaff was found to be the best with more than 70 per cent of survival rate by HaiWang *et al*. (2010).

2.9 Evaluation of mutated plantlets

The evaluation of *in vitro* mutated plants can be done at three stages, viz., *in vitro* culture conditions under tissue culture lab, response to acclimatization under green house conditions and performance after transplanting under field conditions. Growth parameters of plantlets are the best indicator for evaluation of *in vitro* mutants, such as number of callus sprouted and or rooting, plantlet height, thickness, number of nodes, number of leaves developed and or senesced, number of roots and total fresh and dry mass. Under green house conditions mutants can be assessed for survival rate, plant height and thickness, number of nodes, number of leaves, leaf length, root number and or length and thickness of roots (Jorge *et al*., 2000; Mapayi *et al*. 2013).

Once the mutants are established in field conditions the International Institute of Tropical Agriculture (IITA) descriptors for cassava genotypes might be used for both characterization and performance evaluation against the original genotype. Since, each mutant plant has potential to become a new clone the data should be recorded at individual basis. The qualitative characters to be considered are stipule colour, petiole colour, emerging leaf colour, leaf shape, internodal length, leaf scar and stem colour. Plant biometrical traits to be recorded are plant height, height of 1st branch, stem girth, number of branches per plant and incidence of pests and diseases. Harvesting data should consist of tuber colour rind colour, tuber length, length of neck, tuber weight, tuber girth and number of commercial tubers (IITA, 1990; Mapayi *et al*., 2013).

When a mutant appears promising, multiplication of planting material for extensive field testing is necessary. Mutants of vegetatively propagated crop such as cassava can be multiplied by the usual method through cuttings. The methods of testing mutants in comparative trials are essentially the same as for any other newly developed strain. The purpose is to find out whether the mutant promises to become a variety surpassing the value of the mother strain and of the best available variety. The mutants should be tested in a wide range of environments, such as, locations, soil, water and nutrient conditions, planting dates and distances, etc. and if the superiority is confirmed the mutant can be released as a new cassava clone.

Materials and Methods

3 MATERIALS AND METHODS

The present investigation was conducted in the Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University (KAU) located 40 m above MSL at 10º31' N latitude and 76º13' E longitude, during 2012-2014. The study was carried out as two major experiments - Experiment I: Assessment of variability in short duration cassava genotypes and Experiment II: Studies on creating variability through *in vitro* mutagenesis.

3.1 Experiment I: Assessment of variability in short duration cassava genotypes

3.1.1 Experimental material

Fourteen cassava genotypes (Table 1) collected from various districts of Kerala (Figure 1) constituted the material for the study. All the genotypes except M4 were of about six month's duration. M4 with duration of ten months is the most popular high yielding variety of cassava cultivated in Kerala. It was used as the check variety.

Table 1. Short duration cassava genotypes collected from Kerala

SI. No.	Accession No.	Source	Duration
1	CC1	Thavannur, Malappuram	6 months
2	CC2	Parappalli, Kollam	6 months
3	CC3	Kottarakkara, Kollam	6 months (unbranched)
4	CC4	Kottarakkara, Kollam	6 months (branched)
5	CC5	Chavakkad, Thrissur	6 months
6	CC6	Chavakkad, Thrissur	6 months
7	CC7	Chittur, Palakkad	6 months
8	CC8	Manjali, Paraur, Ernakulam	6 months
9	CC9	Mannuthy, Thrissur	6 months
10	CC10	Vellanikkara, Thrissur	6 months
11	Vellayani Hraswa	KAU, COA*, Vellayani	6 months, high yielding
12	Sree Prakash	CTCRI**, Trivandrum	7 months, 30-35 t ha^{-1}
13	Sree Jaya	CTCRI, Trivandrum	7 months, 26-30 t ha^{-1}
14	M4 (Check)	CTCRI, Trivandrum	10 months, 18-23 t ha^{-1}

*COA – College of Agriculture **CTCRI – Central Tuber Crops Research Institute

Site no., locality, district:

1. Thavannur, Malappuram
2. Parappalli, Quilon
3. Kottarakkara, Quilon
4. Chavakkad, Thrissur
5. Chittur, Palakkad
6. Manjali, Paraur, Ernakulam
7. Mannuthy, Thrissur
8. Vellanikkara, Thrissur
9. COA, KAU, Vellayani, Trivandrum
10. CTCRI, Sreekariyam, Trivandrum

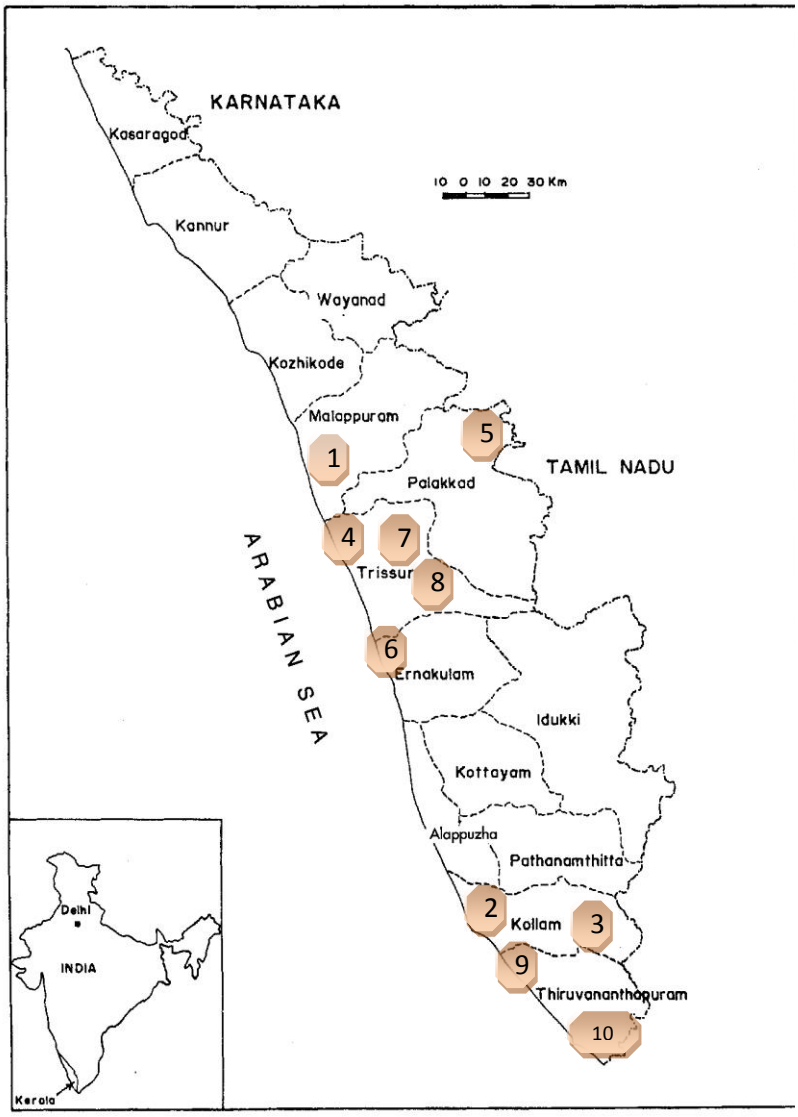


Figure 1: Cassava germplasm collection sites in Kerala

Ten genotypes were collected from farmers' field, three from Central Tuber Crop Research Institute (CTCRI) in Trivandrum and one from Kerala Agricultural University (KAU). The genotypes collected from the farmer's fields were renamed as Cassava Collection and abbreviated as CC and serially numbered.

3.1.2 Methods

Fourteen cassava genotypes collected from various parts of Kerala were evaluated in a randomised block design (RBD) with three replications in plots of size 25 m^2 at a spacing of 1 m x 1 m. The trial was conducted between June and December, 2012. Recommended agronomic practices, as per package of practices

of Kerala Agricultural University – KAU (2011) were followed during the crop growth period.

3.1.3 Observations recorded

3.1.3.1 Morphological and biometrical traits

The data were collected for qualitative and quantitative traits from 25 plants in each replication. This was carried out according to general guidelines as suggested by International Institute of Tropical Agriculture - IITA (Fukuda *et al*., 2010) for selected morphological and agronomic descriptors. The scales and references followed for each trait is given in Table 2.

3.1.3.2 Disease scoring

Scoring was done for Cassava Mosaic Disease (CMD) and Cassava Leaf Spot (CLS) as described by Hahn *et al.* 1980 and 1989. The difference on scoring system is due to the different nature of symptoms observed for each disease, as explained below.

Five plants per genotype per plot were scored for the incidence of CLS disease. Two leaves from top, middle and bottom of each plant were marked and each leaf was scored separately according to the symptoms of brown spots using 0 to 5 scale: 0 - no infection; 1 – less than ten spots; 2 - between 11 and 20 spots; 3 - between 21 and 40 spots; 4 - Between 41 and 60 spots; 5 - more than 60 spots.

All the plants per genotype per plot were scored for CMD. Twenty-five leaves fully opened for each plant were examined and a scale 1 to 5 was assigned according to symptoms: 1 – no symptom; 2 - mild chlorotic pattern over entire leaflets, mild distortion at the base of the leaflets, remaining portion green and healthy; 3 - moderate mosaic pattern over the leaf, narrowing and distortion of leaflets; 4 - Severe mosaic, leaflet distortion, leaf size reduction; 5 - severe mosaic distortion of entire leaf.

Table 2. Descriptor for evaluation of qualitative and biometrical characters in cassava

Sl. No.	Character	Score	Time of observation (MAP*)	Reference
1	Stipule colour	Score 1 – 9	3 MAP	IITA-2010-Fukuda *et al*., 2010
2	Petiole colour	Score 1 – 9		
3	Emerging leaf colour	Score 1 – 9		
4	Leaf shape (central lobe)	Score 1 – 10	6 MAP	
5	Stem colour (exterior)	Score 1 – 9		
6	Flowering	Score 0 – 1		
7	Root external colour (rind)	Score 1 – 4		
8	Root pulp colour	Score 1 – 5		
9	Root cortex colour	Score 1 – 4		
_	**Biometrical character**	_	_	
10	Plant height	cm	6 MAP	IITA-2010 Fukuda *et al*., 2010
11	Height at which 1st branch appears on main stem	cm		
12	Stem girth	cm		Mahangu *et al*. 1983
13	Internodal length	cm		IITA-2010 Fukuda *et al*., 2010
14	Leaf scars	Number		
15	Number of branches plant^{-1}	Number		
16	Number of tuber plant^{-1}	Number		
17	Tuber length	cm		
18	Length of neck	mm		Mahangu *et al*. 1983
19	Tuber weight plant^{-1}	g		_
20	Tuber girth	mm		Mahangu *et al*. 1983
21	Tuber fresh yield per ha	t ha^{-1}		Rai *et al*., 1986
22	Incidence of CMD	Score 1 – 5	3 MAP	Hahn *et al*., 1989
23	Incidence of CLS	Score 0 – 5		Hahn *et al*., 1980
24	Dry matter content plant^{-1}	kg	6 MAP	Mahangu *et al*. 1983
25	Upper ground biomass	kg		Fukuda *et al*., 2010
26	Harvest index (HI)	per cent		
27	Selection index (SI)	_		Ojulong *et al*., 2010

*MAP - Months after planting

3.1.3.3 Dry matter (DM) content of tuber per plant (kg)

It was estimated based on the principle of its linear relationship with specific gravity. Thus, as in the formula given below, where x = specific gravity, which was measured according to the following methodology for five plants per plot (Kawano *et al.*, 1987).

$$DM = 158x - 142$$

Cleaned tuber sample was placed in a sturdy wire basket. The weight of the sample in air (Wa) was measured using a balance. The same sample was weighed in water (Ww) (Plate 1). The specific gravity of tuber was computed using the formula below:

$$x = \frac{Ww}{Wa - Ww}$$

Dry matter (of tuber) was computed using formula enumerate and expressed as per cent and then converted to unit of kg.

3.1.3.4 Harvest index (HI)

It was calculated using formula below (Kawano, 1990):

$$HI = \frac{weight\ of\ roots}{weight\ of\ roots\ +\ weight\ of\ aboveground\ biomass}$$

3.1.3.5 Selection index (SI)

It was calculated according to the formula as enumerated below.

$$SI = [FRY * 10] + [DMC * 8] + [HI * 5] - [CMD * 3] - [CLS * 2] + [TG * 2]$$

Where, FRY- fresh root yield, DMC - dry matter content, HI - harvest index, CMD- cassava mosaic disease, CLS - *Cercospora* leaf spot and TG - tuber girth (Ojulong *et al*., 2010).

Plate 1. Estimation of dry matter in tubers of cassava by gravimetric method

3.1.3.6 Biochemical analysis of cassava

Biochemical analysis and organoleptic evaluation of the cassava genotypes were done immediately after harvest. The references and the scores followed in biochemical analysis and organoleptic evaluation are enumerated in Table 3.

Table 3. Biochemical and organoleptic evaluation of cassava genotypes at harvest

SI. No.	Bio-chemical trait	Unit/score*	Reference
1	Cyanide content	mg kg^{-1}	Sadasivam and Manickam, 1997
2	Total starch content	per cent	
3	Amylose content	per cent	
4	Amylose/amylopectin ratio	_	
5	Fibre content	per cent	
	Organoleptic trait		
6	Colour before cooking	Score 1 – 5	Gonzalez and Johnson, 2009; Fukuda *et al*., 2010
7	Easiness in peeling	Score 1 – 5	
8	Sweetness on chewing	Score 1 – 5	Frank *et al*., 2011; Fukuda *et al*., 2010
9	Colour after cooking	Score 1 – 5	_
10	Texture after cooking	Score 1 – 5	Gonzalez and Johnson, 2009
11	Taste after cooking	Score 1 – 5	
12	Fibreness	Score 1 – 5	
13	Cooking ability	Score 1 – 5	

* at six months after planting

About 100 g of peeled fresh tuber was dried in electric oven at 60 ºC for 72 hours. Two samples were prepared for each of the 14 cassava genotypes. Samples for each biochemical analysis were taken from this base sample.

3.1.3.6.1 Estimation of cyanide content (mg kg^{-1})

Cyanogenic glucosides release hydrogen cyanide (HCN) by an endogenous enzymatic reaction. HCN forms a red colour compound with sodium picrate (Plate 2). The cyanide content in tuber was then calculated through standard graph method.

Plate 2. Evaluation of HCN content in powdery cassava sample

The homogenous solution was placed in 500 ml conical flask and whatman no.1 paper strip saturated with alkaline picrate solution was suspended in the conical flask using cork stopper and thread. The closed experimental set up was incubated at room temperature for 24 hours. The reddish compound that developed in the filter paper proportional to the amount of HCN evolved was eluted by placing the paper in a clean test tube with 10 ml distilled water, and immediately the colour absorbance was read in the spectrophotometer at 625 nm.

A standard solution of potassium cyanide (KCN) was prepared containing 100 µg ml^{-1} of hydrogen cyanide. To prepare standard curve, 5 ml of alkaline picrate solution and 5 ml of potassium cyanide were taken in test tubes and heated for 5 minutes in water bath. 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml volumes of the above warm solution were poured to different test tubes and in each tube volume was made up to 10 ml with distilled water. After putting rubber stoppers to tubes kept for 5 minutes in a cool place and absorbance was measured at 625 nm.

The quantity of hydrogen cyanide in the above 6 tubes will be 5, 10, 20, 30, 40 and 50 µg respectively. 10 ml water was taken as blank. Standard graph was plotted with absorbance and hydrogen cyanide concentration. The hydrogen cyanide in the cassava sample was calculated from the standard graph plotted (Sadasivam and Manickam, 1997).

3.1.3.6.2 Estimation of total starch (per cent)

The total starch in tuber was estimated through anthrone reagent method. One gram of cassava powder was washed and homogenised with 80 per cent hot ethanol to remove sugars. It was centrifuged and the residue retained, washed repeatedly with anthrone reagent until no colour was formed and dried well over water bath for one hour. Starch was extracted with 6.5 ml of 52 per cent perchloric acid in hot acidic medium from the residue after adding 5 ml of water for half of an hour at 0 ºC, followed by centrifugation and the supernatant was saved. Ten milliliters of the supernatant was taken and made up to 100 ml with water in a standard flask. 0.1 ml of the supernatant was pipetted out to make up the volume to 1 ml with water. Standard glucose stock was prepared diluting 100 mg glucose in 100 ml water.

The working standard was prepared by diluting 10 ml of stock solution to 100 ml water. Standard solutions were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard and volume was made up to 1 ml in each tube with water. Four milliliters of anthrone reagent was added to each tube and heated for eight minutes in a boiling water bath, then cooled rapidly and the intensity of green colour was read at 630 nm. The glucose content in the sample is calculated using

standard graph. The starch content was estimated by multiplying the value by a factor 0.9.

3.1.3.6.3 Estimation of amylose content (per cent)

Amylose is one of the components of starch together with amylopectin, exists as a linear and non-branched polymer of glucose in a coiled form with six glucose residues. Thus, the principle for its determination is based on iodine adsorption within helical coils. A blue-coloured complex is formed and is measured colorimetrically.

One millilitre of distilled ethanol and 10 ml of 1 N NaOH was added to 100 mg of cassava powder and left overnight. The volume was made up to 100 ml. 2.5 ml of the extract was then added to 20 ml of distilled water along with few drops of phenolphthalein. 0.1 N HCl was added drop by drop to the mixture until the pink colour disappears and then 1.0 ml of iodine reagent added and the volume was made up to 50 ml. Standard amylose solution was prepared by diluting 100 mg of amylose in 10 ml 1 N NaOH and volume made up to 100 ml with water. Then 0.2, 0.4, 0.6, 0.8 and 1 ml of standard amylose solution were taken and colour developed as in the previous step. 1.0 ml of iodine reagent was then taken and the volume made up to 50 ml with distilled water to use as blank. The colour of sample, standards and blank were read at 590 nm and the amount of amylose present in the sample calculated using standard graph.

3.1.3.6.4 Calculation of ratio of amylose/amylopectin

The amount of amylopectin is obtained by subtracting the amylose content from that of starch. Then the ratio amylose/amylopectin was calculated.

3.1.3.6.5 Estimation of fibre content (per cent)

Two gram of cassava powder was boiled in 100 ml of 0.4 per cent hydrochloric acid for one hour and then filtered through pre weighted filter crucible fitted with filter paper. The filter crucible fitted with filter paper was washed with hot water, and then dried at 105 to 110 ^{o}C in an oven for two hours to get constant weight. Weigh the residue, *i.e.*, the gain in weight of the crucible was

divided by the weight of the test sample to determine the percentage of fibre in the sample.

3.1.3.7 Organoleptic evaluation

The organoleptic trait evaluation of freshly harvested tubers - both cooked and uncooked tubers were done at the College of Horticulture, Vellanikkara by a group of 16 randomly selected individuals among the teaching, non-teaching, research and students category. Scoring (Table 4) was done using a score card prepared as per Gonzalez and Johnson (2009), Fukuda *et al*. (2010) and Frank *et al*. (2011).

1) Colour before cooking (scale 1 to 5) – observation of the external colour of the fresh tuber

2) Easiness in peeling (scale 1 to 5) – observation on how easy is to remove the peel of the fresh tuber.

3) Sweetness on chewing (scale 1 to 5) – observation on sweetness of the flesh of fresh tuber when chewed.

4) Colour after cooking (scale 1 to 5) – observation on the colour of the cooked fresh tuber.

5) Texture after cooking (scale 1 to 5) – observation on the level of hardiness in chewing the cooked fresh tuber

6) Taste after cooking (scale 1 to 5) – observation on how tasty is the flesh of cooked fresh tuber

7) Fibreness (scale 1 to 5) – observation on how much cellulosic fibre is present in flesh of cooked fresh tuber when chewed.

Table 4. Score card for organoleptic evaluation of tuber trait in cassava

Assessor:_____

Please, **score** accordingly

Score	1 - Very Bad	2 - Bad	3 - Medium	4 - Good	5 - Very Good

	Item\Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Colour before cooking															
2	Peeling response: easiness															
3	Chewing: sweetness															
4	Colour after cooking															
5	Texture after cooking: softness															
6	Taste after cooking															
7	Fiber: less = good															
8	Cooking time/boiling (minutes): less = good															
9	Other:															

Signature

8) Cooking ability (scale 1 to 5) – the cooking ability was evaluated accordingly to the cooking time. One kilogram of peeled fresh cassava tubers was cooked by boiling in water, using normal cooking pans, on gas stove. The duration in minutes needed until suitable softness is attained was recorded using stop a watch. Thus, up to five minutes a score 5 was given as 'very good'. Between five and ten minutes, score 4, as 'good'. Between 11 and 15 minutes, score 3 as 'medium'. Between 16 and 20 minutes, score 4, as 'bad'. And for more than 20 minutes of boiling time duration, a score 1 was given, as 'very bad'.

3.1.4 Statistical Analysis

3.1.4.1 Analysis of variance

The data collected for all the biometrical traits were subjected to an analysis of variance suggested by Panse and Sukatme (1954) (Table 5). Duncan test, based on minimal critical difference, was performed in order to identify the actual difference among genotypes for each particular trait and respective ranking order.

Table 5. Analysis of variance of Randomized Block Design

Source of variation	**Degrees of freedom (df)**	**Sum of Squares (SS)**	**Mean Squares (MS)**	**Expectations of MS**
Replications	r – 1	–	–	–
Between genotypes	t – 1	SS_1	MS_1	$\sigma^2_e + r\sigma^2_g$
Within varieties or error	(r – t)(t – 1)	SS_2	MS_2	σ^2_e
Total	(rt – 1)			

Where: r = number of replications; t = number of genotypes;
Environmental variance = σ^2_e ; Genotypic variance (σ^2_g) = $(MS_1 – MS_2)/r$;
Phenotypic variance (σ^2_p) = $\sigma^2_g + \sigma^2_e$

Phenotypic, genotypic and environmental coefficients of variation were estimated by following the formula as suggested by Burton and De Vane (1953):

Phenotypic coefficient of variation (PCV) = (σ_p/Mean)*100

Genotypic coefficient of variation (GCV) = (σ_g/Mean)*100

Environmental coefficient of variation (ECV) = (σ_e/Mean)*100

Where σ_p, σ_g and σ_e are phenotypic, genotypic and environmental standard deviations, respectively. According to Sivasubramanian and Madhava Menon (1973), PCV and GCV are classified as low if less than 10per cent, moderate if is between 10 and 20 per cent, and high if is more than 20 per cent.

3.1.4.2 Heritability

Heritability (Broad sense) for all the traits were computed by the formula suggested by Lush (1940):

$$H = \frac{\sigma_g^2}{\sigma_p^2}$$

It plays an important role in the selection process for crop improvement and as suggested by Johnson *et al*. (1955), heritability values are categorized as low (< 30per cent), moderate (30 to 60per cent) and high (> than 60per cent).

3.1.4.3 Genetic advance

Genetic advance is a measure of genetic gain under selection. The expected genetic gain is estimated as

$$GA = \sigma^2_g / \sigma^2_p * K$$

Where σ^2_g - Genotypic variance and

σ^2_p - Phenotypic variance

K – Selection differential at a particular level of selection intensity

Genetic advance usually expressed as percentage of mean as:

Genetic advance per cent = Genetic advance / Mean * 100

3.1.4.4 Data analysis for organoleptic traits

The organoleptic data were analysed using the Kruskal-Wallis analysis of variance. This is a non-parametric method for testing whether samples originate from the same distribution (Kruskal, 1952).

Kruskal-Wallis statistics (K):

$$K = (N-1)\frac{\sum_{i=1}^{g} n_i(\bar{r_{i.}} - \bar{r})}{\sum_{i=1}^{g}\sum_{j=1}^{n_i}(r_{ij} - \bar{r})}$$

Where:

n_i is the number of observations in group i

r_{ij} is the rank (among all observations) of observation j from group i

N is the total number of observations across all groups

$\bar{r_{i.}} = \frac{\sum_{j=1}^{ni} r_{ij}}{n_i}$ and $\bar{r} = \frac{1}{2}(N+1)$ is the average of all r_{ij}

3.1.4.5 Correlation analysis

Correlation was estimated between different biometrical traits and yield as well as biochemical and organoleptic traits with yield.

Simple correlations based on RBD were worked out using variances and covariances between any two traits as follows:

1) Phenotypic correlation:

$$r_p = \frac{\sigma_p(x,y)}{\sqrt{\sigma_{px}^2 * \sigma_{py}^2}}$$

2) Genotypic correlation:

$$r_g = \frac{\sigma_g(x,y)}{\sqrt{\sigma_{gx}^2 * \sigma_{gy}^2}}$$

3) Environmental correlation:

$$r_e = \frac{\sigma_e(x,y)}{\sqrt{\sigma_{ex}^2 * \sigma_{ey}^2}}$$

Where: $\sigma_p\,(x,y)$, $\sigma_g\,(x,y)$, $\sigma_e\,(x,y)$ are phenotypic, genotypic and environmental covariances respectively between the variables *x* and *y*.

σ_p^2, σ_g^2, σ_e^2 are phenotypic, genotypic and environmental variances respectively.

Test of significance:

The calculated r was tested for its significance (i.e., whether greater than zero) by comparing it with the table value of r available in a standard book at d.f. = N – 2. Alternatively, the test of significance was accomplished by t-test as follows:

$$t = \frac{r}{SE_r}$$

Where:

$$SE_r = \sqrt{\frac{1 - r^2}{N - 2}}$$

3.1.4.6 Path coefficient analysis

Path analysis was used to measure the direct and indirect effect of various independent characters on yield, according to Singh and Chaudhary (1985). Variances σ^2_x and covariances σ_{xy} were estimated as in correlation studies. And correlation coefficients among traits *r* (x_1, x_2), and between traits and yield *r* (x_i, *Y*). Where yield is taken as *Y* and factors as x_i, are shown below.

$$r(x_1,x_2) = \frac{\sigma(x_1,x_2)}{\sqrt{\sigma_{x1}^2 * \sigma_{x2}^2}}$$

$$r(x_1,y) = \frac{\sigma(x_1,y)}{\sqrt{\sigma_{x1}^2 * \sigma_y^2}}$$

Where: x_1 and x_2 – independent factors affecting yield

Y – dependent variable (yield)

Assuming $\sigma_y^2 = \sigma_{x1}^2 + \sigma_{x2}^2 + \sigma_{x3}^2$ and dividing the equation by σ_y^2, the path coefficients *a* and *b* are obtained as follows:

$$\frac{\sigma_{x1}^2}{\sigma_y^2} + \frac{\sigma_{x2}^2}{\sigma_y^2} + \frac{\sigma_{x3}^2}{\sigma_y^2} = 1$$

Where: $= \frac{\sigma_{x1}^2}{\sigma_y^2}$, $b = \frac{\sigma_{x2}^2}{\sigma_y^2}$ and $c = \frac{\sigma_{x3}^2}{\sigma_y^2}$

the equation is written as $a^2 + b^2 + c^2 = 1$, by definition:

a = the path coefficient from x_1 to Y
b = the path coefficient from x_2 to Y
c = the path coefficient from x_3 to Y

Thus, $r(x_1, y) = a + r(x_1\ x_2)b + r(x_1\ x_3)c$

$$r(x_2, y) = r(x_1\ x_2)a + b + r(x_2\ x_3)c$$

$$r(x_3, y) = r(x_1\ x_3)a + r(x_2\ x_3)b + c$$

Besides *x1*, *x2*, and *x3* some undefined factors designed by *R* also contribute to the yield. Then *a*, *b*, *c* and *h* are the path coefficients due to respective variables. Thus, *r(R, Y) = h*. Considering only the first three factors, the above given simultaneous equations in matrix notation as follows.

$$\begin{bmatrix} rx_1y \\ rx_2y \\ rx_3y \end{bmatrix} = \begin{bmatrix} rx_1x_1 & rx_1x_2 & rx_1x_3 \\ rx_1x_2 & rx_2x_2 & rx_2x_3 \\ rx_1x_3 & rx_2x_3 & rx_3x_3 \end{bmatrix} * \begin{bmatrix} a \\ b \\ c \end{bmatrix}$$

Equally, $A = B*C$

The solution for the vector *C* is obtained by multiplying both sides by inverse of *B* matrix, i.e., B^{-1}.

Thus, $C = A*B^{-1}$

The contribution of residual is:

$$h^2 = 1 - a^2 - b^2 - c^2 - 2r(x_1x_2)ab - 2r(x_1x_3)ac - 2r(x_2x_3)bc$$

3.1.4.7 Cluster analysis

Clustering analysis was performed according to D^2 statistics developed by Mahalanobis (1928) cited by Singh and Chaudhary (1985). This method is based on multiple characters giving a measure for group distance. Given x_1, x_2, x_3,..., x_p

as multiple measurements on each individual and d_1, d_2, d_3,..., d_p as $\bar{x}_1^1 - \bar{x}_1^2$, $\bar{x}_2^1 - \bar{x}_2^2$,..., $\bar{x}_p^1 - \bar{x}_p^2$, respectively, being the difference in the means of two populations, Mahalanobis' D^2-statistics as defined as follows:

$$_pD^2 = b_1d_1 + b_2d_2 + \cdots + b_pd_p$$

Where: b_i – value to be estimated such that ratio of variance between the populations to variance within the populations is maximized;

In terms of variances and covariances, the D^2 value is obtained as follows:

$$_pD^2 = W^{ij} * (\bar{x}_i^1 - \bar{x}_i^2) * (\bar{x}_j^1 - \bar{x}_j^2)$$

Where: W^{ij} – inverse of estimated variance co-variance matrix

The various steps involved in the estimation of D^2 values are given below:

1) **Collection of data**: considering v population and in each case p characters

Population	*Trait 1*	*Trait 2*	...	*Trait p*
1	X^1_1	X^2_1	...	X^p_1
2	X^1_2	X^2_2	...	X^p_2
...	...	...	...	
V	X^1_v	X^2_v	...	X^p_v

2) **Test of significance**: from the data variances and covariances are calculated, using *V* statistic which utilizes Wilk's criteria, a simultaneous test of differences between mean values of a number of correlated variables is performed according to Rao (1948) cited by Singh and Chaudhary (1985). For the purpose error and error + variety variance and covariance matrix are required. Using pivotal condensation method, the determinants of error and error + variety matrix are calculated. So:

$$\Lambda = \frac{W}{S} = \frac{|Determinat\ of\ error\ matrix|}{|determinat\ of\ error+variety\ matrix|}$$

$$V_{stat} = -m \log_e \Lambda = -\left(n - \frac{p+q+1}{2}\right) \log_e \Lambda$$

Where: $m = n – (p+q+1)/2$

p = number of variables or traits

q = numbers of varieties – 1 (i.e., d.f. for populations)

n = d.f. for error + varieties

e = 2.7183

V is distributed as χ^2 with $p*q$ d.f.

Analysis of dispersion Table: for significance of V statistic

Source	**d. f.**	**S.S.**	**M.S.**	**F**
Varieties	q or $(S – W)$	SSV	$MS_1 = SSV/(S – W)$	MS_1/MS_e
Error	$n – q$ or W	SSE	$MS_e = SSE/W$	
Total	n or S	TSS		

The significance of of *Vstat* shows that the difference between the means in respect of the pooled effect of p traits between different populations is significant. Hence, further analysis can be made to estimate D^2 values.

3) **Transformation of correlated variables**: the computation is simplified when the traits under study are independent and are expressed in terms of their respective standard errors. In this case, computation of D^2 value is simply summation of the differences in mean value of the various traits of the two populations, i.e., $\sum d^2_i$. Thus, first transformation of correlated variables into uncorrelated ones is performed using pivotal condensation method.
4) **Computation of D^2 values**: for each combination the mean deviation, i.e., $Y^1_i – Y^2_i$ with $i = 1, 2,..., p$, is computed and the D^2 is calculated as sum of the squares of these deviations, i.e., $D^2 = \sum(Y^1_i – Y^2_i)^2$.
5) **Testing the significance of D^2 values**: The D^2 value obtained for a pair of population is taken as the calculated value of x^2 and is tested against the tabulated value of x^2 for p d. f., where p is the number of trait considered.
6) **Contribution of individual traits towards divergence**: in all combinations each trait is ranked on the basis of $d_i = Y^j_i – Y^k_i$ values. Rank *1* is given to the highest mean difference and rank p to the lowest mean difference, where p is the number of the traits.

7) **Grouping the varieties into various clusters**: this can be done according to the Tocher method or to the Canonical roots methods (Rao, 1952 cited by Singh and Chaudhary (1985)).
8) **Average intra-cluster distances**: the formula to be used is $D^2 = \frac{\sum D_i^2}{n}$
9) **Average inter-cluster distances**: clusters are taken one by one and their distances from other clusters are calculated.
10) **Cluster diagram**: with the help of D^2 values between and within clusters, the diagram showing the relationship between different populations can be sketched. Such diagrammatic draw is not exactly to the scale.

The data were analyzed using the statistical package SPSS.

3.2 Experiment II: Standardization of *in vitro* propagation methods in cassava

3.2.1 Experimental Method

3.2.1.1 Preparation of stock solutions

MS nutrient medium (Murashige and Skoog, 1962) was used along with the appropriate type and concentration of plant growth regulators, according to the treatments. Stock solutions were prepared as stock I: macronutrients, stock II: calcium stock, stock III: iron stock, stock IV: micronutrients (Table 6). Vitamins stocks were also prepared, separately: 1) niacin (Nicotinic Acid), 2) pyridoxine-HCl and 3) thiamine-HCl and glycine at the concentration of 25 mg l^{-1}. Stocks for plant growth regulators were prepared at the concentration of 25 mg per 100 ml of distilled water.

3.2.1.2 Preparation of culture medium

Alliquots from all stock solutions were pippeted in proportionate volume into glass into glass beaker and reuired quantities of sucrose and inositol were added and dissolved. The desired volume was made by adding double distilled water.

Table 6. Ingredients and concentration of MS media and stock solutions

Ingredient	Formula	MS media ($mg\ l^{-1}$)	Stock (g)	50x 1L	100x 1L
1) Amonium nitrate	NH_4NO_3	1650	Stock I: Macro-nutrients	82.5	_
2) Potassium nitrate	KNO_3	1900		95	_
3) Potassium phosphate	KH_2PO4	170		8.5	_
4) Magnesium sulphate	$MgSO_4.7H_2O$	370		18.5	_
5) Calcium chloride	$CaCl_2.2H_2O$	440	Stock II	22	_
6) Ferrous sulphate	$FeSO_4.7H_2O$	27.8	Stock III: Iron	_	2.78
7) Sodium EDTA	Na_2EDTA	37.2		_	3.72
8) Manganese sulphate	$MnSO_4.4H_2O$	22.3	Stock IV: Micronutrients	_	2.23
9) Zinc sulphate	ZnSO4.2H2O	8.6		_	0.86
10) Boric Acid	H_3BO_3	6.2		_	0.62
11) Potassium Iodide	KI	0.83		_	0.083
12) Sodium molybdate	$NaMoO_4$	0.25		_	0.025
13) Copper sulphate	$CuSO_4.5H_2O$	0.025		_	0.0025
14) Cobalt Chloride	CoCl2	0.025		_	0.0025
15) Nicotinic acid (B_3)	$C_6H_5NO_2$	0.5	Vitamin stocks	_	0.05
16) Pyridoxine-HCl (B_6)	$C_8H_{11}NO_3$	0.5		_	0.05
17) Thiamine-HCl (B_1)	$C_{12}H_{17}ClN_4OS$	0.1		_	0.01
18) Glycine (aminoacid)	$C_2H_5NO_2$	2.0		_	0.20
19) Myo-inositol	$C_6H_{12}O_6$	100		_	10.0
20) Sucrose (sugar)	$C_{12}H_{22}O_{11}$	30,000	_	_	_
21) Agar (solidifying agent)	$(C_{12}H_{18}O_9)_n$	8,000 – 10,000	_	_	_
22) pH		Adjusted to 5.7	1 N HCl and 1 N NaOH		

The pH of the media was adjusted between 5.5 and 5.8 and using 5.5 and 5.8 using 0.1 N NaOH/HCl. Agar was used at a concentration of 0.8 per cent. The autoclaving was done after distributing the melted media into containers (tubes/flasks or bottles) at 121 ºC with 100 kPa (15 psi) pressure for 15 minutes (Berhanu, 2011). Basal Murashige and Skoog medium with 3 per cent sucrose is referred in this study as MS3.

3.2.1.3 Explant preparation

The standardisation of *in vitro* methods was attempted using the genotypes, M4 and Vellayani Hraswa. Fresh stem cuttings of two varieties of cassava namely, M4 and Vellayani Hraswa were planted in pots containing a red loam soil and the plants were maintained in a net house at KAU, College of Horticulture (COH), Department of Plant Breeding and Genetics to be used as source of explant for tissue culture work.

The average temperature was around 30 ºC under natural light and relative humidity. When Sree Jaya and CC1 were identified as the best two varieties, based on the first experiment by the criteria of selection index, they were also planted in pots under same conditions for collection of explants for work on *in vitro* mutagenesis. Thus, the rest of standardization was done considering CC1 and Sree Jaya genotypes.

3.2.2 Propagation through somatic embryogenesis from leaf explants

The leaf explants, their surface sterilisation and inoculation in cassava were standardised. Leaves were collected from newly sprouted shoot tips about 5-10 cm of M4 and Vellayani Hraswa varieties every four weeks from the net house and their surface was sterilized as follows:

1. Shoots of size 2-3 cm were taken to the lab and washed once to remove dirt and mud with tap water;
2. Immature leaf lobes of about 1x1 cm long were excised from shoot tips with scissors, ensuring that the cut portion contains the nerve and kept in distilled water;
3. Leaf explants were placed in 5 per cent Teepol solution prepared with distilled water for 4 – 5 minutes with gentle shaking;
4. Washed again and rinsed well with tap water until all the detergent was removed and taken to laminar air flow cabinet (LAF)
5. Under the LAF the leaf explants were wiped or immersed in 70 per cent ethanol for one or two minutes, according to the treatment, and rinsed three times with sterile distilled water

6. Then further disinfected with mercuric chloride 0.05 per cent for one or two minutes according to the treatment followed by three times rinsing with sterile distilled water.
7. The inoculation was carried out by placing each leaf explant into culture tubes containing agar solid media with the abaxial facing the media;
8. The test tubes were transferred to tissue culture room and kept under controlled conditions of about 26-30 ºC and nine hours light (8am to 5pm);
9. Test tubes with signs of contamination were removed from the culture room.

3.2.2.1 Treatment for sterilisation in leaf explants of cassava

A total of twenty-four leaf sterilization treatments were carried out, using M4 and Hraswa cassava genotypes, as described in Table 7. The media MS3 + 2 mg l^{-1} 2,4-D was used. The number of cultures per replication was two with two replications and the total number of observations was 96. The explants were evaluated twice a week. Explant survival was scored from zero to two as: 0-contaminated, 1- necrotic cultures, 2- callusing cultures.

3.2.2.2 Effect of leaf maturity on sterilisation of leaf explants in cassava

A total of four treatments were carried out for sterilization of leaf, using M4 and Hraswa, as presented in Table 8. The media MS3 + 2 mg l^{-1} 2,4-D was used. The number of cultures per replication was six with four replications and the total number of observations was 96. The explants were evaluated twice a week. Explant survival was scored from zero to two as in the previous experiment.

3.2.2.3 Effect of 2,4 –D on callusing of leaf explants in cassava

A total of five treatments with 2,4-D at concentrations ranging from 2 mg l^{-1} to 10 mg l^{-1} at an interval of 2 mg l^{-1} were carried out, using M4 and Hraswa (Table 9). The basal media MS3 was used. The number of cultures per replication was six with three replications and the total number of observations was 90.

Table 7. Treatment for sterilisation in leaf explants of cassava

Treatment code	Treatment			
	Chopping of leaf	**Teepol (5 per cent)**	**Ethanol (75 per cent)**	**$HgCl_2$ (0.1 per cent)**
1	Before	One minute	Wiping	One minute
2	Before	One minute	Wiping	Two minutes
3	Before	One minute	One minute	One minute
4	Before	One minute	One minute	Two minutes
5	Before	One minute	Two minutes	One minute
6	Before	One minute	Two minutes	Two minutes
7	Before	Two minutes	Wiping	One minute
8	Before	Two minutes	Wiping	Two minutes
9	Before	Two minutes	One minute	One minute
10	Before	Two minutes	One minute	Two minutes
11	Before	Two minutes	Two minutes	One minute
12	Before	Two minutes	Two minutes	Two minutes
13	After	One minute	Wiping	One minute
14	After	One minute	Wiping	Two minutes
15	After	One minute	One minute	One minute
16	After	One minute	One minute	Two minutes
17	After	One minute	Two minutes	One minute
18	After	One minute	Two minutes	Two minutes
19	After	Two minutes	Wiping	One minute
20	After	Two minutes	Wiping	Two minutes
21	After	Two minutes	One minute	One minute
22	After	Two minutes	One minute	Two minutes
23	After	Two minutes	Two minutes	One minute
24	After	Two minutes	Two minutes	Two minutes

Basal media – MS3 + 2 mg l^{-1} 2,4-D

Observations were taken twice a week. Explant survival was scored from zero to five as enumerated below.

0 - Cultures showing contamination; 1 - Cultures showing necrosis

2 - Cultures showing greening; 3 - Cultures showing hard callusing

4 - Cultures showing friable embryogenic callusing;

5 – Somatic embryos initiation

Table 8. Effect of leaf maturity on sterilisation of leaf explants in cassava

Treatment Code	Factors	
	Leaf maturity	**Duration of treatment of $HgCl_2$ (0.05 per cent)**
T1	Immature	One minute
T2	Immature	Two minutes
T3	Mature	One minute
T4	Mature	Two minutes

Basal media – MS3 + 2 mg l^{-1} 2,4-D

Table 9. Effect of 2,4-D on callusing of leaf explants in cassava

Treatment Code	Concentration of 2,4-D
T1	2 mg l^{-1}
T2	4 mg l^{-1}
T3	6 mg l^{-1}
T4	8 mg l^{-1}
T5	10 mg l^{-1}

Basal media – MS3

3.2.2.4 Effect of light on embryogenesis of leaf explants in cassava

Leaf explants were cultured under continuous dark or under normal tissue culture condition of nine hours of light, using Hraswa genotype and MS3 + 5 mg l^{-1} BA + 10 mg l^{-1} 2,4-D media. The number of cultures per replication was five with two replications and the total number of observations was 20. The cultures were evaluated twice a week. Embryogenesis of the cultures was evaluated as scores ranging from zero to five as in previous experiment.

3.2.2.5 Effect of BA and NAA on somatic embryogenesis in cassava

A total of sixteen treatments for induction of somatic embryogenesis were carried out with M4 and Hraswa genotypes, as presented on Table 10. The number of cultures per replication was six with three replications and the total number of observations was 288. The cultures were evaluated twice a week. Embryogenesis of the cultures was evaluated as scores ranging from zero to five as enumerated in the previous treatment.

Table 10. Effect of BA and NAA on somatic embryogenesis in cassava

Treatment code	Growth regulator	
	BA (mg l^{-1})	**NAA (mg l^{-1})**
T1	10.0	0.10
T2	10.0	0.20
T3	10.0	0.30
T4	10.0	0.50
T5	1.00	0.10
T6	1.00	0.20
T7	1.00	0.30
T8	1.00	0.50
T9	3.00	0.10
T10	3.00	0.20
T11	3.00	0.30
T12	3.00	0.50
T13	6.00	0.10
T14	6.00	0.20
T15	6.00	0.30
T16	6.00	0.50

Basal media – MS3

3.2.2.6 Effect of explants on somatic embryogenesis in cassava

Leaf and nodal explants were cultured in MS medium containing 3.0 per cent sucrose + 10 mg l^{-1} Picloram + 3 mg l^{-1} $CuSO_4$ using Hraswa genotype. The number of cultures per replication was eight with three replications and the total number of observations was 48. Observations were taken twice a week. Embryogenesis of the cultures was evaluated as scores ranging from zero to five as in the previous experiment.

3.2.2.7 Effect of picloram on somatic embryogenesis in cassava

The friable embryogenic callus cultures of the genotypes Sree Jaya and CC1 were cultured in MS3 media with two levels of picloram, 8 and 10 mg l^{-1}, as given in Table 11. Due to the shortage of cultures CC1 was tested at one level. The number of cultures per replication was 10 with three replications and the total number of observations was 90. The cultures were evaluated twice a week. Regeneration of the cultures was evaluated as scores ranging from zero to six as enumerated below:

0- Callus showing contamination

1- Callus showing necrosis

2- Callus showing no changes

3- Hard callus formation

4- Friable embryogenic callus

5- Somatic Embryo initiation

6- Somatic embryos.

Table 11. Effect of picloram on somatic embryogenesis in cassava

	Factors	
Treatment code	**Genotype**	**Picloram**
T1	Sree Jaya	8.00 mg l^{-1}
T2	Sree Jaya	10.0 mg l^{-1}
T3	CC1	8.00 mg l^{-1}

Basal media – MS3

3.2.2.8 Effect of TDZ and BA on germination of somatic embryos in cassava

Germination of somatic embryos was attempted in MS3+ 0.25 mg l^{-1} TDZ and MS3 + 4.00 mg l^{-1} BA using Sree Jaya genotype. The number of cultures per replication was 11 with three replications and the total number of observations was 66. The cultures were evaluated twice a week. Scoring on 0 - 5 scale as done as enumerated below.

0- Callus showing contamination

1- Callus showing necrosis

2- Callus showing green colour

3- Rooting of somatic embryos

4- Shoot emergence of somatic embryos

5- Root and shoot emergence of somatic embryos

3.2.3 Propagation through morphogenesis from nodal explants

Nodes were also used for micropropagation in order to provide an alternative and easy source of plantlet cultures for *in vitro* cassava mutagenesis. Thus, newly sprouted shoot tips of about 5-10 cm were collected from CC1 and Sree Jaya cassava genotypes at four weeks interval from the net house and its

surface was pre-sterilized and sterilized according to IITA Cassava Gene Bank Manual (Dumet *et al.*, 2007) as given below.

1. Washed to remove dirt and mud with tap water;
2. Pre cleaned nodal cuttings of about one cm were prepared with scisors/scapel and kept in distilled water;
3. Placed in 5 per cent Teepol solution for 5 minutes with gentle shaking;
4. Washed again and rinsed well with tap water until all the detergent was removed;
5. Placed for disinfection in carbendazim solution (0.2 per cent) for 5 minutes with gentle shaking;
6. Rinsed thrice with distilled water and taken to tissue culture lab;
7. The explant was immersed in 70 per cent ethanol for one minute and rinsed three times with sterile distilled water;
8. Further disinfected with mercuric chloride 0.05 per cent for 1 minute followed by three times rinsing with sterile distilled wate;
9. The inoculation was carried out planting one explant in to the agar in the test tube and closed the mouth with cotton plug;
10. The test tubes were transferred to tissue culture room and kept under controlled conditions of 26-30 ºC and nine hours light;
11. Test tubes with signs of contamination were removed from the culture room;
12. About 1 – 2 months after inoculation, node explants that successfully developed into plantlets (3 – 6 cm of height with 4 – 6 new nodes) were subjected to *in vitro* multiplication. New explants were excised from those for micropropagation.

3.2.3.1 Surface sterilisation of nodal explants

For sterilization of nodal explants 8 treatments were used, involving CC1 and Sree Jaya cassava genotypes, on MS3 + 0.25 mg l^{-1} TDZ, as shown in Table 12. The number of cultures per replication was 10 with four replications and the

total number of observations was 320. The cultures were evaluated twice a week. Explant survival was evaluated as score zero, one or two for contaminated, necrotic or green/callusing, respectively.

Table 12. Treatments for surface sterilization of nodal explants

Treatment code	Factors	
	Genotype	**$HgCl_2$ as sterilant (per cent)**
1	CC1	0.05 for 1 minute
2	CC1	0.05 for 2 minutes
3	CC1	0.1for 1 minute
4	CC1	0.1 for 2 minutes
5	Sree Jaya	0.05 for 1 minute
6	Sree Jaya	0.05 for 2 minutes
7	Sree Jaya	0.1 for 1 minute
8	Sree Jaya	0.1 for 2 minutes

Basal media – MS3

3.2.3.2 Effect of BA and kinetin on morphogenesis of nodal explants in cassava

The effect of BA and kinetin on the morphogenesis of the nodal explants in two genotypes in cassava, namely M4 and Hraswa, was studied as shown in Table 13. The treatments consisted of MS3 alone, MS3 + 1.0 mg l^{-1} BAP, MS3 + 2.0 mg l^{-1} BAP, MS3 + 1.0 mg l^{-1} Kinetin and MS3 + 2.0 mg l^{-1} Kinetin. The number of cultures per replication was six with three replications and the total number of observations was 180. The cultures were evaluated twice a week. Scoring on 0 - 5 scale as done as enumerated below:

0- Cultures showing contamination

1- Cultures showing necrosis

2- Cultures showing green colour/ callusing

3- Explants showing rooting

4- Explants showing shoot emergence

5- Explants showing rooting and shoot emergence

Table 13. Effect of BA and kinetin on morphogenesis of nodal explants in cassava

	Factors	
Treatment code	**Genotype**	**Media**
T1	V. Hraswa	Basal MS3
T2	V. Hraswa	MS3 + 1.0 mg l^{-1} BA
T3	V. Hraswa	MS3 + 2.0 mg l^{-1} BA
T4	V. Hraswa	MS3 + 1.0 mg l^{-1} kinetin
T5	V. Hraswa	MS3 + 2.0 mg l^{-1} kinetin
T6	M4	Basal MS3
T7	M4	MS3 + 1.0 mg l^{-1} BA
T8	M4	MS3 + 2.0 mg l^{-1} BA
T9	M4	MS3 + 1.0 mg l^{-1} kinetin
T10	M4	MS3 + 2.0 mg l^{-1} kinetin

Basal media – MS3

3.2.3.3 Effect of TDZ and of BA on morphogenesis of nodal explants

The treatments tried to find out the effect of TDZ and BA on morphogenesis of nodal explants in cassava genotypes M4, Hraswa, CC1 and Sree Jaya were based on two types of media: MS3 + 0.25 mg l^{-1} TDZ and MS3 + 2.0 mg l^{-1} BA. The number of cultures per replication was eight with four replications and the total number of observations was 192. M4 and Hraswa were tested only for TDZ media. Observations were taken twice a week as score from zero to five as in the previous experiment.

3.2.3.4 Effect of liquid and solid media on morphogenesis of nodal explants

Two treatments involving basal MS3 media in liquid and solid state were considered for morphogenesis of nodal explants of cassava genotype M4. The number of cultures per replication was nine with two replications and the total number of observations was 36. Observations were recorded twice a week as scores from zero to five as in the previous experiment.

3.2.3.5 Regeneration from *in vitro* nodal explants of CC1 and Sree Jaya

In order to assess the regeneration, the *in vitro* nodal explants of CC1 and Sree Jaya cassava genotypes were cultured in MS3 + 0.25 mg l^{-1} TDZ. The number of cultures per replication was 13 with two replications and the total

number of observations was 52. Observations were recorded twice a week for the number of shoots produced after four weeks of inoculation.

3.2.3.6 Effect of reduction in strength of MS media and sugar on *in vitro* regeneration of cassava plantlets

The effect of reduction in strength of MS media on *in vitro* regeneration of cassava plantlets was tried as shown in Table 14. Nodal derived plantlets for CC1 and somatic embryo derived plantlets for Sree Jaya genotype were used. This effect was studied on separated genotypes for the easiness of statistical analysis and interpretation of results. For CC1 the number of cultures per replication was 7 and the total number of observation was 35. For Sree Jaya the number of cultures per replication was 23 and the total number of observations 46. Five different media were tried T1: MS3 + 0.25 mg l^{-1} TDZ, T2: ½ MS2, T3: ½ MS1, T4: ¼ MS1 and T5: ¼ MS0 for CC1. Two media, namely T1 and T3, were tried for Sree.

Table 14. Effect of reduction in strength of MS media and sugar on *in vitro* regeneration in cassava

Treatment code	Media
T1	MS3 + 0.25 mgl^{-1} TDZ
T2	½ MS +2 per cent sucrose or ½ MS2
T3	½ MS+ 1 per cent sucrose or ½ MS1
T4	¼ MS+ 1 per cent sucrose or ¼ MS1
T5	¼ MS + no sucrose or MS0

3.2.3.7 Effect of rooting media on the *in vitro* root development in cassava

Effect of rooting media on the *in vitro* root development of cassava plantlets were tried with three treatments as shown in Table 15. Nodal derived plantlets for CC1 and somatic embryo derived plantlets for Sree Jaya genotype were used. This effect was studied on separated genotypes for the easiness of statistical analysis and interpretation of results. For CC1 the number of cultures per replication was 9 and the total number of observations was 27. For Sree Jaya the number of cultures per replication was 13 and the total number of observations 39.

Table 15. Effect of rooting media on *in vitro* regeneration of cassava plantlets

Treatment code	Rooting media
T1	MS3 + 0.25 mg l^{-1} TDZ
T2	1 minute dip in 1 per cent NAA solution, then inoculated into MS3 + 0.25 mg l^{-1} TDZ
T3	MS3 + 0.25 mg l^{-1} TDZ + 0.5 mg l^{-1} NAA

3.3 Standardization of LD50 for *in vitro* mutagenesis in cassava

The best two genotypes from the field experiment, namely CC1 and Sree Jaya were selected for *in vitro* mutagenic treatment. Each genotype was treated with gamma radiation (gamma rays) and ethyl methane sulfonate (EMS), physical and chemical mutagen, respectively. The gamma rays exposure varied from 10 to 110 Gy (at 10 Gy intervals) and the EMS concentration incorporated into solid media from 0.30 per cent to 1.50 per cent for 72 hours of exposure at an interval of 0.30 per cent. Three types of cultures for each genotype were subjected to *in vitro* mutagenesis treatment, namely, friable embryogenic callus (FEC), somatic embryos (SE) and plantlets.

In order to perform *in vitro* gamma irradiation work the respective three cultures were inoculated into small test tubes of 25x100 mm size and 45 ml capacity with 15 ml corresponding MS solid media. The test tubes were organized in bunches of seven and tied with rubber bands for each treatment exposure. The treatment was initially conducted in Radio Tracer Lab (RTL), at KAU, Vellanikkara and completed in Centert of Plant Breeding and Genetics, TNAU, Coimabtore. The source machine of gamma rays (Co 60 isotope) was Gamma Chamber 1200, built by Board of Radiation and Isotope Technology (BRIT), DAE, Mumbai, India. Soon after the treatment subculturing was carried out into fresh media.

The estimation of the respective LD50 was done for both gamma rays and EMS based on survival of the treated cultures.

3.3.1 Effect of gamma irradiation on the *in vitro* cultures of cassava genotypes CC1 and Sree Jaya

Evaluation of the effect of gamma rays on *in vitro* cultures of cassava was done by taking observations twice a week and recorded as number of survival of inoculated cultures over total number of cultures treated.

The number of *in vitro* callus cultures per replication was seven with two replications and the total number of observations was 154. The number of treatments was 11, *i.e.*, from 10 Gy to 110 Gy with 10 Gy intervals, and the media used was MS3 + 8.0 mg l^{-1} Picloram.

The number of *in vitro* somatic embryo cultures (only Sree Jaya genotype) per replication was seven with two replications and the total number of observations was 98. The number of treatments was seven, *i.e.*, from 10 Gy to 70 Gy with 10 Gy intervals, and the media used was MS3 + 0.25 mg l^{-1} TDZ.

The number of *in vitro* nodal plantlets cultures (only CC1 genotype) per replication was seven with two replications and the total number of observations 140. The number of treatments was 10, *i.e.*, from 10 Gy to 100 Gy with 10 Gy intervals, and the media used MS3 + 0.25 mg l^{-1} TDZ.

3.3.2 Effect of EMS on *in vitro* cultures of cassava

Evaluation of the effect of EMS on *in vitro* cultures of cassava was done by taking observations twice a week and recorded as number of survival of inoculated cultures over total number of cultures treated.

The number of *in vitro* callus cultures per replication was 18 (nine for each genotype), the number of treatments 5 (0.30, 0.60, 0.90, 1.20 and 1.50 percent EMS) and the media used was MS3 + 8 mg l^{-1} Picloram.

The number of *in vitro* somatic embryo cultures per replication (only Sree Jaya genotype) was 18, the number of treatments 5 (0.30, 0.60, 0.90, 1.20 and 1.50 percent EMS) and the media used was MS3 + 0.25 mg l^{-1} TDZ.

The number of *in vitro* nodal plantlets cultures per replication (only CC1 genotype) was 29, the number of treatments 5 (0.30, 0.60, 0.90, 1.20 and 1.50 percent EMS) and the media used was MS3 + 0.25 mg l^{-1} TDZ.

3.4 Evaluation of *in vitro* regenerated plantlets

In vitro regenerated plantlets of the genotypes CC1 and Sree Jaya under different treatments were evaluated, just before the planting out, based on the following traits.

1. Height (cm) – measured using scale in centimetres from the media level up to the top most leaf;
2. Number of shoots per culture – counted all the emerging shoots;
3. Number of leaves per culture – counted all the leaves, both old and new, small and big alike;
4. Number of roots per culture – counted all the roots, both old and new, small and big alike;
5. Length of roots per culture (cm) – measured from the insertion point up to the farthest tip;
6. Size of the roots (score: 1 to 10) – most prevalent size is considered, the score one corresponds to the thinnest (less than a 1 mm) and score ten to the thickest (more than 5 mm).

3.4.1 Evaluation of somatic embryos of Sree Jaya treated with EMS

The number of *in vitro* somatic embryo cultures per replication was 14, the number of treatments 5 (Control, 0.30, 0.60, 0.90 and 1.20 percent EMS) and the media used was MS3 + 0.25 mg l^{-1} TDZ.

3.4.2 Evaluation of somatic embryo derived plantlets of Sree Jaya treated with EMS

The number of *in vitro* somatic embryo derived plantlet cultures per replication was 9, the number of treatments 5 (Control, 0.30, 0.60, 0.90 and 1.20 percent EMS) and the media used was MS3 + 0.25 mg l^{-1} TDZ.

3.4.3 Effect of treatment of different concentrations of EMS on in vitro cultured plantlets of CC1

The number of *in vitro* nodal derived plantlet cultures per replication was 31, the number of treatments 4 (Control, 0.30, 0.60 and 0.90 percent EMS) and the media used was MS3 + 0.25 mg l^{-1} TDZ.

3.4.4 Effect of gamma irradiation on somatic embryos of Sree Jaya

The number of *in vitro* somatic embryo cultures per replication was 9, the number of treatments 3 (Control, 40 and 50 Gy) and the media used was MS3 + 0.25 mg l^{-1} TDZ. Other doses of gamma rays were not tried because of shortage of cultures.

3.4.5 Effect of gamma irradiation on somatic embryo derived plantlets of Sree Jaya

The number of *in vitro* somatic embryo derived plantlet cultures per replication was 9, the number of treatments 3 (Control, 40 and 50 Gy) and the media used was MS3 + 0.25 mg l^{-1} TDZ. Other doses of gamma rays were not tried because of shortage of cultures.

3.4.6 Effect of irradiation on in vitro derived plantlets of CC1

The number of *in vitro* nodal derived plantlet cultures per replication was 9, the number of treatments 8 (Control, 20, 30, 40, 50, 60, 70 and 80 Gy) and the media used was MS3 + 0.25 mg l^{-1} TDZ.

3.5 Hardening of *in vitro* derived plantlets in cassava

In vitro regenerated plantlets in cassava having at least 2.0-4.0 cm of height and with roots number of 3-5, as minimal growth parameters (Jorge *et al.*, 2000), were shifted to external environment by following steps (Dumet *et al.*, 2007):

1. Paper cups of 2½ inch having pores for water drainage was filled up ¾ of its volume with the sandy soil;
2. *in vitro* plantlets from the test tube or jam bottle were gently removed and its root portion was rinsed with a solution of distilled water and

fungicide at 0.10 per cent (Bavistin) to remove agar and prevent fungal diseases;

3. One plantlet per cup was planted and sprinkled with water up to field capacity;
4. The cups with transplanted plantlets were transferred in a big plastic tray and covered with transparent plastic bags to prevent the loss of humidity and placed in the transparent humidity box (480x355x250 mm or bigger). Excess water accumulated in the trays were removed periodically, water was sprinkle regularly to ensure humid environment for the plantlets and the humidity box was kept tightly closed to prevent loss of humidity.

3.5.1 Hardening of *in vitro* derived plantlets inside tissue culture laboratory

Plantlets were carefully taken out and transplanted into small cups as per the procedure explained and maintained in the tissue culture laboratory with temperature 26.6 – 30.2 ºC. The plantlets consisted of *in vitro* nodal derived for the genotypes M4, Hraswa and CC1, and somatic embryo derived for Sree Jaya genotype. The total number of 110 plantlets was transplanted originated from various treatments, *viz.*, control (untreated), gamma ray and EMS.

3.5.2 Hardening of *in vitro* derived plantlets inside conventional green house

Inside the conventional green house, the *in vitro* plantlets were transplanted following the same procedure as in the tissue culture laboratory. The temperature inside humidity chamber in the green house was 23.4 – 31.4 ºC and the humidity was 55 – 99 per cent. Under green house conditions, four planting substrates were also tested as in Table 16. The number of plantlets per replication was 7.

After four weeks once plantlets started elongation the transparent plastic cover was removed but the humidity chamber is maintained. Later the survived plants were shifted to fan and pad green house.

Table 16. Treatments of soil substrate for acclimatization of plantlets

Treatment Code	Factor Soil type (proportion)
T1	Sand (full)
T2	Soilrite™ (full)
T3	Coco peat (full)
T4	Mixture (½ Sand : ½ Soilrite)

The plantlets consisted of *in vitro* nodal derived for the genotypes M4 and CC1, and somatic embryo derived for Sree Jaya genotype. The total number of 79 plantlets was transplanted originated from various treatments, *viz.*, control (untreated), gamma ray, EMS and rooting media treatments.

3.5.3 Hardening of *in vitro* derived plantlets inside pad and fan green house

Plantlets hardened in the conventional green house after one week, as well as few plants directly from the *in vitro* system were transferred to pad and fan green house. The planting substrate consisted of sandy soil mixed with Soilrite™ (commercial product consisting on perlite, Irish peat moss and vermiculite) at the proportion of 1:1. The plantlets were transplanted according to the steps described section 3.5 above.

Three to four weeks later (once plantlets start elongation) the transparent plastic cover was removed but the plantlets were maintained inside pad and fan green house. Once plantlets reach 30 cm height, application of nutrients was done with commercial preparation Greencare™ (N-P-K: 30-10-10, *i.e.*, primary and secondary macro and micro nutrients) at 0.5 per cent concentration gradually increasing the frequency of application. In the first week it was applied at once in a week, and during second and third week thrice a week and from fourth week onwards the nutrient was applied on a daily basis.

Similarly, plantlets of more than 30 cm height were gradually exposed to sunlight as follows: First week - one hour at 8-9 am; Second week –two hours at 8-10 am; Third week three hours at 8-11 am and; Fourth week - four hours from 8 am to 12 pm.

3.6 Data analysis of standardization of *in vitro* techniques, mutation and hardening

Analysis of variance (ANOVA) was performed on the data on standardization of *in vitro* techniques, mutation and hardening as Completely Randomized Design (CRD) with 15 observations replicated thrice (Table17).

Table 17. Analysis of variance of Completely Randomized Design

Source of variation	Degrees of freedom (df)	Sum of Squares (SS)	Mean Squares (MS)	Expectations of MS
Between treatments	t – 1	SS_1	MS_1	$\sigma^2_e + n\sigma^2_t$
Within treatments or error	t*(n – 1)	SS_2	MS_2	σ^2_e
Total	n*t – 1			

Where:

t = number of treatments

n = number of observations per treatment

N = n*t

Environmental variance = σ^2_e

Treatment variance (σ^2_g) = ($MS_1 – MS_2$)/r

Total variance (σ^2_p) = $\sigma^2_g + \sigma^2_e$

Results

4 RESULTS

Fourteen genotypes of cassava were evaluated in a randomized blocks design. The observation on qualitative and quantitative traits were analysed to assess the variability. The results obtained are presented below.

4.1 Assessment of variability in short duration cassava genotypes

4.1.1 Variability in qualitative traits

Eight qualitative traits comprising of five shoot traits and three tuber traits were evaluated and the results presented in Table 18 and Table 19.

4.1.1.1 Stipule colour

The stipule colour consisted of two categories, green purple and light green, with 57 per cent and 43 per cent of the genotypes, respectively belonging to each category.

4.1.1.2 Petiole colour

The petiole colour could be grouped into six classes. Half of the genotypes exhibited purple petiole colour. Fourteen per cent each of the genotypes showed red and green petiole colour while, pink, reddish green and greenish red petiole were observed in 7 per cent each of the tested genotypes.

4.1.1.3 Emerging leaf colour

The colour of emerging leaves among the genotypes was either purplish green or green. Majority of the genotypes (71 per cent) exhibited purplish green colour, while 29 per cent of the genotypes had green coloured emerging leaf.

4.1.1.4 Central lobe shape

Four different shapes were observed for the central lobe in leaf. In half of the genotypes, the central lob shape was lanceolate, while in 21 per cent of the genotypes it was oblanceolate. Elliptic and linear central lob was seen in 14 per cent of the genotypes each.

Table 18. Evaluation of qualitative traits of cassava genotypes

Genotype/ Trait	Stipule colour	Petiole colour	Emerging Leaf colour	Central lobe shape	Stem Exterior colour	Flowering	Root external colour	Root pulp colour	Root cortex colour
CC1	Light green	Pink	Purplish green	Lanceolate	Golden	Present	Light Brown	Cream	Pink
CC2	Light green	Reddish green	Purplish green	Oblanceolate	Golden	Present	Dark Brown	White	Pink
CC3	Light green	Purple	Green	Elliptic	Light brown	Present	Dark Brown	White	Yellow
CC4	Green purple	Greenish red	Purplish green	Elliptic	Golden	Absent	Dark Brown	White	Pink
CC5	Green purple	Red	Green	Oblanceolate	Golden	Absent	Dark Brown	White	Yellow
CC6	Light green	green purple	Green	Linear	Grey	Absent	White	White	Yellow
CC7	Green purple	Purple	Purplish green	Lanceolate	Light brown	Present	Dark Brown	White	Pink
CC8	Green purple	green purple	Purplish green	Oblanceolate	Greenish yellow	Absent	Dark Brown	White	Pink
CC9	Green purple	Purple	Purplish green	Lanceolate	Light brown	Absent	Dark Brown	White	Pink
CC10	Green purple	Purple	purplish green	Lanceolate	Light brown	Absent	Light Brown	Yellow	Yellow
Vellayani Hraswa	Green purple	Purple	Green	Lanceolate	Light brown	Absent	Dark Brown	White	Pink
Sree Prakash	Light green	Red	Purplish green	Linear	Greenish yellow	Absent	Light Brown	Cream	Cream
Sree Jaya	Light green	Purple	Purplish green	Lanceolate	Light brown	Absent	Dark Brown	White	Purple
M4	Green purple	Purple	Purplish green	Lanceolate	Silver	Present	Dark Brown	White	Purple

Table 19. Categories of qualitative traits in cassava genotypes

Trait	Category	Number of genotypes	Per cent distribution
1. Stipule colour	1. Light green	6	43
	2. Green purple	8	57
	Total	**14**	**100**
2. Petiole colour	1. Pink	1	7
	2. Reddish green	1	7
	3. Purple	7	50
	4. Greenish red	1	7
	5. Red	2	14
	6. Green purple	2	14
	Total	**14**	**100**
3. Emerging Leaf colour	1. Purplish green	10	71
	2. Green	4	29
	Total	14	**100**
4. Central lobe shape	1. Lanceolate	7	50
	2. Oblanceolate	3	21
	3. Elliptic	2	14
	4. Linear	2	14
	Total	**14**	**100**
5. Stem exterior colour	1. Golden	4	29
	2. Light brown	6	43
	3. Grey	1	7
	4. Greenish yellow	2	14
	5. Silver	1	7
	Total	**14**	**100**
6. Flowering	1. Present	5	36
	2. Absent	9	64
	Total	**14**	**100**
7. Root external colour	1. Light Brown	3	21
	2. Dark Brown	10	71
	3. White	1	7
	Total	**14**	**100**
8. Root pulp colour	1. Cream	2	14
	2. White	11	79
	3. Yellow	1	7
	Total	**14**	**100**
9. Root cortex colour	1. Pink	7	50
	2. Yellow	4	29
	3. Cream	1	7
	4. Purple	2	14
	Total	**14**	**100**

4.1.1.5 Stem exterior colour

Five categories of exterior colour of the stem were observed. Forty-three percent of genotypes possessed light brown stem colour. Golden stem and greenish yellow stem were observed in 29 per cent and 14 per cent genotypes respectively. Grey and silver colour stem was observed in 7 per cent each of the tested genotypes.

4.1.1.6 Flowering

Five genotypes flowered (36 per cent), namely CC1, CC2, CC3, CC7 and M4. The rest of the genotypes (64 per cent) did not show signs of flowering.

4.1.1.7 Root external colour

The root external colour consisted of three categories and they were dark brown which comprised 71 per cent of the genotypes, while light brown comprised 21per cent and white consisted 7 per cent of the genotypes.

4.1.1.8 Root pulp colour

Most of the tested genotypes (79 per cent) had white root pulp colour. While and cream root pulp colour was observed in 14 per cent and yellow in 7 per cent of the genotypes.

4.1.1.9 Root cortex colour

The root cortex colour exhibited four categories namely, pink in half of the genotypes, and red, purple and cream in 29, 14 and 7 per cent of the genotypes, respectively.

4.1.2 Variability in biometrical traits in cassava genotypes

4.1.2.1 Analysis of variance

The analysis of variance revealed that there existed significant differences among the genotypes for most of the yield attributes studied.

4.1.2.2 Mean performance of cassava genotypes

Mean performance of cassava genotypes for the various biometrical traits are given in Table 20.

4.1.2.2.1 Plant height (cm)

Plant height varied between 313.33 cm (M4) and 181.00 cm (Vellayani Hraswa) with an overall mean of 239.28 cm, the SE of 7.19 cm, and range of 224.40 to 254.15 cm. Its PV and GV, were 1019.32 and 879.22, respectively.

4.1.2.2.2 Branch height (cm)

The height at which the first branch arose ranged from 36.67 cm (CC6) to 196.67 cm (CC4) with a grand mean of 113.60 cm, S.E. of 18.78 and range of 74.75 to 152.45 cm. Its PV and GV were 3377.07 and 2421.01, respectively.

4.1.2.2.3 Stem girth (cm)

Genotypes CC5, Sree Jaya and M4 had thickest stem (28.45, 26.56 and 29.23 mm) while, CC10 had the thinnest stem (18.87 mm). The overall mean stem girth was 22.81 cm, S.E. of 0.87 mm and range of 21.01 and 24.62 mm. Its PV and GV were 10.87 and 8.70, respectively.

4.1.2.2.4 Inter nodal length (cm)

Longer intermodal lengths (3.10 and 2.93 cm) were observed in the genotypes CC7 and CC9 whereas in Vellayani Hraswa, Sree Prakash, CC4 and CC1, inter nodal lengths were shorter (1.46, 1.63, 1.64 and 1.68 cm). The mean intermodal length was 2.23 cm, S.E. of 0.22 and range of 1.77 to 2.69 cm. Its PV and GV were 0.36 and 0.23, respectively.

4.1.2.2.5 Number of scars in 30 cm at 1 m height from ground level

The genotypes, Vellayani Hraswa and Sree Prakash had higher number of leaf scars per meter length of stem (21.07 and 20.33) and CC7 and CC9 had lower number (10.27 and 9.80). The overall mean of the number of scars was 14.69, S.E. of 1.62 cm and range of 11.33 to 18.04. Its PV and GV were 17.82 and 10.70, respectively.

Table 20. Assessment of biometrical traits in cassava genotypes

	Genotype													
Trait	CC1	CC2	CC3	CC4	CC5	CC6	CC7	CC8	CC9	CC10	Vellayani Hraswa	Sree Prakash	Sree Jaya	M4
Plant height (cm)	231.25 ^{cde}	253.33 ^{ef}	245.33 ^{def}	257.33 ^{f}	256.44 ^{f}	211.33 ^{bc}	250.67 ^{ef}	223.33 ^{cd}	245.33 ^{def}	198.00 ^{ab}	181.00 ^{a}	212.00 ^{bc}	282.92 ^{g}	313.33 ^{h}
Branch height (cm)	151.88 ^{efg}	123.67 ^{cdef}	119.67 ^{cde}	196.67 ^{g}	145.11 ^{defg}	36.67 ^{ab}	136.33 ^{defg}	0.00 ^{a}	138.33 ^{defg}	95.67 ^{bcde}	83.00 ^{bcd}	67.22 ^{bc}	107.50 ^{cde}	185.00 ^{fg}
Stem girth (mm)	25.71 ^{ef}	23.24 ^{cde}	21.98 ^{bcd}	24.00 ^{def}	28.45 ^{g}	19.56 ab	22.15 ^{bcd}	20.00 ^{ab}	20.52 ^{abc}	18.87 ^{a}	19.52 ^{ab}	20.56 ^{abc}	26.56 ^{fg}	29.23 ^{g}
Inter nodal length (cm)	1.68 ^{a}	1.89 ^{ab}	1.90 ^{ab}	1.64 ^{a}	2.40 ^{bcd}	2.11 abc	2.93 ^{d}	2.59 ^{bcd}	3.10 ^{d}	2.52 ^{bcd}	1.46 ^{a}	1.63 ^{a}	2.82 ^{cd}	2.60 ^{bcd}
Scar no. in 30cm @1m	17.88 ^{cd}	15.93 ^{bcd}	16.07 ^{bcd}	18.27 ^{cd}	12.60 ^{ab}	14.27 abc	10.27^{a}	11.73 ^{ab}	9.80 ^{a}	12.07 ^{ab}	21.07 ^{d}	20.33 ^{d}	11.00 ^{ab}	12.50 ^{ab}
Branch no.s	3.25 ^{d}	3.00 ^{d}	3.27 ^{d}	0.60^{ab}	3.18 ^{d}	2.27 cd	2.93 ^{d}	0.00 ^{a}	3.00 ^{d}	3.13 ^{d}	3.27 ^{d}	1.32 ^{bc}	2.25 ^{cd}	2.83 ^{d}
Tuber no. plant^{-1}	5.38 ^{bcd}	5.93 ^{cd}	5.53 ^{bcd}	5.33 ^{bcd}	6.16 ^{cd}	4.33 abc	4.93 ^{abcd}	6.60 ^{d}	5.53 ^{bcd}	5.40 ^{bcd}	3.67 ^{ab}	3.28 ^{a}	4.42 ^{abc}	3.33 ^{a}
Tuber length (cm)	47.31 ^{c}	43.79 ^{bc}	35.65 ^{ab}	41.95 ^{bc}	40.29 ^{abc}	35.17 ab	39.73 ^{abc}	39.15 abc	38.42 ^{abc}	41.57 ^{bc}	31.32 ^{a}	35.26 ^{ab}	43.36 ^{bc}	43.09 ^{bc}
Tuber neck (mm)	43.13 ^{bc}	30.86 ^{abc}	33.87 ^{abc}	31.10 ^{abc}	35.47 ^{abc}	26.93 ab	45.77 ^{c}	34.27 ^{abc}	40.99 ^{bc}	38.93 ^{abc}	22.75 ^{a}	41.11 ^{bc}	40.17 ^{bc}	62.17 ^{d}
Tuber fresh weight (kg plant^{-1})	3.31 ^{fg}	2.87 ^{efg}	2.21 ^{bcde}	2.50 ^{cde}	2.70 ^{def}	1.39 a	1.79 ^{abc}	2.20 ^{bcde}	1.82 ^{abc}	1.88 ^{abc}	1.53 ^{ab}	1.48 ^{ab}	3.49 ^{g}	2.01 ^{abcd}
Tuber girth (mm)	52.58 ^{e}	44.73 ^{cd}	42.57 ^{bc}	44.78 ^{cd}	39.90 ^{abc}	40.36 abc	38.37 ^{ab}	38.02 ^{ab}	36.19 ^{a}	35.16 ^{a}	48.39 ^{de}	44.27 ^{cd}	43.71 ^{bcd}	40.51 ^{abc}
Tuber yield (t ha^{-1})	33.13 ^{fg}	28.87 ^{efg}	22.05 ^{bcde}	24.97 ^{cde}	27.04 ^{def}	13.93 a	17.91 ^{abc}	22.01 ^{bcde}	18.21 ^{abc}	18.76 ^{abc}	15.33 ^{ab}	14.82 ^{ab}	34.92 ^{g}	20.12 ^{abcd}
CMD Score (1-5)	1.67 ^{a}	2.56 ^{abcd}	3.40 de	2.12 ab	2.43 abcd	4.04 ^{ef}	3.10 ^{bcd}	2.43 ^{abcd}	3.33 ^{de}	3.12 ^{cd}	4.67 ^{fg}	5.33 ^{g}	2.21 ^{abc}	2.88 ^{bcd}
CLS Score (0-5)	0.65 ^{bc}	0.47 ^{abc}	0.27 abc	0.63 bc	0.38 abc	0.30 ^{abc}	0.22 ^{ab}	0.09 ^{a}	0.38 ^{abc}	0.73 ^{bc}	0.26 ^{ab}	0.00 ^{a}	0.78 ^{c}	0.66 ^{bc}
Dry matter of tubers (kg plant^{-1})	1.12 ^{fg}	1.06 ^{efg}	0.85 bcde	0.95 def	0.91 cdef	0.52 ^{a}	0.67 ^{abc}	0.80 ^{bcde}	0.71 ^{abcd}	0.72 ^{abcd}	0.63 ^{ab}	0.50 ^{a}	1.25 ^{g}	0.70 ^{abcd}
Plant upper biomass (kg)	3.14 ^{de}	2.90 ^{bcde}	2.05 ^{a}	2.36 ^{abcd}	3.04 ^{cde}	1.97 a	2.19 ^{ab}	1.63 ^{a}	1.94 ^{a}	1.63 ^{a}	1.60 ^{a}	2.31 ^{abc}	3.39^{e}	3.11 ^{de}
Harvest Index (HI)	0.51 ^{cde}	0.50 ^{bcde}	0.51 ^{cde}	0.51 ^{cde}	0.47 ^{abcd}	0.42 ^{ab}	0.44 ^{abc}	0.57 ^{e}	0.48 ^{bcd}	0.53 ^{de}	0.48 ^{bcd}	0.39 ^{a}	0.52 ^{cde}	0.40 ^{a}

a, b,... - Means followed by same letter are not significantly different; CMD – cassava mosaic disease; CLS – *Cercospora* leaf spot

4.1.2.2.6 Branch numbers

It was recorded as the number of divisions of branching (most frequent occurrence), with zero (0) for no branching. The genotype CC8 did not have any branches while most of the genotypes were having nearly three branches per plant. The grand mean was 2.42, S.E. of 0.34 and range of 1.72 to 3.13. Its PV and GV were 1.31 and 1.00, respectively.

4.1.2.2.7 Tuber number

The CC8 recorded the highest number of tubers per plant (6.60) while, Sree Prakash and M4 had the lowest number of tubers per plant (3.28 and 3.33). The overall mean tuber no. was 4.92, S.E of 0.56 and range of 3.77 to 6.08. Its PV and GV were 1.62 and 0.78, respectively.

4.1.2.2.8 Tuber length (cm)

Longest tuber (47.31 cm) was observed in genotype CC1 while, Vellayani Hraswa had the shortest tubers (31.32 cm). The mean tuber length was 39.82 cm, S.E. of 2.70 and range of 34.24 to 45.29 cm. Its PV and GV were 29.49 and 9.80, respectively.

4.1.2.2.9 Tuber neck

The longest tuber neck was observed in M4 (62.17 mm) while Vellayani Hraswa recorded the shortest (22.75 mm). The grand mean was 37.76 mm, S.E. of 5.04 and range of 27.33 to 48.18 mm. Its PV and GV were 119.51 and 50.67, respectively.

4.1.2.2.10 Tuber fresh weight (kg plant^{-1})

The tuber fresh weight varied between 1.39 kg (CC6) to 3.49 kg (Sree Jaya). The overall mean was 2.20 kg, S.E. of 0.23 and range of 1.71 to 2.68 kg. Its PV and GV were 0.43 and 0.28, respectively.

4.1.2.2.11 Tuber girth (mm)

The thicker tubers were observed in the genotypes CC1 (55.58 mm) and thinner tubers were observed in CC9 and CC10 (35.16 and 36.19 mm). The mean

tuber girth was 42.05 mm, S.E. 1.75 mm and range of 38.44 to 45.67 mm. Its PV and GV were 24.73 and 16.47, respectively.

4.1.2.2.12 Tuber yield (t ha^{-1})

The fresh tuber yield per ha varied between 13.93 t ha-1 of the genotype CC6, to 34.92 t ha-1 of Sree Jaya with a grand mean of 21.97 t ha-1, S.E. of 2.35 and range of 17.11 to 26.83 t ha-1. Its PV and GV were 43.00 and 28.05, respectively.

4.1.2.2.13 Cassava mosaic disease (CMD) score

The score for ranged from 5.33 (Sree Prakash) to 1.67 (CC1) with an overall score of 3.07, S.E. of 0.29 and the range of 2.47 to 3.68. Its PV and GV were 1.14 and 0.91, respectively.

4.1.2.2.14 Cercospora leaf spot (CLS) score

The *Cercospora* leaf spot (CLS) score was high for the genotype Sree Jaya (0.78) while Sree Prakash did not have any infection. The mean score was 0.42, S.E. of 0.15 and range of 0.10 to 0.74. Its PV and GV were 0.10 and 0.03, respectively.

4.1.2.2.15 Dry matter (kg)

The dry matter content was the highest in Sree Jaya (1.25 kg), whereas Sree Prakash (0.50 kg) possessed the lowest dry matter content. The overall mean dry matter was 0.8 kg, S.E. 0.08 and range of 0.63 and 0.97 kg. Its PV and GV were 0.05 and 0.03, respectively.

4.1.2.2.16 Plant upper biomass (kg)

The mean value of the upper ground plant biomass showed that the genotypes Sree Jaya had the highest value for biomass. It ranged from 1.60 kg (Vellayni Hraswa) to 3.39 kg (Sree Jaya) with a grand mean of 2.35 kg, S.E. of 0.24 and range of 1.84 to 2.85 kg. Its PV and GV were 0.39 and 0.23, respectively.

4.1.2.2.17 Harvest index

The harvest index (HI) ranged from 0.39 (Sree Prakash) to 0.57 (CC8) with a grand mean of 0.48, S.E. of 0.03 and range of 0.43 to 0.53. Its PV and GV were zero each.

4.1.2.3 Estimation of genetic parameters

Variability and genetic parameter estimates for the seventeen biometrical traits studied at are enlisted in Table 21. The results are detailed below.

4.1.2.3.1 Phenotypic and genotypic coefficient of variation

In general, the phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) for all the yield attributes studied. Phenotypic coefficient of variation ranged from 11.83 per cent to 51.16 per cent, corresponding to the traits tuber girth and branch height. Plant height, stem girth, tuber length, tuber girth and harvest index recorded moderate PCV, while all other traits registered high values of PCV. No traits exhibited low PCV.

Genotypic coefficient of variation (GCV) ranged from 7.86 per cent (tuber length) to 43.47 per cent (CLS). Low estimates of GCV were also observed in harvest index (9.28 per cent) and tuber girth (9.65 per cent). Plant height (12.39 per cent), stem girth (12.93 per cent), tuber number (17.91per cent) and tuber neck (18.85 per cent) exhibited moderate GCV, while all other traits registered high GCV estimates.

4.1.2.3.2 Heritability and genetic advance as per cent of mean

Heritability estimates ranged from 33.22 per cent (tuber length) to 86.26 per cent (plant height). Traits tuber length (33.22 per cent), *Cercospora* leaf spot score (34.63 per cent), tuber neck (42.40 per cent), harvest index (52.55 per cent), Tuber number (48.01 per cent) and plant biomass (59.39 per cent) recorded moderate estimates of heritability, while all other traits exhibited high heritability estimates. None of the traits registered low heritability.

Table 21. Mean, range and estimates of genetic parameters of biometrical traits in cassava

Trait	Mean	S.E.	Min	Max	PV	GV	PCV (per cent)	GCV (per cent)	H^2 (per cent)	GA	GG (per cent)
Plant height (cm)	239.28	7.19	224.40	254.15	1019.32	879.22	13.34	12.39	86.26	56.73	23.71
Branch height (cm)	113.60	18.78	74.75	152.45	3377.07	2421.01	51.16	43.31	71.69	85.82	75.55
Stem girth (mm)	22.81	0.87	21.01	24.62	10.76	8.70	14.38	12.93	80.81	5.46	23.94
Inter nodal length (cm)	2.23	0.22	1.77	2.69	0.36	0.23	27.00	21.49	63.34	0.79	35.23
Scar no. in 30cm @1m	14.69	1.62	11.33	18.04	17.82	10.70	28.75	22.27	60.04	5.22	35.55
Branch numbers	2.42	0.34	1.72	3.13	1.31	1.00	47.19	41.16	76.10	1.79	73.97
Tuber number	4.92	0.56	3.77	6.08	1.62	0.78	25.84	17.91	48.01	1.26	25.56
Tuber length (cm)	39.82	2.70	34.24	45.39	29.49	9.80	13.64	7.86	33.22	3.72	9.33
Tuber neck (mm)	37.76	5.04	27.33	48.18	119.51	50.67	28.95	18.85	42.40	9.55	25.29
Tuber fresh weight (kg plant^{-1})	2.20	0.23	1.71	2.68	0.43	0.28	29.85	24.11	65.24	0.88	40.11
Tuber girth (mm)	42.05	1.75	38.44	45.67	24.73	16.47	11.83	9.65	66.58	6.82	16.22
Tuber yield (t ha^{-1})	21.97	2.35	17.11	26.83	43.00	28.05	29.85	24.11	65.24	8.81	40.11
CMD score (1-5)	3.07	0.29	2.47	3.68	1.14	0.91	34.66	30.95	79.72	1.75	56.92
CLS score (0-5)	0.42	0.15	0.10	0.74	0.10	0.03	73.86	43.47	34.63	0.22	52.69
Dry matter tuber (kg plant^{-1})	0.80	0.08	0.63	0.97	0.05	0.03	27.95	22.23	63.28	0.29	36.44
Plant upper biomass (kg)	2.35	0.24	1.84	2.85	0.39	0.23	26.77	20.63	59.39	0.77	32.75
Harvest Index (HI)	0.48	0.03	0.43	0.53	0.00	0.00	12.81	9.28	52.55	0.07	13.86

PV – phenotypic variation; GV – genotypic variation; PCV – phenotypic coefficient of variation; GCV – genotypic coefficient of variation; H^2 – heritability (broad sense); GA – genetic advance; GG – genetic gain (GA as percentage of mean)

Genetic advance as per cent of mean estimates varied between 9.33 per centand 75.55 per cent for branch height, respectively. High estimates were also observed for branch numbers (73.97 per cent). Other traits recorded low to moderate estimates of genetic advance as per cent of mean.

4.1.2.4 Correlation studies

The phenotypic and genotypic correlations of biometrical traits in cassava genotypes are shown in Table 22 and are detailed below.

4.1.2.4.1 Association of biometrical traits with tuber fresh weight

Tuber fresh yield recorded significant to high significant positive correlation at phenotypic level and genotypic level with dry matter (rp = 0.97, rg = 0.98), biomass (rp = 0.66, rg = 0.66), stem girth (rp = 0.64, rg = 0.63), harvest index (rp = 0.49, rg = 0.53), plant height (rp = 0.47, rg = 0.46), tuber length (rp = 0.47, rg = 0.50); tuber no. (rp = 0.44, rg = 0.52), CLS (rp = 0.41, rg = 0.40) tuber girth (rp = 0.37, rg = 0.41) and branch height (rg = 0.27). High significant negative correlation at phenotypic and genotypic level was estimated for (rp = -0.54, rg = -0.61).

4.1.2.4.2 Inter-correlation among yield attributes

4.1.2.4.2.1 Plant height

The plant height had highly significant and positive phenotypic correlation with branch height (0.53) and stem girth (0.77). It also had positive correlation to internode length (0.44) and negatively correlation to scar numbers (-0.41). Plant height exhibited highly significant and positive genotypic correlation with branch height (0.53), stem girth (0.77) and internode length (0.42). It also had negative correlation to scar numbers (-0.41).

4.1.2.4.2.2 Branch height

The branch height had highly significant and positive correlation to stem girth (0.56) and positive correlation to branch numbers (0.26).

Table 22. Genotypic and phenotypic correlation of biometrical traits in cassava genotypes

Traits	Plant height	Branch height	Stem girth	Internode length	Scar no.	Branch no.	Tuber no.	Tuber length	Tuber neck	Tuber fresh Weight	Tuber girth	CMD (1-5)	CLS (0-5)	Dry matter	Biomass	Harvest Index
Plant height	1.00	0.53**	0.77**	0.42**	-0.40**	0.06	0.04	0.47**	0.48**	0.47**	-0.13	-0.51**	0.33*	0.45**	0.70**	-0.19
Branch height	0.53**	1.00	0.56**	-0.04	0.02	0.26*	-0.13	0.29	0.33	0.21	0.06	-0.37*	0.42*	0.23	0.28	-0.08
Stem girth	0.77**	0.56**	1.00	0.1	-0.12	0.1	0.03	0.50**	0.46**	0.63**	0.16	-0.49**	0.32*	0.57**	0.79**	-0.11
Internode length	0.44*	0.01	0.13	1.00	-0.95**	0.15	0.24	0.29	0.36*	0.09	-0.61**	-0.40*	0.17	0.11	0.19	-0.04
Scar no.	-0.41*	0	-0.13	-0.94**	1.00	-0.14	-0.28	-0.28	-0.33	-0.12	0.63**	0.41*	-0.16	-0.14	-0.15	-0.04
Branch no.	0.05	0.25	0.09	0.12	-0.15	1.00	-0.07	-0.01	0.13	0.03	0.1	0.11	0.19	0.03	0.19	-0.19
Tuber no.	0.06	-0.03	0.07	0.11	-0.21	-0.03	1.00	0.02	-0.26*	0.44**	0.06	-0.37**	0.07	0.53**	0.01	0.62**
Tuber length	0.47**	0.32*	0.50**	0.22	-0.25	0.01	-0.08	1.00	0.40**	0.47**	-0.06	-0.62**	0.44**	0.49**	0.45**	0.15
Tuber neck	0.44**	0.25	0.40**	0.40**	-0.35*	0.09	-0.16	0.49**	1.00	0.21	-0.19	-0.19	0.17	0.03	0.38*	-0.22
Tuber fresh Weight	0.46**	0.27*	0.63**	-0.01	-0.08	0.06	0.52**	0.50**	0.09	1.00	0.41**	-0.61**	0.40**	0.98**	0.66**	0.53**
Tuber girth	-0.11	0.1	0.18	-0.63**	0.63**	0.11	-0.04	-0.11	-0.13	0.36*	1.00	0.01	0.08	0.39**	0.25	0.15
CMD (1-5)	-0.48**	-0.42**	-0.49**	-0.23	0.32*	0.06	-0.22	-0.60**	-0.36*	-0.54**	0.13	1.00	-0.45**	-0.52**	-0.25	-0.43*
CLS (0-5)	0.33*	0.41**	0.32*	0.15	-0.16	0.19	0.06	0.44*	0.18	0.41*	0.08	-0.49**	1.00	0.45**	0.38*	0.14
Dry matter	0.44**	0.29*	0.57**	0.01	-0.1	0.07	0.45**	0.452*	0.15	0.97**	0.34*	-0.60**	0.43**	1.00	0.59**	0.54**
Biomass	0.70**	0.30*	0.79**	0.14	-0.14	0.2	0.07	0.46**	0.32*	0.66**	0.27*	-0.29*	0.38**	0.60**	1.00	-0.26*
Harvest Index	-0.17	-0.02	-0.07	-0.1	-0.01	-0.16	0.59**	0.11	-0.16	0.49**	0.1	-0.48**	0.14	0.58**	-0.31	1.00

4.1.2.4.2.3 Stem girth

The stem girth had highly significant positive genotypic correlation with biomass (0.79), plant height (0.77), branch height (0.56) and tuber length (0.51), and significant negative correlation with CMD (-0.49).

4.1.2.4.2.4 Intermodal length

Internodal length had positive correlation with plant height (0.42) and tuber neck (0.40). However, the negative association of intermodal length was with scar number (-0.94) and tuber girth (-0.63).

4.1.2.4.2.5 Scar numbers

The scar numbers had highly significant and negative phenotypic correlation to internode length (-0.95). The scar number exhibited highly significant and negative genotypic correlation with internode length (-0.95).

4.1.2.4.2.6 Branch number

Branch number exhibited positive correlation only with branch height (0.26) for all other traits the correlation of branch height was non significant.

4.1.2.4.2.7 Tuber number

Tuber numbers registered highly significant and positive phenotypic correlation with HI (0.59) and dry matter (0.45). The number of tubers per plant had highly significant and positive genotypic correlation to harvest index (0.62) and dry matter (0.54), and negative correlation to (-0.37) and tuber neck (-0.26).

4.1.2.4.2.8 Tuber length

The tuber length had highly significant and positive phenotypic correlation to tuber neck (0.49), dry matter (0.45), plant height (0.47) and stem girth (0.50), and negative correlation to (-0.60). It was also positively correlated to CLS (0.44). The tuber length was highly significantly and positively correlated to tuber neck (0.40), dry matter (0.49), CLS (0.44), plant height (0.47) and stem girth (0.51), and negatively correlated to CMD (-0.62). It was also positively correlated to branch height (0.32).

4.1.2.4.2.9 Tuber neck

The tuber neck had highly significant and positive phenotypic correlation to plant height (0.48) and stem girth (0.46). It is also had positive correlation with internode length (0.36) and negative correlation with (-0.36). There was highly significant and positive genotypic correlation of tuber neck to plant height (0.44), stem girth (0.46) and internode length (0.44). It also negatively correlated to (-0.35).

4.1.2.4.2.10 Tuber fresh weight

The phenotypic correlation of the tuber fresh weight was highly significant (0.01) and positively correlated to biomass (0.66), tuber numbers (0.44), tuber length (0.49), harvest index (0.49), dry matter (0.97) and stem girth (0.64) and negatively correlated to (-0.54). It was also positively correlated to CLS and plant height.

Genotypic correlation among the biometrical traits in cassava revealed that the tuber fresh weight had highly significant and positive correlation to biomass (0.66), tuber numbers (0.52), tuber length (0.50), tuber girth (0.41), harvest index (0.53), dry matter (0.98), CLS (0.40), plant height (0.46) and stem girth (0.63) and negatively correlated to (-0.61) (Table 6). It also had positive correlation to branch height (0.27).

4.1.2.4.2.11 Tuber girth

The tuber girth showed highly significant and positive phenotypic correlation to number of scars per meter length of plant (0.63) and negative correlation to internode length (-0.61). It also had positive correlation to dry matter content (0.43). The tuber girth had highly significant and positive genotypic correlation to dry matter (0.39) and scar numbers (0.63) and negative correlation to internode length (-0.63).

4.1.2.4.2.12 CMD

The CMD had highly significant and negative phenotypic correlation to CLS (-0.49), plant height (-0.51) and stem girth (-0.49). It was also negatively

correlated to branch height (-0.37) and internode length (-0.40), and positively correlated to scars numbers (0.41). The CMD had highly significant and negative genotypic correlation to CLS (-0.45), plant height (-0.48), branch height (-0.42) and stem girth (-0.49). It also had positive correlation to scars numbers (0.32).

4.1.2.4.2.13 CLS

The CLS was positively correlated to plant height (0.33), branch height (0.42) and stem girth (0.32) at phenotypic level. The CLS had highly significant and positive genotypic correlation with branch height (0.42) and significant correlation to plant height (0.33) and stem girth (0.32).

4.1.2.4.2.14 Upper ground biomass

The upper ground biomass had registered highly significant and positive phenotypic correlation with tuber length (0.45), tuber dry matter content (0.59), plant height (0.71), and stem girth (0.79). It also had positive correlation to tuber neck (0.38) and CLS (0.38). The number of tubers per plant had highly significant and positive correlation with harvest index (0.59) and dry matter (0.45).

At genotypic level, upper ground plant biomass had recorded highly significant and positive genotypic correlation to tuber length (0.47), dry matter (0.60), CLS (0.38), plant height (0.70) and stem girth (0.79). It also registered positive correlation to tuber neck (0.32), tuber girth (0.28) and branch height (0.30), and negative correlation to harvest index (-0.26) and CMD (-0.29).

4.1.2.4.2.15 Dry matter content

The dry matter content had highly significant and positive phenotypic correlation to CLS (0.45), plant height (0.45) and stem girth (0.57) and negative correlation to (-0.54). The dry matter had highly significant (0.01) and positive genotypic correlation to CLS (0.43), plant height (0.44) and stem girth (0.57) and negative correlation to CMD (-0.60). It also had positive correlation to branch height (0.29).

4.1.2.4.2.16 Harvest index

There was highly significant and positive phenotypic correlation of harvest index with dry matter (0.55) and negative correlation with CMD (-0.43). At genotypic level, the harvest index was highly significantly and positively correlated to dry matter (0.58) and negatively correlated to CMD (-0.48).

4.1.2.4.2.17 Correlation of tuber fresh yield with qualitative traits

Correlation of tuber fresh yield with biochemical and qualitative traits are presented in the Table 23.

4.1.2.4.3 Tuber fresh yield

The tuber fresh yield was highly and positively correlated to starch content (0.36), amylose content (0.22), chewing sweetness (0.22) and to the taste (0.19) and texture (0.18) after cooking. However, the tuber fresh yield was negatively correlated to crude fibre (-0.34), fast cooking ability (-0.33) and peeling response (Table 23). The rest of the traits were not correlated to tuber fresh yield.

4.1.2.4.4 Colour before cooking

The colour before cooking was positively correlated to colour after cooking (0.28), chewing sweetness (0.26), peeling easiness (0.24), texture (0.19), taste (0.19) and organoleptic fibre (0.13).

4.1.2.4.5 Peeling easiness

The peeling easiness was positively correlated to colour before cooking (0.24), chewing sweetness (0.23), taste (0.23), texture (0.15), amylose content (0.13) and to colour after cooking (0.11), fibre organoleptic (0.11) and cookability (0.11). And was negatively correlated to yield (-0.13).

4.1.2.4.6 Chewing sweetness

The chewing sweetness was positively correlated to colour before (0.26), peeling (0.23), yield (0.22), taste (0.23), texture (0.21), colour after cooking (0.21), fibre organoleptic (0.20) and starch content (0.19). Also it was negatively correlated to crude fibre (-0.12).

Table 23: Genotypic correlation of tuber fresh yield with tuber qualitative traits

Traits	Tuber fresh yield (t ha^{-1})	Colour before cooking	Peeling easiness	Chewing sweetness	Colour after cooking	Texture after cooking	Taste after cooking	Fibre organo-leptic	Fast cooking ability	Cumulative organoleptic scoring	Starch content	HCN content	Amylose content	Crude fibre content
Tuber fresh yield (t ha^{-1})	1.00	0.11 ns	-0.13*	0.22**	0.10 ns	0.18**	0.19**	0.05 ns	-0.33**	0.11 ns	0.36**	-0.10 ns	0.22**	-0.34**
Colour before cooking		1.00	0.24**	0.26**	0.28**	0.19**	0.19**	0.13*	0.06ns	0.53**	-0.01 ns	0.08 ns	0.00 ns	-0.06 ns
Peeling easiness			1.00	0.23**	0.11*	0.15*	0.20**	0.11*	0.11*	0.47**	0.02 ns	0.02 ns	0.13*	-0.07 ns
Chewing sweetness				1.00	0.21**	0.21**	0.23**	0.20**	0.02 ns	0.53**	0.19**	-0.01 ns	0.09 ns	-0.12*
Colour after cooking					1.00	0.46**	0.40**	0.32**	0.07 ns	0.62**	0.07 ns	-0.04 ns	-0.08 ns	-0.05 ns
Texture after cooking						1.00	0.63**	0.45**	0.16**	0.72**	0.16**	-0.15**	0.02 ns	-0.06 ns
Taste after cooking							1.00	0.37**	0.16**	0.71**	0.19**	-0.11*	0.09ns	-0.04 ns
Fibre organoleptic								1.00	0.12*	0.62**	0.05 ns	-0.02 ns	-0.11*	0.00 ns
Fast cooking ability									1.00	0.32**	0.24**	0.12*	-0.19**	0.14*
Cumulative organoleptic scoring										1.00	0.19**	-0.03 ns	0.00 ns	-0.07 ns
Starch content											1.00	0.04 ns	-0.01 ns	-0.15*
HCN content												1.00	0.10 ns	-0.50**
Amylose content													1.00	-0.58**
Crude fibre content														1.00

4.1.2.4.7 Colour after cooking

The colour after cooking was positively correlated to texture (0.46), taste (0.40), fibre organoleptic (0.32) and to colour before cooking (0.28), chewing (0.21) and peeling (0.11).

4.1.2.4.8 Texture after cooking

The texture was positively correlated to taste (0.63), colour after cooking (0.46), fibre organoleptic (0.45), chewing (0.21), colour before (0.19), yield (0.18), cookability (0.16), starch content (0.16) and peeling (0.15). It was also negatively correlated to HCN content (-0.15).

4.1.2.4.9 Taste after cooking

The taste was positively correlated to texture (0.63), colour after cooking (0.40), fibre organoleptic (0.37), chewing (0.23), peeling (0.20), colour before cooking (0.19), yield (0.19), starch content (0.19) and cookability (0.16). It was also negatively correlated to HCN content (-0.11).

4.1.2.4.10 Fibre organoleptic

The fibre organoleptic was positively correlated to texture (0.45), taste (0.37), colour after cooking (0.32), chewing (0.20), colour before cooking (0.13), cooking ability (0.12) and peeling (0.11). It was negatively correlated to amylose content (-0.11).

4.1.2.4.11 Fast cooking ability

The fast cooking ability was positively correlated to starch content (0.24), taste (0.16), texture (0.16), crude fibre (0.14), HCN content (0.12), organoleptic fibre (0.12) and peeling (0.11). And negatively correlated to yield (-0.33) and amylose content (-0.19).

4.1.2.4.12 HCN content

The HCN content was positively correlated to cookability (0.12) and negatively correlated to crude fibre (-0.50), texture (-0.15) and taste (-0.11).

4.1.2.4.13 Starch content

The starch content was positively correlated to yield (0.36), cookability (0.24), chewing (0.19), taste (0.19) and texture (0.16). It was negatively correlated to crude fibre (-0.15).

4.1.2.4.14 Amylose content

The amylose content was positively correlated to tuber fresh yield (0.22) and peeling easiness (0.13). It is also negatively correlated to crude fibre (-0.58), cookability (-0.19) and fibre organoleptic (-0.11).

4.1.2.4.15 Crude fibre

The crude fibre was positively correlated to cookability (0.14) and negatively correlated to amylose (-0.58), HCN (-0.50), yield (-0.34), starch (-0.15) and chewing sweetness (-0.12).

4.1.2.5 Path coefficient analysis

The estimates of direct and indirect effects of the biometrical traits on tuber fresh yield are shown in Table 24. The residual effect was 0.11.

The direct and indirect effects were classified based on the scale given by Lenka and Mishra (1973), as more than 1.0 – very high, between 0.3-0.99 – high, between 0.2-0.29 – moderate, between 0.10-0.19 – Low and between 0.00-0.09 – negligible.

4.1.2.5.1 Direct effects

High, positive and direct effect was exerted by biomass (0.56), dry matter (0.44) and harvest index (0.38) on yield, whereas, negative and low direct effect was exerted by plant height (-0.10). The rest of the traits, direct effects were negligible.

Table 24. Path analysis with direct and indirect effects of tuber fresh yield components on cassava genotypes

	Plant height	Branch height	Stem girth	Scar no	Branch no.	Tuber no.	Tuber length	Tuber neck	Tuber girth	CMD (1-5)	CLS (0-5)	Dry matter	Biomass	Harvest Index	Pearson correlation with yield (r_g)
Plant height	**-0.10**	0.02	0.02	0.00	0.00	0.00	0.01	0.01	0.00	0.03	-0.02	0.19	0.39	-0.06	0.46**
Branch height	-0.05	**0.03**	0.01	0.00	0.00	0.00	-0.01	0.01	0.00	0.03	-0.03	0.13	0.17	-0.01	0.27*
Stem girth	-0.08	0.02	**0.02**	0.00	0.00	0.00	-0.01	0.01	0.00	0.04	-0.02	0.25	0.44	-0.03	0.63**
Scar no.	0.04	0.00	0.00	**0.01**	0.00	0.00	0.01	-0.01	0.01	-0.02	0.01	-0.04	-0.08	-0.01	-0.08ns
Branch no.	-0.01	0.01	0.00	0.00	**-0.01**	0.00	0.00	0.00	0.00	0.00	-0.01	0.03	0.11	-0.06	0.06ns
Tuber no.	-0.01	0.00	0.00	0.00	0.00	**0.00**	0.00	-0.01	0.00	0.03	-0.01	0.24	0.04	0.24	0.52**
Tuber length	-0.05	0.01	0.01	0.00	0.00	0.00	**-0.02**	0.01	0.00	0.04	-0.03	0.21	0.26	0.06	0.50**
Tuber neck	-0.05	0.01	0.01	0.00	0.00	0.00	-0.01	**0.02**	0.00	0.01	-0.01	0.01	0.18	-0.08	0.09ns
Tuber girth	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	**0.02**	0.00	-0.01	0.17	0.15	0.06	0.41**
CMD (1-5)	0.05	-0.01	-0.01	0.00	0.00	0.00	0.01	0.00	0.00	**-0.07**	0.03	-0.27	-0.16	-0.18	-0.61**
CLS (0-5)	-0.03	0.01	0.01	0.00	0.00	0.00	-0.01	0.00	0.00	0.03	**-0.07**	0.19	0.21	0.05	0.40**
Dry matter	-0.05	0.01	0.01	0.00	0.00	0.00	-0.01	0.00	0.01	0.04	-0.03	**0.44**	0.33	0.22	0.98**
Biomass	-0.07	0.01	0.02	0.00	0.00	0.00	-0.01	0.01	0.00	0.02	-0.03	0.26	**0.56**	-0.1	0.66**
Harvest Index	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	-0.01	0.26	-0.15	**0.38**	0.53**

Significant at ** - 1 per cent and * - 5 per cent levels; Direct effects in bold; residual = **0.11**

4.1.2.5.2 Indirect effects

4.1.2.5.2.1 Dry matter

Moderate indirect and positive effect was exerted by biomass (0.26), harvest index (0.26), stem girth (0.25), tuber number (0.24) and tuber length (0.21) though dry matter. Also, moderate negative and indirect effect was exerted by scoring (-0.27) through dry matter. Low and positive effect was exerted by plant height (0.19), CLS (0.19), tuber girth (0.17) and branch height (0.13) through dry matter. The rest of the indirect effects were negligible.

4.1.2.5.2.2 Biomass

High indirect and positive effect was exerted by stem girth (0.44), plant height (0.39) and dry matter (0.33) through biomass. Moderate indirect and positive effect was exerted by tuber length (0.26) and CLS (0.21) through biomass. Low significant and indirect effect was exerted by tuber neck (0.18), branch height (0.17), tuber girth (0.15) and branch number (0.11). The other effects registered negligible values.

4.1.2.5.2.3 Harvest index

Moderate indirect and positive effect was exerted by tuber number (0.24) and dry matter through harvest index (0.22). Low, negative indirect effect was exerted by (-0.18) and biomass (-0.10) through harvest index. The rest of the indirect effects were negligible.

4.1.2.6 Cluster analysis

The dissimilarity matrix based on squared Euclidean distance for the biometrical traits in cassava genotypes is given in Table 25.

According to the relative magnitude of squared Euclidean distance values, 14 genotypes were grouped into five clusters and the values varied from 63 to 43236. The cluster 1 comprised of seven genotypes, viz. CC1, CC2, CC3, CC5, CC7, CC9 and Sree Jaya.

Table 25: Dissimilarity matrix and clustering of cassava genotypes

	Squared Euclidean Distance													
Genotypes	CC1	CC2	CC3	CC4	CC5	CC6	CC7	CC8	CC9	CC10	Vellayani Hraswa	Sree Prakash	Sree Jaya	M4
CC1	0.00													
CC2	1538.50	0.00												
CC3	1703.30	208.70	0.00											
CC4	2998.80	5375.60	6151.90	0.00										
CC5	1023.80	567.80	883.50	2769.70	0.00									
CC6	14662.20	9686.50	8177.00	27965.90	14157.10	0.00								
CC7	1195.00	601.60	534.60	4072.80	351.50	11900.60	0.00							
CC8	23698.80	16358.30	14874.20	39970.10	22269.60	1649.90	19502.10	0.00						
CC9	1057.70	643.70	505.20	3871.30	369.60	11759.30	65.30	19698.20	0.00					
CC10	4913.50	4144.60	2969.20	13987.50	6060.10	3902.80	4503.60	9854.30	4084.00	0.00				
Vellayani Hraswa	8341.30	7354.20	5741.50	19081.90	10167.40	3213.80	8536.00	9140.60	7876.30	1091.50	0.00			
Sree Prakash	8150.40	5313.80	4003.30	19093.10	8412.40	1192.80	6473.50	4897.10	6379.70	1234.20	1586.50	0.00		
Sree Jaya	4797.30	1302.90	1882.30	8857.00	2235.10	10906.90	2260.10	15417.70	2770.70	7759.30	12010.20	7265.70	0.00	
M4	8579.20	8490.50	9828.70	4352.60	5606.30	33850.10	6643.70	43236.00	7384.80	21967.90	29868.00	24834.10	7658.40	0.00

Cluster 3 comprised of four genotypes, viz. CC6, CC10, Vellayani Hraswa and Sree Prakash, and the clusters 2, 4 and 5 comprised of one genotype each, namely CC4, CC8 and M4, respectively.

At rescaled distance of 15 and 20 there were only two clusters the first cluster consisted of genotypes CC7,CC9,CC5,CC2,CC3,CC1, Sree Jaya,CC4,and M4. The second cluster consisted of CC10, Hraswa, CC6, Sree Prakash, and CC8.

4.1.3 Variability in biochemical traits in cassava genotypes

4.1.3.1 Analysis of variance

The analysis of variance revealed that there existed high significant differences among the genotypes for all the biochemical traits studied.

4.1.3.2 Mean performance of cassava genotypes for biochemical traits

The mean values of estimated biochemical traits in cassava genotypes are detailed in Table 26.

4.1.3.2.1 HCN (mg kg^{-1})

The HCN content of the genotype CC7 was highest (41.00 mg kg^{-1}) where as CC3 and CC9 recorded the lowest HCN content. The grand mean was 19.57 mg kg^{-1}.

4.1.3.2.2 Starch (per cent)

Starch content of the genotypes ranged from 56.51 per cent (Vellayani Hraswa) to 87.86 per cent (CC7 and Sree Jaya). The grand mean was 75.20 per cent.

4.1.3.2.3 Amylose (per cent)

The amylose content varied between 12.85per cent (Vellayani Hraswa) and 26.87 (M4) and overall mean of 20.94 per cent.

4.1.3.2.4 Amylose/Amylopectin ratio

The ratio varied between 0.24 (Sree Jaya) and 0.71 (CC5) with the overall mean of 0.40.

Table 26. Biochemical traits of cassava genotypes on dry weight basis

	Genotype													
Trait	**CC1**	**CC2**	**CC3**	**CC4**	**CC5**	**CC6**	**CC7**	**CC8**	**CC9**	**CC10**	**Vellayani Hraswa**	**Sree Prakash**	**Sree Jaya**	**M4**
Starch (per cent)	72.05 cd	80.22 ef	73.92 cde	78.21 def	64.68 b	74.46 cde	87.72 g	76.34 de	75.67 de	84.24 fg	56.51 a	72.45 cd	87.86 g	68.43 c
Amylose (per cent)	23.68 c	25.36 c	25.78 c	18.05 b	26.78 c	18.81 c	18.98 b	16.71 bc	23.51 c	23.09 c	12.85 a	16.20 bc	17.13 b	26.87 c
Amylose / amylopectin ratio	0.49 d	0.46 cd	0.54 d	0.30 ab	0.71 e	0.34 ab	0.28 ab	0.28 ab	0.45 cd	0.38 bc	0.29 ab	0.29 ab	0.24 a	0.65 e
Fibre (per cent)	7.00 a	7.00 a	8.50 cd	8.00 bc	9.00 d	8.50 cd	8.50 cd	8.00 bc	9.00 d	7.00 a	8.00 bc	8.50 cd	9.00 d	7.50 a
HCN (mg kg^{-1})	33.86 ef	25.29 d	8.14 a	13.86 abc	15.29 abc	12.43 ab	41.00 f	9.57 ab	8.14 a	33.86 ef	26.71 de	21.00 cd	8.14 a	16.71 bc

a, b,... - Means followed by same letter are not significantly different at 0.05 level

4.1.3.2.5 Fibre (per cent)

The genotypes CC5, CC9, and Sree Jaya recorded the highest fibre content (9.00per cent) while CC1, CC2, CC10 and M4 registered the lowest fibre content (7.50per cent). The overall mean was 8.11 per cent.

4.1.3.3 Variability studies

Variability and genetic parameter estimates for the four biochemical traits are enlisted in Table 27. The results are detailed below.

Table 27. Genetic parameters of biochemical traits in cassava genotypes

	Genetic parameters								
Trait	Mean	S.E.	Minimum	Maximum	PV	GV	PCV (per cent)	GCV (per cent)	H^2 (per cent)
Starch (per cent)	75.20	2.08	70.69	79.70	78.20	69.50	11.76	11.09	88.88
Amylose (per cent)	20.94	1.23	18.28	23.58	22.38	19.34	22.59	21.01	86.45
Amylose/Amylopectin ratio	0.40	0.03	0.33	0.48	37.59	35.66	90.01	0.28	69.69
Fibre (per cent)	8.11	0.24	7.59	8.63	0.60	0.49	9.58	8.61	80.76
HCN (mg kg^{-1})	19.57	2.31	14.58	24.56	124.99	114.31	57.12	54.63	91.45

SE – standard error; PV – phenotypic variability; GV – Genotypic variability; PCV – Phenotypic coefficient of variation; GCV – Genotypic coefficient of variation; H^2 – Broad sense heritability

4.1.3.3.1 Phenotypic and genotypic coefficient of variation

In general, the phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) for all the yield attributes studied. Phenotypic coefficient of variation ranged from 9.58 per cent to 57.12 per cent, corresponding to the traits fibre and HCN content. Amylose content (22.59 per cent) also recorded high PCV, while starch (11.76 per cent) registered moderate values of PCV. Genotypic coefficient of variation ranged from 8.61 per cent to 54.63 per cent, corresponding to the traits fibre per cent and HCN content. Amylose content (21.01 per cent) also recorded high PCV, while starch (11.09 per cent) registered moderate values of PCV.

4.1.3.3.2 Heritability and genetic advance as per cent of mean

Heritability estimates ranged from 80.76 per cent (fibre) to 91.45 per cent (HCN content). Other traits viz., starch (88.88 per cent) and amylose (86.45 per cent) content also registered high heritability estimates.

4.1.4 Organoleptic evaluation of cassava genotypes

The means values of of organoleptic evaluation of cassava genotypes are detailed in Table 28. Its test of significance by Kruskal Wallis Test is shown in Table 29.

The easiness in peeling and sweetness on chewing both tested before cooking, texture and taste after cooking and cooking time differed significantly among the cassava genotypes. Genotypes did not differ for all other tested traits.

Organoleptic evaluation of the genotypes showed that M4 peeled easily and CC5 had sweet fresh tubers. The CC5 had soft tubers on cooking while the tubers of M4 had good taste after cooking. The varieties Sree Prakash, Sree Jaya and M4 required lesser time for cooking.

4.2 Standardization of *in vitro* propagation method in cassava

4.2.1 Propagation through somatic embryogenesis from leaf explants

4.2.1.1 Sterilization of leaf explant

The effect of the leaf sterilization treatments of M4 and Hraswa cassava genotypes on MS media is shown in Table 30. The best treatments were T21 and T23, with mean survival value of two. The treatment T21 was a combination of Teepol (five per cent) washing for two minutes, with leaf chopping after sterilization and one minute exposure in ethanol (75 per cent), and one minute exposure in mercuric chloride (0.1 per cent) solutions. The T23 is the same as T21 except the ethanol exposure was for two minutes, in both cases 100 per cent survival was observed.

Table 28. Organoleptic evaluation of cassava genotypes

	Genotype													
Trait	**CC1**	**CC2**	**CC3**	**CC4**	**CC5**	**CC6**	**CC7**	**CC8**	**CC9**	**CC10**	**Vellayani Hraswa**	**Sree Prakash**	**Sree Jaya**	**M4**
Colour before cooking	4.06	3.56	3.44	3.94	4.19	3.81	4.13	3.38	3.63	3.00	4.19	3.88	4.13	4.00
Eeasiness in peeling response:	3.56	2.94	4.44	4.13	3.63	3.88	4.13	4.06	3.50	4.19	4.25	3.94	4.00	4.56
Sweetness on chewing:	3.44	2.75	2.63	3.19	3.94	3.81	3.50	3.06	3.25	3.00	3.06	3.19	3.69	3.75
Colour after cooking	4.00	3.75	3.25	3.75	4.38	3.81	3.69	3.56	3.44	3.81	3.75	3.56	4.06	4.00
Texture after cooking:	3.88	3.00	3.00	3.88	4.38	3.44	3.63	3.81	3.69	3.75	3.50	3.44	4.25	4.19
Taste after cooking	3.81	2.44	2.81	3.25	4.38	3.50	3.81	3.56	3.75	3.63	3.44	3.44	4.19	4.44
Fibre content:	4.00	3.94	3.75	3.56	4.31	3.69	3.50	3.69	3.69	3.63	3.94	3.56	4.25	4.19
Cooking time	3.00	3.00	1.00	3.00	3.00	2.00	2.00	3.00	3.00	3.00	3.00	4.00	4.00	4.00

Table 29: Kruskal Wallis Test of significance for organoleptic traits in cassava genotypes

Trait	**Chi-Square**	**Df**	**Asymp. Sig.**	**sig.**
Colour before cooking	19.55	13	0.11	Ns
Eeasiness in peeling response:	40.44	13	0.00	**
Sweetness on chewing:	32.21	13	0.00	**
Colour after cooking	20.83	13	0.08	Ns
Texture after cooking:	37.69	13	0.00	**
Taste after cooking	50.77	13	0.00	**
Fibre content:	12.03	13	0.52	Ns
Cooking time	223.00	13	0.00	**

** - significant at 1 per cent level; ns - non significant

Table 30. Standardisation of sterilisation methods in *in vitro* cultures of cassava

Treatment code	Chopping of leaf	Teepol (5 per cent)	Ethanol (75 per cent)	$HgCl_2$ (0.1 per cent)	Mean score for the response of culture*
T1	Before	One minute	Wiping	One minute	0.00[a]
T2	Before	One minute	Wiping	Two minutes	0.00[a]
T3	Before	One minute	One minute	One minute	0.00[a]
T4	Before	One minute	One minute	Two minutes	0.00[a]
T5	Before	One minute	Two minutes	One minute	0.00[a]
T6	Before	One minute	Two minutes	Two minutes	0.00[a]
T7	Before	Two minutes	Wiping	One minute	0.00[a]
T8	Before	Two minutes	Wiping	Two minutes	0.00[a]
T9	Before	Two minutes	One minute	One minute	0.00[a]
T10	Before	Two minutes	One minute	Two minutes	0.00[a]
T11	Before	Two minutes	Two minutes	One minute	0.00[a]
T12	Before	Two minutes	Two minutes	Two minutes	0.00[a]
T13	After	One minute	Wiping	One minute	0.00[a]
T14	After	One minute	Wiping	Two minutes	0.00[a]
T15	After	One minute	One minute	One minute	0.00[a]
T16	After	One minute	One minute	Two minutes	0.00[a]
T17	After	One minute	Two minutes	One minute	0.00[a]
T18	After	One minute	Two minutes	Two minutes	0.00[a]
T19	After	Two minutes	Wiping	One minute	0.00[a]
T20	After	Two minutes	Wiping	Two minutes	0.00[a]
T21	After	Two minutes	One minute	One minute	2.00[d]
T22	After	Two minutes	One minute	Two minutes	0.75[a]
T23	After	Two minutes	Two minutes	One minute	2.00[d]
T24	After	Two minutes	Two minutes	Two minutes	1.50[c]

Basal media: MS3 + 2 mg l^{-1} 2,4 D; * at two weeks after culturing (2 WAC);

4.2.1.2 Effect of leaf maturity on sterilization of leaf explants

The effect of leaf maturity on culture establishment of cassava leaf explant is shown in Table 31. There was no significant difference among the treatments.

4.2.1.3 Effect of 2,4-D on callusing of leaf explants in cassava

The effect of 2,4-D on callusing of leaf explants is shown in Table 32 and the best treatment were T3 and T4 which, consisted of 6 and 8 mg l^{-1} of 2,4-D incorporated into MS media. The percentage of callusing was 66.67 and 88.89 per cent, respectively.

Table 31. Effect of leaf maturity on sterilisation of leaf explants in cassava

Treatment code	Leaf maturity	Duration of treatment of $HgCl_2$ (0.05per cent)	Mean score for the response of culture*
1	Immature	One minute	1.20[a]
2	Immature	Ttwo minutes	1.50[a]
3	Mature	One minute	1.08[a]
4	Mature	Two minutes	1.38[a]

Basal media: MS3 + 2 mg l^{-1} 2,4 D; * at 2 WAC

Table 32. Effect of 2,4-D on callus induction in leaf explants in cassava

Treatment code	Concentration of 2,4-D	Mean score for the response of culture	Percentage of callusing*
T1	2 mg l^{-1}	2.22[a]	5.56
T2	4 mg l^{-1}	2.33[a]	16.67
T3	6 mg l^{-1}	2.94[ab]	66.67
T4	8 mg l^{-1}	3.61[b]	88.89
T5	10 mg l^{-1}	2.56[a]	61.11

Basal media: MS + 3 per cent sucrose, *i.e.*, MS3; * at 4 WAC

4.2.1.4 Effect of light on callusing of leaf explants in cassava

There was no significant difference among the treatments when the leaf explants were cultured on nine hours of light or continues dark condition.

4.2.1.5 Effect of BA and NAA on callus induction in cassava

The effect of BA and NAA on callus induction is shown in Table 33. The best treatments for callus induction were T5, T6, T7, T8 and T12. The percentage of callusing was 88.89, 77.78, 94.44, 61.11 and 66.67, respectively. The treatments T5 to T8 consisted of a combination of 1 mg l^{-1} BA with 0.1 mg l^{-1}, 0.2 mg l^{-1}, 0.3 mg l-1 and 0.5 mg l^{-1} of NAA, respectively. Treatment T12 consisted of combination of 3 mg l^{-1} BA with 0.5 mg l^{-1} NAA.

4.2.1.6 Effect of Picloram and CuSO4 on somatic embryognesis in cassava

The effects of Picloram and CuSO4 on somatic embryognesis of leaf explants showed that there was no significant difference among the treatments.

Table 33: Effect of BA and NAA on callus induction in leaf explants in cassava

Treatment code	Growth regulator		Effect	
	BA (mg l^{-1})	NAA (mg l^{-1})	Mean score of the response of culture*	Callusing (per cent)
T1	10.0	0.10	1.67^{bc}	11.11
T2	10.0	0.20	1.89^{c}	22.22
T3	10.0	0.30	1.39^{ab}	11.11
T4	10.0	0.50	1.56^{bc}	0.00
T5	1.00	0.10	2.83^{de}	88.89
T6	1.00	0.20	2.67^{de}	77.78
T7	1.00	0.30	2.89^{e}	94.44
T8	1.00	0.50	2.56^{de}	61.11
T9	3.00	0.10	2.39^{d}	44.44
T10	3.00	0.20	1.89^{c}	22.22
T11	3.00	0.30	1.56^{bc}	5.56
T12	3.00	0.50	2.61^{de}	66.67
T13	6.00	0.10	1.33^{ab}	0.00
T14	6.00	0.20	1.06^{a}	0.00
T15	6.00	0.30	1.72^{bc}	0.00
T16	6.00	0.50	1.39^{ab}	0.00

Basal media: MS + 3 per cent sucrose, *i.e.*, MS3; * at 4 WAC

4.2.1.7 Effect of Picloram on somatic embryogenesis from FEC of leaf explants of cassava

The effect of Picloram treatments on somatic embryogenesis has shown that there was no significant difference among the treatments.

4.2.1.8 Effect of TDZ and BA on regeneration of somatic embryos

The effect of TDZ and BA treatments on regeneration of somatic embryos has shown that there was no significant difference among the treatments.

4.2.2 Propagation through morphogenesis from nodal explants

4.2.2.1 Surface sterilization of nodal explants

The effect of treatments for surface sterilization of nodal explants is shown in Table 34. The best treatments were, T6 and T7 which consisted of exposure of nodal explant to 0.05 per cent solution of mercuric chloride for two minutes and 0.1 per cent solution of mercuric chloride for one minute.

Table 34. Effect of treatments for surface sterilization of nodal explants in cassava

Treatment code	Factors		Mean score of the response of culture*
	Genotype	$HgCl_2$ as sterilant	0.77^{bc}
T1	CC1	0.05per cent for 1 minute	0.65^{abc}
T2	CC1	0.05per cent for 2 minutes	0.43^{a}
T3	CC1	0.1per cent for 1 minute	0.55^{ab}
T4	CC1	0.1per cent for 2 minutes	0.60^{abc}
T5	Sree Jaya	0.05per cent for 1 minute	0.77^{bc}
T6	Sree Jaya	0.05per cent for 2 minutes	0.87^{c}
T7	Sree Jaya	0.1per cent for 1 minute	0.87^{c}
T8	Sree Jaya	0.1per cent for 2 minutes	0.70^{abc}

Basal media: MS + 3 per cent sucrose, *i.e.*, MS3; * at 2 WAC

4.2.2.2 Effect of BA and kinetin on morphogenesis from nodal explants

The effect of BA and kinetin on morphogenesis from nodal explants is shown in Table 35. The best treatment was T8 which consisted of MS + 2 mg l-1 BA.

Table 35. Effect of BA and kinetin on morphogenesis of nodal explants in cassava

Treatment code	Media	Mean score of the response of culture*
T1	Basal MS3	0.44^{ab}
T2	MS3 + 1.0 mg l^{-1} BA	0.44^{ab}
T3	MS3 + 2.0 mg l^{-1} BA	0.17^{a}
T4	MS3 + 1.0 mg l^{-1} kinetin	0.11^{a}
T5	MS3 + 2.0 mg l^{-1} kinetin	0.33^{ab}
T6	Basal MS3	0.83^{bc}
T7	MS3 + 1.0 mg l^{-1} BA	0.33^{ab}
T8	MS3 + 2.0 mg l^{-1} BA	1.17^{c}
T9	MS3 + 1.0 mg l^{-1} kinetin	0.00^{a}
T10	MS3 + 2.0 mg l^{-1} kinetin	0.33^{ab}

Basal media: MS+3 per cent sucrose, *i.e.*, MS3; * at 7 WAC

4.2.2.3 Effect of TDZ and BA on morphogenesis from nodal explants

The effects of TDZ and BA on morphogenesis of nodal explants showed that there was no significant difference in the treatments.

4.2.2.4 Effect of solid or liquid media on *in vitro* morphogenesis from nodal explants

The effect of liquid and solid media on morphogenesis from nodal explants showed that there was no significant difference among the treatments

4.2.2.5 Effect of genotype on *in vitro* morphogenesis

The effect of the genotype on morphogenesis has shown that the genotype CC1 exhibited better response with a mean score of two while Sree Jaya had mean score response of 0.88. In a period of four weeks time CC1 has added 26 new nodes out of 26 subcultured, while Sree Jaya add none out of 26 (but three got lost by necrosis).

4.2.2.6 Effect of reduction in MS salt strength on *in vitro* cultures in cassava

4.2.2.6.1 Effect of reduction in MS salt strength on cassava genotype CC1

Effect of reduction in MS salt strength on genotype CC1 is given in Table 36. Treatments did not differ significantly with respect to number of shoots and number of leaves per treatments. In this experiment MS3, MS2, MS1 and MS0 stands for basal MS media with 3.0, 2.0, 1.0 and 0.0 per cent sucrose.

Table 36. Effect of reduction in MS salt strength on cassava genotype CC1

	Mean of treatments at 9 WAC				
Trait	**T1**	**T2**	**T3**	**T4**	**T5**
Height of the longest plantlet (cm)	3.43^{a}	5.00^{a}	4.57^{a}	9.57^{b}	11.43^{b}
No. of Shoots per culture	2.57^{a}	3.29^{a}	4.14^{a}	2.57^{a}	2.57^{a}
No. of leaves per culture	11.14^{a}	11.57^{a}	9.00^{a}	10.29^{a}	12.71^{a}
No. of roots per culture	1.57^{a}	8.29^{b}	2.14^{a}	9.14^{b}	8.29^{b}
Length of longest root (cm)	1.00^{a}	4.43^{b}	1.29^{a}	6.14^{b}	5.00^{b}
Size of roots (score 1 - 10)	0.57^{a}	2.14^{bc}	1.00^{ab}	1.57^{ab}	3.00^{c}

Media: T1: MS3 + 0.25 mgl^{-1} TDZ; T2: ½ MS + 2 per cent sucrose; T3: ½ MS + 1 per cent sucrose; T4: ¼ MS + 1 per cent sucrose; T5: ¼ MS + no sucrose.

In the case of height of plantlet the best treatments were T4 and T5. It produced plants up to 11.43 cm height after ten weeks of culturing. The treatments T5 and T4 were of ¼ MS0 and ¼ MS1, respectively. Numbers of roots up to 9.14 per culture were produced in T2, T4 and T5. Longest roots up to

6.14cm were observed in cultures in the treatment T2, T4 and T5. Biggest roots were observed in the treatment T5. The treatment T2 was 1/2 MS2.

4.2.2.6.2 Effect of reduction in strength of MS salt on cassava genotype Sree Jaya

The effect of reduction in MS salt strength on genotypes Sree jaya is shown in Table 37. Treatments did not differ significantly with respect to number of roots per culture, length and size of the roots. Tallest plantlets of 5.35 cm were observed in media containing ½ MS2 (T2). High number of shoots and leaves were observed in media MS3 + 0.25 mg l^{-1} TDZ (T1).

Table 37. Effect of reduction in strength of MS salt on cassava genotype Sree Jaya

	Mean of treatments (9 WAC)	
Trait	**T1**	**T2**
Height of the longest plantlet (cm)	3.37[a]	5.35[b]
No. of Shoots per culture	7.09[b]	2.35[a]
No. of leaves per culture	15.48[b]	8.00[a]
No. of roots per culture	3.39[a]	2.43[a]
Length of longest root (cm)	2.96[a]	2.09[a]
Size of roots (score 1 - 10)	1.61[a]	0.91[a]

Media: T1: MS3 + 0.25 mg l^{-1} TDZ; T2: ½ MS + 2 per cent sucrose

4.2.2.7 Effect of rooting media on *in vitro* plantlets

4.2.2.7.1 Effect of rooting media on *in vitro* plantlets of cassava genotype CC1

Effect of rooting media on *in vitro* cultured plantlets in CC1 is presented in Table 38. Treatment T3 (MS3 + 0.25 mg l^{-1} TDZ + 0.5 mg l^{-1} NAA) produced longest shoots of 13.00 cm, more number of shoots and longest roots (5.89cm). Less number of shoots and leaves and larger sized roots were observed in T2 (one minute dipping the lower portion of stem in 1.0 percent NAA and culturing in MS3 +0.25 mg l^{-1} TDZ).

4.2.2.7.2 Effect of rooting media on *in vitro* plantlets of cassava genotype Sree Jaya

Effect of rooting media on plantlets in Sree Jaya is presented in Table 39. The tallest plantlets were produced in media T3 (MS3 + 0.25 mg l^{-1} TDZ + 0.50

mg l^{-1} NAA). While more number of shoots, leaves, roots, longer and larger roots were produced in treatment T1 (MS3 + 0.25 mg l^{-1} TDZ).

Table 38. Effect of rooting media on *in vitro* cultured plantlets of cassava genotype CC1

	Mean of treatments (9 WAC)		
Trait	**T1**	**T2**	**T3**
Height of the longest plantlet (cm)	7.67[a]	6.22[a]	13.00[b]
No. of Shoots per culture	4.00[b]	2.11[a]	4.78[b]
No. of leaves per culture	11.78[b]	8.33[a]	13.22[b]
No. of roots per culture	8.67[b]	1.67[a]	5.22[ab]
Length of longest root (cm)	4.11[ab]	2.11[a]	5.89[b]
Size of roots (score 1 - 10)	2.00[b]	1.00[a]	1.44[ab]

Media: T1: MS3 + 0.25 mg l^{-1} TDZ; T2: 1 minute dip into 1 per cent NAA solution, then culturing in MS3 + 0.25 mg l^{-1} TDZ; MS3 + 0.25 mg l^{-1} TDZ + 0.5 mg l^{-1} NAA)

Table 39. Effect of rooting media on *in vitro* cultured plantlets of cassava genotype Sree Jaya

	Man of treatments (9 WAC)		
Trait	**T1**	**T2**	**T3**
Height of the longest plantlet (cm)	5.69[a]	5.62[a]	13.15[b]
No. of Shoots per culture	22.00[c]	1.08[a]	5.46[b]
No. of leaves per culture	43.38[c]	7.92[a]	19.92[b]
No. of roots per culture	5.92[b]	2.31[a]	8.38[b]
Length of longest root (cm)	5.31[b]	2.38[a]	4.46[b]
Size of roots (score 1 - 10)	1.85[c]	1.15[a]	1.38[ab]

Media: T1: MS3 + 0.25 mg l^{-1} TDZ; T2: 1 minute dip into 1 per cent NAA solution, then culturing in MS3 + 0.25 mg l^{-1} TDZ; MS3 + 0.25 mg l^{-1} TDZ + 0.5 mg l^{-1} NAA)

4.3 Standardization of *in vitro* mutagenesis in cassava

In vitro cultures in cassava were exposed to two mutagens, *viz.*, gamma irradiation and EMS to induce mutation. There was a shortage of somatic embryo cultures for CC1 and shortage of nodal plantlets for Sree Jaya. Therefore, the results presented in this study on somatic embryos to Sree Jaya and results on plantlets refer and CC1. The effect of treatment of mutagens on i*n vitro* cultures, seven weeks after culturing (WAC), is presented here.

4.3.1 Effect of gamma radiation on *in vitro* cultures of cassava genotypes CC1 and Sree Jaya

The gamma radiation at 40 Gy resulted in 57 per cent survival rate for callus cultures. Observations were recorded four weeks after subculturing. The mean value for the survival of the gamma irradiated callus was 34 per cent. The gamma irradiation at 30 Gy resulted in 43 per cent survival rate for somatic embryos (Sree Jaya). The Gamma irradiation at 50 Gy resulted in 36 per cent survival rate for *in vitro* derived plantlets (CC1) (Table 40).

Table 40. Effect of gamma radiation on *in vitro* cultures of cassava genotypes CC1 and Sree Jaya

Treatment code	Dose of gamma radiation	Survival of cultures 7 WAC in percentage		
		Callus*	Somatic embryos†	Plantlets‡
T1	10Gy	86.00^{e}	100.00^{e}	100.00^{c}
T2	20Gy	64.00^{de}	71.00^{de}	100.00^{c}
T3	30Gy	57.00^{cde}	57.00^{cd}	79.00^{bc}
T4	40Gy	43.00^{bcd}	30.06^{bc}	71.00^{bc}
T5	50Gy	36.00^{abcd}	29.00^{abc}	64.00^{b}
T6	60Gy	29.00^{abc}	14.00^{ab}	29.00 ^{a}
T7	70Gy	29.00^{abc}	00.00 ^{a}	29.00 ^{a}
T8	80Gy	21.00^{ab}	00.00 ^{a}	14.00 ^{a}
T9	90Gy	14.00^{ab}	0.00 ^{a}	07.00 ^{a}
T10	100Gy	00.00^{a}	00.00 ^{a}	00.00 ^{a}
T11	110Gy	00.00^{a}	00.00^{a}	00.00 ^{a}

Media: MS3 + 0.25 mg l^{-1} TDZ; WAC: weeks after culturing; * - both genotypes; † - Sree Jaya; ‡ - CC1

4.3.2 Effect of EMS treatment on *in vitro* cultures of cassava genotypes CC1 and Sree Jaya

The treatment of EMS at 1.20 per cent, incorporated into the solid media for 72 hours, resulted in 37 per cent of survival rate in *in vitro* derived callus. Survival rate of 56 per cent was observed in somatic embryos (Sree Jaya) treated with 1.20 per cent EMS. *In vitro* derived plantlets (CC1) on treatment with 0.9 per cent EMS exhibited a survival per cent of 41 (Table 41).

Table 41. Effect of EMS treatment on *in vitro* cultures of CC1 and Sree Jaya cassava genotypes

Treatment code	Concentration of EMS (per cent)	Survival of cultures 7 WAC in percentage		
		Callus*	Somatic embryoid†	Plantlets‡
T1	0.30	89.00[b]	89.00[c]	93.00[c]
T2	0.60	83.00[b]	72.00[bc]	76.00[bc]
T3	0.90	67.00[b]	61.00[bc]	59.00[b]
T4	1.20	22.00[a]	44.00[b]	14.00[a]
T5	1.50	11.00[a]	0.00[a]	0.00[a]

Media: MS3 + 0.25TDZ; * - both genotypes; † - Sree Jaya; ‡ - CC1; WAC: weeks after culturing

4.4 Evaluation of *in vitro* cultures in cassava treated with mutagen

4.4.1 Evaluation of somatic embryos of Sree Jaya treated with EMS

The mean effect of Sree Jaya *in vitro* mutagenesis through EMS treatment on somatic embryos (SE) in terms of height of the longest plantlet in centimeters, number of shoots per culture, number of leaves per culture, number of roots per culture, length of the longest root in centimeter and size of roots (in score of 1-10) is shown in Table 42. Results indicated that there was no significant difference among the treatments with respect to number of roots produced per culture and the score for the size of the roots. Taller plantlets, more number of roots and leaves, and long roots were observed in control, *i.e.*, media without EMS incorporated.

Table 42. Evaluation of somatic embryos of Sree Jaya cassava genotype treated with EMS

Trait	Mean of treatments (7 WAC)				
	Control	Concentration of EMS in percentage			
		0.3	0.6	0.9	1.2
Height of the longest plantlet (cm)	3.36[b]	1.18[a]	1.02[a]	0.94[a]	0.36[a]
No. of shoots per culture	11.36[b]	4.57[a]	5.50[a]	4.00[a]	5.00[a]
No. of leaves per culture	20.36[b]	9.64[a]	10.50[a]	8.00[a]	9.71[a]
No. of roots per culture	2.93[a]	0.50[a]	0.71[a]	0.86[a]	1.86[a]
Length of longest root (cm)	2.64[b]	0.50[a]	0.71[a]	0.64[a]	1.07[a]
Size of roots (score 1 - 10)	1.14[a]	0.50[a]	0.71[a]	0.57[a]	0.71[a]

Media: MS3 + 0.25 mg l^{-1} TDZ; WAC: weeks after culturing

4.4.2 Evaluation of somatic embryo derived plantlets of Sree Jaya cassava genotype treated with EMS

Results of evaluation of plantlets derived from somatic embryos treated with EMS at various concentrations are presented in Table 43. Effect of EMS treatment on somatic embryo derived plantlets showed that the number of shoots and leaves per culture were high in medium without EMS, *i.e.*, control. Taller plantlets, high number of roots per culture, and longer roots were observed in both control and 0.3 per cent EMS treatments. Lowest number of roots per culture (3.44) and shorter roots (2.33 mm) were observed at 1.2 per cent concentration of EMS.

Table 43. Evaluation of somatic embryo derived plantlets of Sree Jaya cassava genotype treated with EMS

Trait	Mean of treatments (7 WAC)				
	Control	Concentration of EMS per cent			
		0.3	0.6	0.9	1.2
Height of the longest plantlet (cm)	8.89^{c}	7.33^{bc}	5.89^{abc}	4.22^{ab}	2.67^{a}
No. of Shoots per culture	13.89^{b}	9.22^{a}	7.44^{a}	6.00^{a}	5.67^{a}
No. of leaves per culture	20.56^{a}	17.11^{b}	14.11^{a}	13.78^{a}	9.78^{a}
No. of roots per culture	7.56^{b}	5.89^{ab}	5.67^{ab}	4.56^{ab}	3.44^{a}
Length of longest root (cm)	5.22^{b}	5.00^{b}	4.78^{ab}	3.11^{ab}	2.33^{a}
Size of roots (score 1 - 10)	2.22^{a}	1.89^{a}	1.78^{a}	1.67^{a}	1.33^{a}

Media: MS3 + 0.25 mg l^{-1} TDZ; WAC: weeks after culturing

4.4.3 Effect of treatment of different concentrations of EMS on *in vitro* cultured plantlets of CC1

Effect of treatment of different concentrations of EMS on *in vitro* cultured plantlets in genotype CC1 is presented in Table 44. The results show that the number of roots and length of roots did not differ significantly between the treatments. Longer plantlets with high number of shoots and leaves were observed in media devoid of EMS. Larger sized roots were observed in media containing zero or 0.3 per cent EMS. Higher concentration of EMS (0.9 per cent) negatively affected expression of all the traits.

Table 44. Effect of EMS on *in vitro* cultured plantlets of CC1 cassava genotype

Trait	Mean of treatments (7 WAC)			
		Concentration of EMS per cent		
	Control	0.3	0.6	0.9
Height of the longest plantlet (cm)	6.80[b]	5.10[ab]	4.65[ab]	3.92[a]
No. of Shoots per culture	7.29[b]	3.32[a]	2.81[a]	2.81[a]
No. of leaves per culture	14.94[b]	11.10[a]	9.10[a]	9.06[a]
No. of roots per culture	5.55[a]	5.32[a]	4.55[a]	4.13[a]
Length of longest root (cm)	4.31[a]	3.61[a]	3.29[a]	2.61[a]
Size of roots (score 1 - 10)	2.77[b]	1.94[ab]	1.65[a]	1.26[a]

Media: MS3 + 0.25 mg l^{-1} TDZ; WAC: weeks after culturing

4.4.4 Effect of gamma irradiation on somatic embryos of Sree Jaya cassava genotype

Effect of gamma irradiation on somatic embryos in Sree Jaya after seven weeks of culture is presented in Table 45. Results indicated that the number of shoots and leaves per culture, and number, length and size of the roots did not differ significantly among the treatments. Cultures which are not irradiated yielded plantlets with longer and more shoots and roots. Other doses of gamma ray were not tried because of shortage of cultures.

Table 45. Effect of gamma irradiation on somatic embryos of Sree Jaya cassava genotype

Trait	Mean of treatments (7 WAC)		
	Control	Dose of gamma ray	
		40Gy	50Gy
Height of the longest plantlet (cm)	1.44[b]	1.00[a]	1.00[a]
No. of Shoots per culture	11.11[a]	8.78[a]	3.78[a]
No. of leaves per culture	18.22[a]	8.33[a]	7.00[a]
No. of roots per culture	0.56[a]	0.00[a]	0.00[a]
Length of longest root (cm)	0.67[a]	0.00[a]	0.00[a]
Size of roots (score 1 - 10)	0.67[a]	0.00[a]	0.00[a]

Media: MS3 + 0.25 mg l^{-1} TDZ; WAC: weeks after culturing

4.4.5 Effect of gamma irradiation on somatic embryo derived plantlets of Sree Jaya

Results of effect of gamma irradiation on somatic embryo derived plantlets of Sree Jaya are shown in Table 46. The size of the roots did not differ between

the treatments. Height of somatic embryo derived plantlets, number of shoots and leaves per culture, were higher in plantlets which were not irradiated. However, less number of roots was formed in cultures irradiated at 50 Gy. Other doses of gamma ray not tried because of shortage of cultures.

Table 46. Effect of gamma irradiation on somatic embryo derived plantlets of Sree Jaya cassava genotype

Trait	Mean of treatments (7 WAC)		
	Control	Dose of gamma ray	
		40Gy	50Gy
Height of the longest plantlet (cm)	5.44[b]	2.89[a]	1.94[a]
No. of Shoots per culture	24.00[b]	9.56[a]	5.67[a]
No. of leaves per culture	19.00[a]	17.78[b]	12.11[a]
No. of roots per culture	13.44[b]	7.22[a]	1.89[a]
Length of longest root (cm)	5.89[b]	2.67[a]	1.44[a]
Size of roots (score 1 - 10)	1.89[a]	1.11[a]	1.00[a]

Media: MS3 + 0.25TDZ; WAC: weeks after culturing

4.4.6 Effect of gamma irradiation on *in vitro* derived plantlets of CC1 cassava genotype

The results on the effect of gamma irradiaton on *in vitro* derived plantlets in the genotype CCI after seven weeks of culture is given in the Table 47. All the observed traits showed a gradual reduction with increase in dose of irradiation. The effect was drastic at higher doses of irradiation (<50 Gy). No roots were produced in plantlets at this higher dose.

4.5 Acclimatization of *in vitro* plantlets

4.5.1 Effect of substrate on acclimatization of plantlets

The effect of soil substrate for acclimatization outside the tissue culture system showed that the best treatment was mixture of Soilrite^{TM} and sandy soil at 50:50 proportions which gave 43 per cent survival of plantlets. Four planting substrates were tested. The number of plantlets per replication was 7.

Table 47. Effect of gamma irradiation on *in vitro* derived plantlets of CC1 cassava genotype

Traits	Mean of treatments (7 WAC)							
	Control	Dose of gamma irradiation						
		20 Gy	30 Gy	40 Gy	50 Gy	60 Gy	70 Gy	80 Gy
Height of the longest plantlet (cm)	10.11^{d}	5.33^{c}	4.56^{c}	3.44^{bc}	2.00^{ab}	1.00^{a}	1.00^{a}	1.00^{a}
No. of Shoots/ culture	5.78^{c}	2.33^{b}	2.22^{ab}	1.67^{ab}	1.67^{ab}	1.00^{a}	1.00^{a}	1.00^{a}
No. of leaves/culture	13.56^{d}	10.67^{cd}	9.33^{cd}	7.33^{bc}	3.44^{ab}	2.89^{a}	2.33^{a}	2.22^{ab}
No. of roots/culture	10.67^{b}	8.33^{b}	7.33^{a}	0.78^{a}	0.67^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Length of longest root(cm)	6.00^{b}	4.44^{b}	4.11^{b}	1.22^{a}	0.78^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Size of roots (score 1 -10)	3.67^{d}	2.56^{c}	1.56^{bc}	0.56^{ab}	0.67^{ab}	0.00^{a}	0.00^{a}	0.00^{a}

Media: MS3 + 0.25TDZ; WAC: weeks after culturing

4.5.2 Hardening of *in vitro* derived plantlets under tissue culture laboratory

None of the plantlets attempted for acclimatization in the tissue culture laboratory were able to establish under the condition (Table 48).

4.5.3 Hardening of *in vitro* derived plantlets under conventional green house

Hardening of *in vitro* derived plantlets in the conventional green house is presented in the Table 49. The results showed that out of 79 plants kept for hardening only three survived under green house condition.

4.5.4 Hardening of *in vitro* derived plantlets under pad and fan green house

Results on hardening of *in vitro* derived plantlets under pad and fan green house is shown in Table 50. Out of 29 plants kept for hardening under fan and pad green house 14 plant got established.

Table 48. Hardening of *in vitro* derived plantlets in tissue culture laboratory

Genotype	Culture source	Treatment	Plantlets (No.s)	Plantlet average			Survival (No.s)
				Height (cm)	Length Roots (cm)	Size of roots (Score)	
CC1	Node	0.3 per cent EMS	5	11	5	3	0
CC1	Node	0.6 per cent EMS	15	31	15	9	0
CC1	Node	0.9 per cent EMS	9	8	6	3	0
CC1	Node	10 Gy	22	7	7	4	0
CC1	Node	20 Gy	17	8	5	3	0
CC1	Node	30 Gy	6	6	2	1	0
CC1	Node	40 Gy	4	6	3	2	0
CC1	Node	Control	17	7	6	3	0
Hraswa	Node	Control	2	7	5	3	0
M4	Node	Control	1	6	7	3	0
Sree Jaya	SE	0.3 per cent EMS	9	5	3	2	0
Sree Jaya	SE	0.9 per cent EMS	2	8	7	3	0
Sree Jaya	SE	Control	1	7	1	1	0
		Total	110				0
				Success rate (per cent)			0

SE: Somatic Embryo

Table 49. Hardening of *in vitro* derived plantlets under conventional green house

Genotype	Culture source	Treatment	Plantlets (No.s)	Plantlet average			Survival rate (No.s)
				Height (cm)	Length Roots (cm)	Size Roots (Score)	
CC1	Node	0.3 per cent EMS	2	9	5	3	0
CC1	Node	10Gy	3	10	6	2	0
CC1	Node	Control	29	9	6	4	2
M4	Node	Control	2	9	3	2	0
Sree Jaya	SE	0.3 per cent EMS	7	12	4	2	0
Sree Jaya	SE	0.6 per cent EMS	2	11	5	3	0
Sree Jaya	SE	0.9 per cent EMS	6	17	3	2	0
Sree Jaya	SE	1.2 per cent EMS	11	12	6	3	1
Sree Jaya	SE	20Gy	7	5	1	1	0
Sree Jaya	SE	Control	10	7	5	3	0
		Total	79				3
				Success rate (per cent)			4

SE: Somatic Embryo

Table 50. Hardening of *in vitro* derived plantlets inside pad and fan green house

Genotype	Culture source	Treatment	Plantlets (No.s)	Plantlet average				Success (No.s)
				Height (cm)	Length Roots (cm)	Size Roots (Score)	Roots (No.s)	
CC1	Node	0.6 per cent EMS	1	12	4	2	10	1
CC1	Node	1.2 per cent EMS	2	10	6	5	6	0
CC1	Node	Control	5	11	6	4	8	2
Sree Jaya	SE	0.3 per cent EMS	7	17	8	3	14	5
Sree Jaya	SE	0.6 per cent EMS	2	17	13	4	13	0
Sree Jaya	SE	0.9 per cent EMS	1	12	7	3	16	0
Sree Jaya	SE	1.2 per cent EMS	11	11	6	3	10	6
		Total	29					14
					Success rate (per cent)			48

SE: Somatic Embryo

4.5.5 Evaluation of the mutagen treated *in vitro* derived plantlets under pad and fan green house

The results on the evaluation of mutagen treated *in vitro* derived plantlets for quantitative traits (Table 51) showed that there are variations in the plant height, number of branches and leaf number. There are 14 succesfully acclimatized plantlets transplanted between July and September of 2014.

Table 51. Evaluation of mutagen treated *in vitro* derived plantlets under pad and fan green house for quantitative traits

No.	Genotype	Treatment	Height (cm)	Shoot no.	Leaf no.
1	Sree Jaya	0.3 per cent EMS	58	1	10
2	Sree Jaya	0.3 per cent EMS	45	1	8
3	Sree Jaya	0.3 per cent EMS	25	4	14
4	Sree Jaya	0.3 per cent EMS	33	1	10
5	Sree Jaya	0.3 per cent EMS	10	1	8
6	Sree Jaya	1.2 per cent EMS	30	1	10
7	Sree Jaya	1.2 per cent EMS	15	1	2
8	Sree Jaya	1.2 per cent EMS	50	1	5
9	Sree Jaya	1.2 per cent EMS	21	1	4
10	Sree Jaya	1.2 per cent EMS	21	1	3
11	Sree Jaya	1.2 per cent EMS	20	2	8
12	CC1	Control	54	1	12
13	CC1	Control	44	3	10
14	CC1	0.6 per cent EMS	20	1	4

The evaluation of qualitative traits showed that there are variations in petiole colour stem colour and emerging leaf colour in both the genotypes as presented in Table 52 and Table 53.

In Sree Jaya, the wild type was having petiole colour purple while in the mutated plants 18 percent were light red, 27 per cent yellowish green, 45 per cent pink and 9 per cent red coloured petiole. Stipule colour changed from light green of the wild type to 36 per cent purplish green of mutated plants while 64 per cent had light green stipule colour. Emerging leaf colour in the wild type was purplish green, while, the mutated plants showed variations as 55 per cent green and 18 per cent light green.

Table 52. Evaluation of qualitative traits of mutagen treated *in vitro* derived plantlets of Sree Jaya under pad and fan green house

Trait	Genotype	Category	Mutagenic treatment	No. of plants	Per cent distribution
1. Stipule colour	Mother plant	0. Light green	_	_	_
	Mutants	1. Light green	0.3 EMS, 1.2 EMS	7	64
		2. Purplish green	1.2 EMS	4	36
		Total		**11**	**100**
2. Petiole colour	Mother plant	0. Purple	_	_	_
	Mutants	1. Light red	0.3EMS	2	18
		2. Yellowish green	0.3EMS	3	27
		3. Pink	1.2EMS	5	45
		4. Red	1.2EMS	1	9
		Total		**11**	**100**
3. Emerging leaf colour	Mother plant	0. Purplish green	_	_	_
	Mutants	1. Purplish green	1.2EMS	3	27
		2. Light green	1.2EMS	2	18
		3. Green	0.3EMS, 1.2EMS	6	55
		Total		**11**	**100**
4. Stem	Mother plant	0. Light brown	_	_	_
	Mutants	1. Light brown	1.2EMS	4	36
		2. Brown	1.2EMS	1	9
		3. Orange	0.3EMS	2	18
		4. Yellow	1.2EMS	1	9
		5. Green yellowish	0.3EMS	1	9
		6. Green	0.3EMS	2	18
		Total		**11**	**100**

In wild type stem colour was light brown, while the mutated plants showed variations as 36 per cent of plantlets light brown, 18 per cent orange, 18 per cent green, 9 per cent greenish yellow, 9 per cent yellow and 9 per cent green.

In case of CC1, the petiole colour in the wild type was pink, while the tissue culture derived plants showed variations as 67 per cent reddish green and 33 per cent red. Stipule colour showed variations as 33 per cent green and 33 per cent purplish green, where the wild type had light green petiole. Emerging leaf colour in the wild type was purplish green, while the tissue culture derived plants had 67 per cent green and 33 per cent purplish green emerging leaf. Lanceolate leaf shape of the wild type it showed variation in 33 per cent plants as ovoid in shape.

Golden stem colour of the wild type was changed to 33 per cent orange, 33 per cent brown and 33 per cent greenish brown.

Table 53. Evaluation of qualitative traits of mutagen treated *in vitro* derived plantlets of CC1 under pad and fan green house

Trait	Genotype	Category	Mutagenic treatment	No. of plants	Per cent distribution
1. Stipule colour	Mother plant	0. Light green	_	_	_
	Control or Mutant	1. Light green	0.6EMS	1	33
		2. Purplish green	Control	1	33
		3. Green	Control	1	33
		Total		**3**	**100**
2. Petiole colour	Mother plant	0. Pink	_	_	_
	Control or Mutant	1. Redish green	Control, 0.6EMS	2	67
		2. Red	Control	1	33
		Total		**3**	**100**
3. Emerging leaf colour	Mother plant	0. Purplish green	_	_	_
	Control or Mutant	1. Purplish green	Control	1	33
		2. Green	Control, 0.6EMS	2	67
		Total		**3**	**100**
4. Leaf shape	Mother plant	0. Lanceolate	_	_	_
	Control or Mutant	1. Lanceolate	Control	2	67
		2. Ovoid	0.6EMS	1	33
		Total		**3**	**100**
5. Stem colour	Mother plant	0. Golden	_	_	_
	Control or Mutant	1. Brown	Control	1	33
		2. Orange	Control	1	33
		3. Greenish brown	0.6EMS	1	33
		Total		**3**	**100**

Discussion

5 DISCUSSION

Cassava (*Manihot esculenta*, Crantz) is a popular crop in tropical and subtropical regions around the world. It constitutes the cheapest source of carbohydrates due to its less demanding agronomical requirements in terms of labour and fertilization. Cassava tubers are commonly consumed after boiling, or processed to obtain large array of by-products.

The CIAT (Centro Internacional de Agricultura Tropical, Colombia), IITA (International Institute of Tropical Agriculture, Nigeria) and CTCRI (Central Tuber Crop Research Institute, India), have contributed largely in dissemination of technical knowledge on cassava. This has promoted the exploitation of the crop, especially for industrial purposes (Cock, 1985; CTCRI, 2013).

Wide array of variability of traits are fuelled by hybridization and spontaneous mutation, from which both natural and farmers' selection can act for crop evolution and adaptability to both environmental conditions and farmers' preference. However, due to difficulties in flowering in most cassava genotypes, the potential of both natural and artificial hybridization in generating variability is highly reduced. Thus, the induction of variability through mutations, either *in vivo* or *in vitro* conditions, becomes critically important in the crop. Hence, in the study an attempt has been made to create variability through *in vitro* mutagenesis, on assessing the existing variability in short duration cassava genotypes. The results of the study are discussed hereunder.

5.1 Assessment of variability in short duration cassava genotypes

The genetic improvement of the traits of any crop depend on three important aspects, *viz*., the propagation method of the crop, the existing genetic variability associated with those particular traits, and the selection method chosen to act on available genetic variability. For this reason, the available genetic variability of the traits, the inter-relation among them and their heritability become important factors for the choice of the most suitable breeding method for a particular crop.

Researchers exploit quantitative traits through biometrical techniques to improve the traits genetically at biometrical, biochemical or organoleptic levels.

5.1.1 Variability in qualitative traits

Qualitative traits are not influenced by the environment, therefore are useful to identify and distinguish genotypes under field conditions. The results on the qualitative traits indicated that the various genotypes studied were distinct from each other. The study also confirmed that there was no duplication in genotypes among the germplasm collected, because all the genotypes were distinctly different from each other. The absence of duplications in the cassava core collection increases the efficiency of germplasm management and use (Ng and Ng, 2002). High variability was observed with respect to petiole colour and root cortex colour (Plate 3).

It can be concluded that the collected genotypes constitute an excellent collection of variability and as a result all the genotypes are worth for biometrical evaluation. Similar result was obtained by Mezette *et al*. (2013), where the stem habit was the less variable morphological trait but the petiole colour and the root cortex colour were the most variable. The practical use of morphological traits reside on their usefulness as a tool for preliminary evaluation of germplasm, hence, they are used as a fast approach for assessing the extent of diversity present among the genotypes. Additionally, the presence of high level of morphological diversity among the genotypes provides a basis for crop improvement through hybridization and selection (Lekha *et al*., 2011).

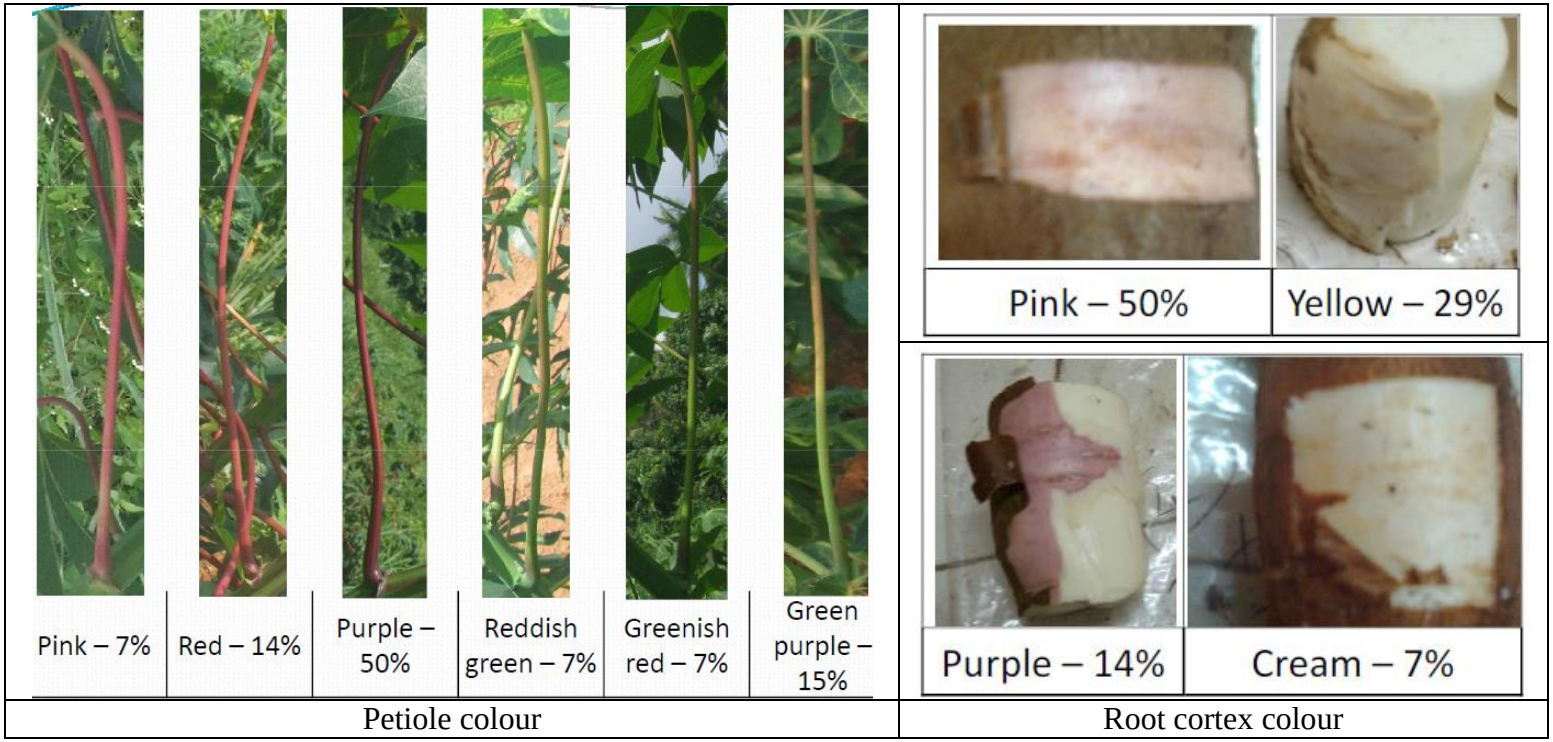


Plate 3. Variability of qualitative traits in short duration cassava genotypes

5.1.2 Biometrical traits

5.1.2.1 Genetic variability

Analysis of variance revealed existence of significant differences among the genotypes for all the biometrical traits studied. This confirmed that the selected germplasm is genetically diverse and appropriate for the study.

In the present study, variety Sree Jaya from CTCRI and genotype CC1 collected from farmers' field in Thavannur, Malappuram district were the best yielding genotypes with a yield performance of 34.92 and 33.13 t ha^{-1}, respectively (Figure 2). CC1 also had the longest tubers of 47.31 cm and the highest tuber girth of 52.58 mm. According to Suja *et al*. (2010), tuber yield of high yielding short duration cassava types ranges between 27.71 – 30.31 t ha^{-1}. Hence, genotypes CC1 and CC2, the farmer varieties having yield more than 28 t ha^{-1} falls to the high yielding short duration category.

However, varieties Sree Prakash and Vellayani Hraswa performed poorly, with about 14.82 and 15.33 t ha^{-1}, respectively, in comparison to reported potential yield (Suja *et al*., 2010) of 27.71 and 29.81 t ha^{-1} respectively, the results also suggested better yield stability of Sree Jaya over the other genotypes across locations and seasons.

The range of variability for other biometric traits of the genotypes evaluated in this study was comparable to the values indicated by Alves (2002). The range for plant height was between 224.40 and 254.15 cm, and the stem girth 21.01 and 24.62 mm. The number of tubers per plant ranged from 3.77 to 6.08, tuber fresh yield, 1.71 to 2.68 kg, tuber length, 34.24 to 45.39 cm and tuber girth, 38.44 and 45.67 mm.

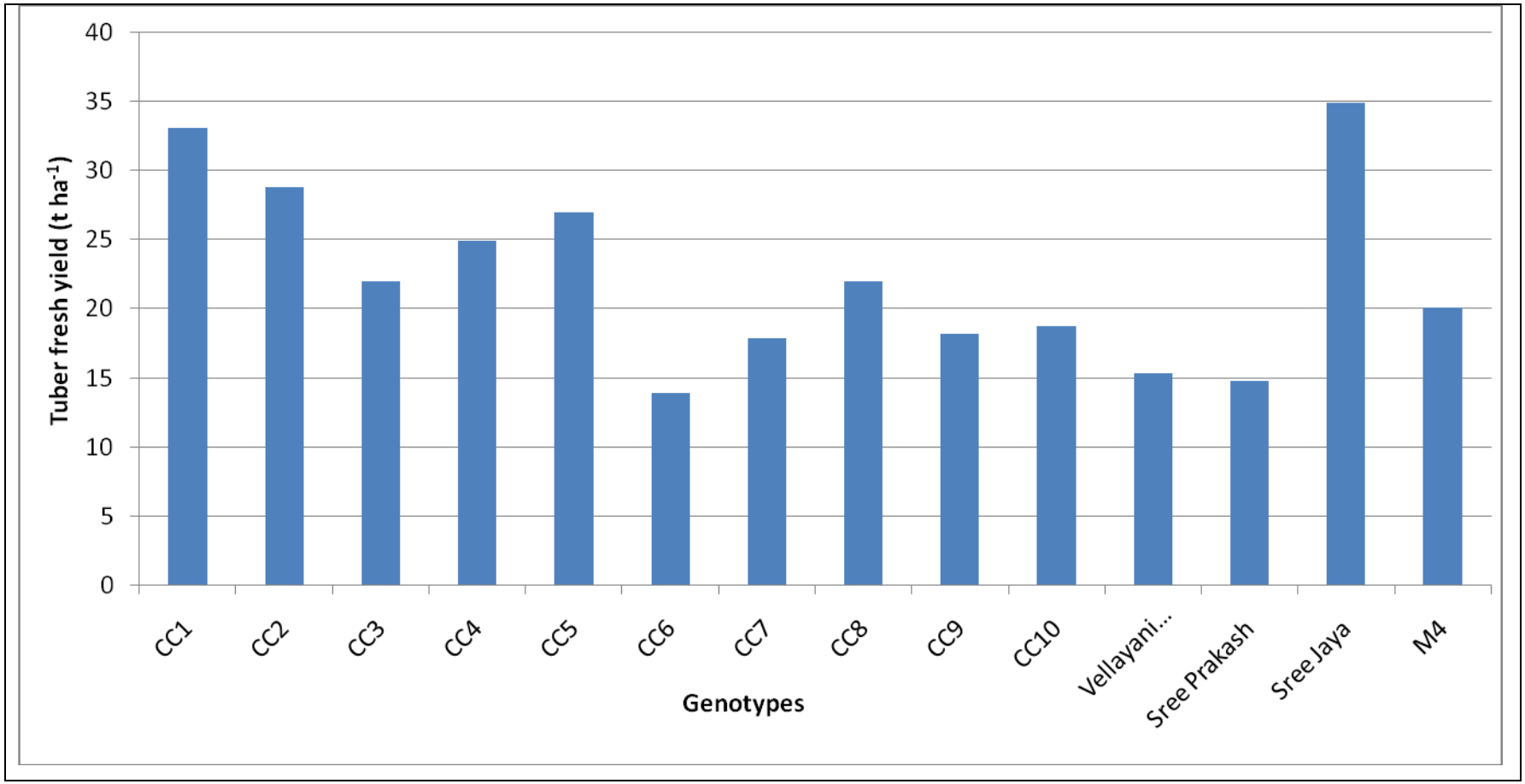


Figure 2: Tuber fresh yield of short duration cassava genotypes

Lebot (2009), considered the ideotype of cassava as a genotype producing one stem per cutting, *i.e.*, preferably non branching type, with short internodes, with harvest index more than 0.50, plant height less than two meters and tuber numbers of eight per plant. Considering the above, CC8, Hraswa, CC10 and Sree Jaya fit most of the criteria of an ideal plant type in cassava. Although the ideotype cassava plant is controversial, it constitutes a useful indication and can be applied to carry out the preliminary selection among genotypes.

5.1.2.2 Heritability, genetic advance and genetic gain

The genetic parameters such as genotypic coefficient of variation (GCV), heritability (H^2) and genetic advance (GA) constitute a reliable guideline for selection.

A valid basis for comparing and assessing the range of genetic diversity for quantitative trait is provided by the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) which measures the extent of total variability. In general, the estimates of PCV were higher than GCV indicating the effect of environment on yield and yield attributes. The difference between the PCV and GCV estimates were low in case of plant height, stem girth and tuber girth indicating predominance of genetic factors controlling variability in these traits i.e., these attributes are less influenced by environment.

All traits, except plant height, stem girth, tuber length, tuber girth and harvest index registered moderate PCV and GCV estimates, indicating presence of ample variability among the genotypes studied and the possibility of improvement through simple selection. High magnitude of variability in cassava has been reported by Naskar *et al.* (1991), Kumar *et al.* (1996), Alam *et a*l. (1998) and Chaudhary *et al.* (1999).

Cassava, being an asexually propagated crop, all the genetic variability can be exploited between asexual generations by means of selection. Thus, the estimation of heritability in broad sense (H^2) is more meaningful (Hogarth, 1971). In the present study high heritability, was exhibited by plant height, stem girth and

score for CMD (Figure 3), indicating that selection would be effective in improving these traits. Heritability studies by Asante and Dixon, 2002, has established that tuber root numbers, tuber weight and fresh tuber yield are under genetic control.

It has been suggested by Burton and DeVane (1953) that the GCV along with heritability estimate could provide a better picture of the amount of advance to be expected by phenotypic selection. Branch height, branch numbers, susceptibility to CMD and CLS had registered high genetic advance as per cent of mean (Figure 3), indicating that improvement could be expected if judicious selection is exercised. However, moderate genetic gain was observed for economic traits tuber number, fresh weight of tubers and tuber yield implying influence of both additive and non-additive gene action on expression of these traits.

5.1.2.3 Correlation of biometrical traits with tuber yield

Correlation helps in determining the relationship of yield with its components which in turn is used to select superior genotypes from diverse genetic populations. The nature and extent of those relationships, their direction and intensity at genotypic level provide a reliable measure to distinguish critical associations between traits to be exploited for breeding purposes (Falconer, 1981).

In the present investigation, all traits except tuber neck, scar number, branch number and internode length were significantly associated with the tuber fresh yield at genotypic level (Figure 4). Dry matter, biomass, tuber number, tuber length, tuber girth, harvest index, CLS score, plant height, branch height and stem girth were positively correlated with tuber fresh yield. This is in conformity with studies of Biradar *et al*. (1978). Asante and Dixon (2002), found that the correlation between economically important traits, *viz*., root number, root weight and root fresh yield was highly significant and positive.

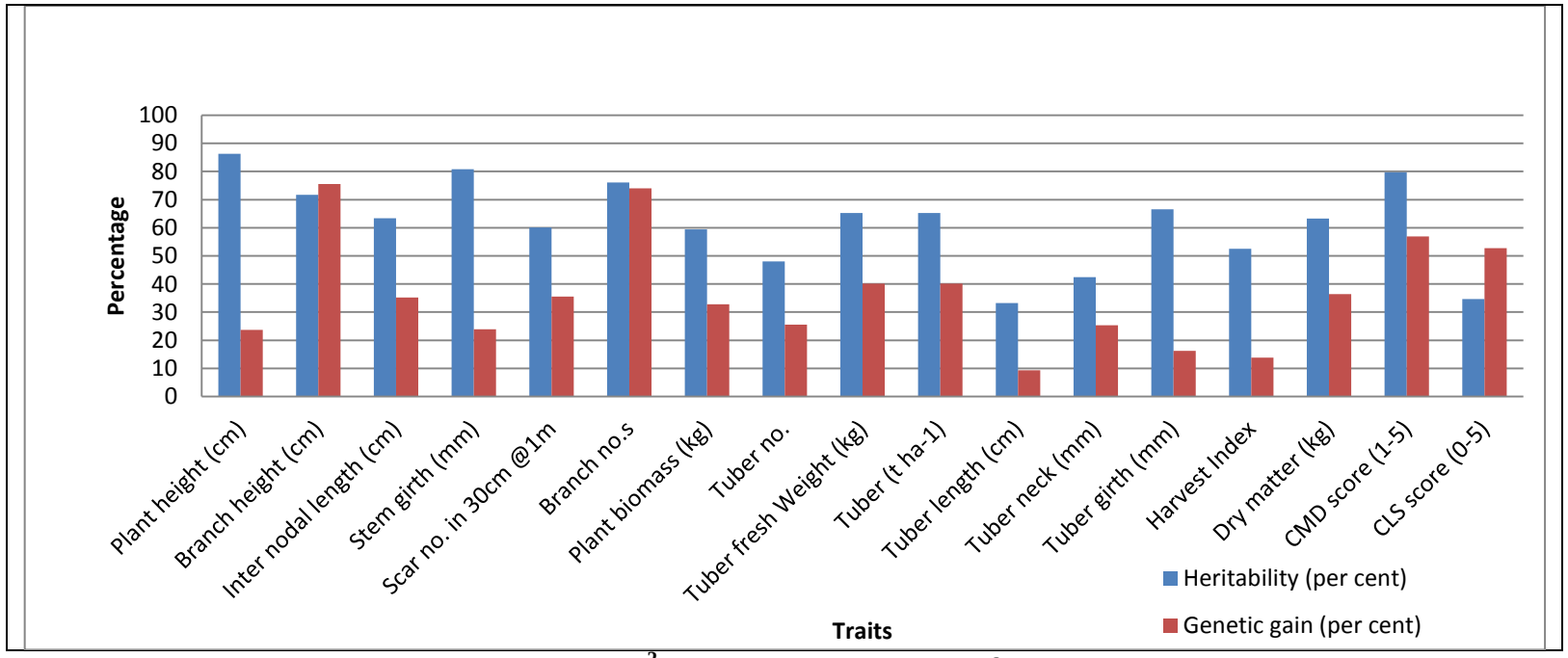


Figure 3: Heritability in broad sense (H^2) and genetic gain (GG) of short duration cassava genotypes

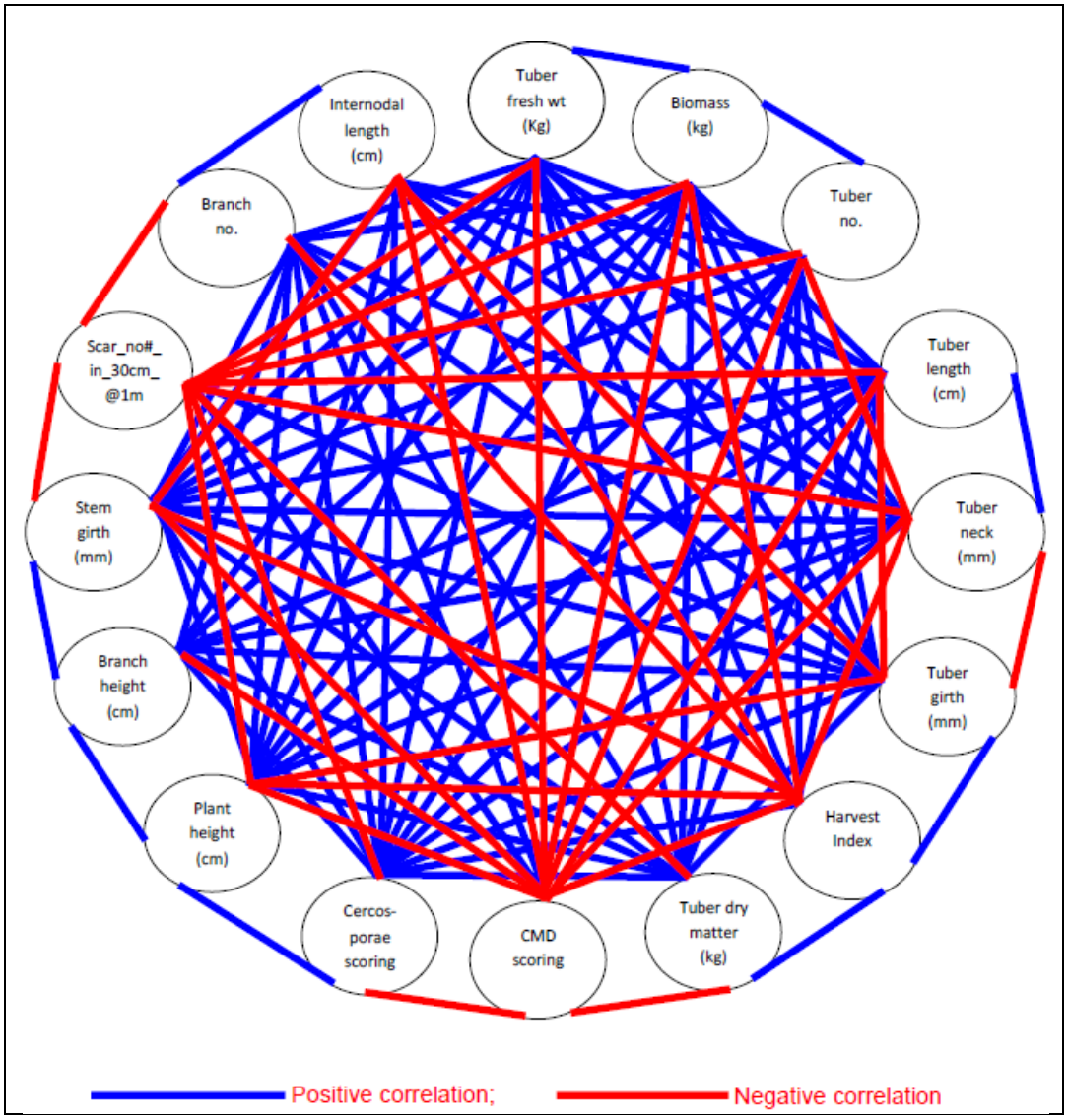


Figure 4: Genotypic correlation of biometrical traits with tuber yield in cassava

According to Padma *et al*. (2009), tuber yield was positively correlated with plant height, tuber weight and tuber width. Fresh root yield in cassava was positively correlated with canopy mass, number of storage roots per plant, plant height and HI. Mulualem and Dagne (2013), observed that the genotypic correlation between fresh root yield and number of vertical stem per plant, root girth, root diameter, weight of above ground plant parts and root dry weight was highly significant, while positive and significant phenotypic correlation was observed between root fresh yield per plot, root girth, root length and diameter.

Interrelationship of traits helps to develop suitable selection criteria for the improvement of complex traits. The study indicated that improvement in yield can be attempted by selecting genotypes with high dry matter content of the tuber, above ground biomass, plant height, tuber number, tuber length and harvest index.

5.1.2.4 Path analysis

Correlation studies are helpful in measuring the association between yield and yield components but they do not provide a clear picture of the direct and indirect causes of such associations. Path coefficient analysis is very much useful in identifying the important yield components which can be utilized for formulating selection parameters.

In the present study, path coefficient analysis was performed considering eleven yield components which were significantly correlated to the tuber fresh yield at genotypic level. The residual effect of path analysis was very low (10.74 per cent), indicating that 89.26 per cent variation in tuber fresh yield was contributed genotypically by the eleven tuber fresh yield components selected for path analysis.

The highest direct contribution to tuber yield was through above ground biomass (0.56) followed by dry matter content. Even though the dry matter has the maximum positive genotypic correlation with yield its direct effect is only second in position indicating that it may have indirect effect on yield through other traits. Dry matter and upper ground biomass are the most influential

characters in increasing tuber fresh yield (Radhakrishnan and Gopakumar, 1984; Rekha *et al.*, 1991; Kumar *et al*., 1996 and Mohankumar *et al.,* 1990).

The next important trait which had the maximum direct effect (0.40) as well as significant positive correlation (0.53) with tuber yield was harvest index, indicating that this character should be taken into account for fresh tuber yield improvement in cassava. The effect of this trait in determining the tuber yield was reported by Rekha *et al.* (1991) in cassava. The plant height had direct and negative effect (-0.10) on tuber yield and it was genetically positively correlated to tuber yield (0.46).

Correlation and path analysis studies conducted in the present investigation revealed that for tuber fresh yield improvement programme in cassava, the breeder should give emphasis on high upper ground biomass, high harvest index, and high dry matter along with optimal plant height. Biotic stresses, such as CMD and CLS should be absent or minimized.

5.1.2.5 Clustering

The extent for improvement in any crop depends on mainly the extent of genetic variability in the population. Hence, the assessment of divergence in the population is of immense importance. Squared Euclidean distances statistics is found to be a powerful tool in the hands of the plant breeder to assess the degree of dissimilarity among the genotypes and consequently to group them based on their phenotypic expression. Squared euclidean distances employing a combined classification approach in respect of selected characters revealed that at rescaled inter cluster distance of five, the genotypes studied can be grouped into five clusters. Few genotypes belonging to same location segregated into different clusters (e.g. CC3 and CC4 from Kottarakkara) while, certain genotypes habituating different locations grouped into the same cluster (e.g. CC1 and CC7, from Thavannor and Chittur, respectively). The clusters accommodating maximum number of accessions (I and II) comprised of genotypes belonging to different districts or eco-geographical conditions (seven and four genotypes,

respectively). This indicated that, factors other than geographical diversity has contributed to genetic diversity and that there is no parallelism between geographic distribution and genetic diversity. A similar observation of non-correspondence of genetic divergence with geographical distribution is reported by Roy and Panwar (1993) and Mannan *et al*. (1993) in colocasia. According to Raut *et al*. (1980) genetic drift and human selection could cause greater diversity than geographic distance.

Clusters II, IV and V had only one genotype each (CC4, CC8 and M4, respectively). Polarisation of genotypes collected from different districts can also be observed from the clustering. Murthy and Arunachalam (1966) explained that such a wide adaptability is possible due to reasons such as heterogeneity, genetic architecture of the populations, past history of selection, developmental factors and degree of combining ability.

At rescaled distance of 15 and 20 there were only two clusters the first cluster consisted of genotypes CC7,CC9,CC5,CC2,CC3,CC1, Sree Jaya ,CC4,and M4. The second cluster consisted of CC10, Hraswa, CC6, Sree Prakash, and CC8. This also indicate that the superiority of farmer varieties falling in to the same cluster as that of varieties.

Estimates of inter and intra cluster distances also supported the above conclusions. The statistical distance represents the index of genetic diversity among clusters. The greater the distance between them the wider will be the genetic distance between the genotypes. The dendrogram of this genetic diversity obtained using the average linkage between groups is shown in Figure 5.

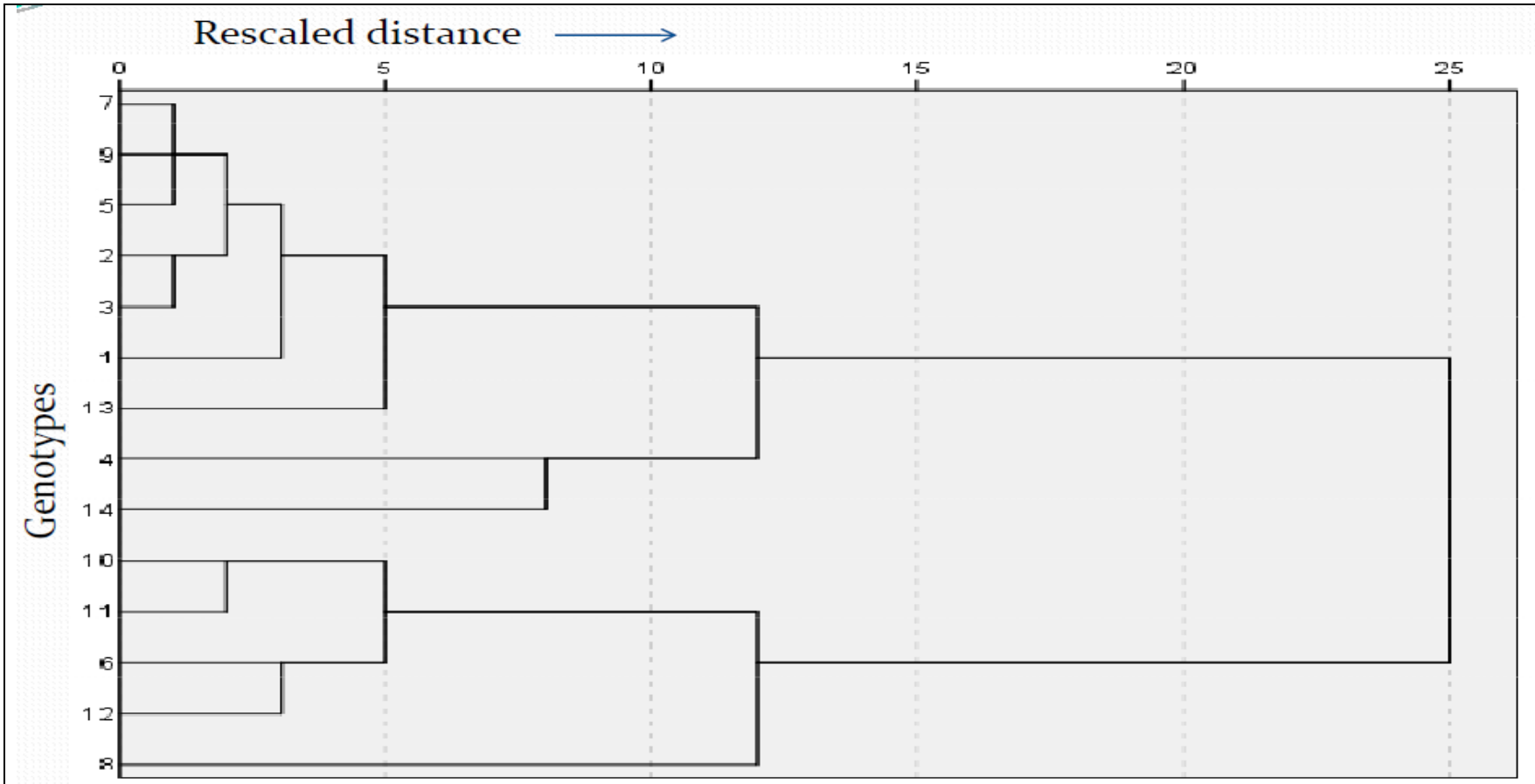
Maximum intra cluster distance was seen in cluster I, indicating that there is considerable variability among the genotypes of this cluster (namely CC1, CC2, CC3, CC5, CC7, CC9, Sree Jaya). Inclusion of farmers' genotypes along with the best variety (Sree Jaya) in the cluster indicated that the farmers' varieties are on par with respect to the traits under consideration. According to Angadi (1976) varieties in a cluster with higher order of divergence among themselves would be

the best breeding material for achieving maximum genetic advance with regard to yield. Hence, selection within a cluster might also be exercised based on the highest mean performance of the genotypes for desirable traits such as fresh tuber yield, dry matter, harvest index and plant height.

5.1.3 Biotic stresses

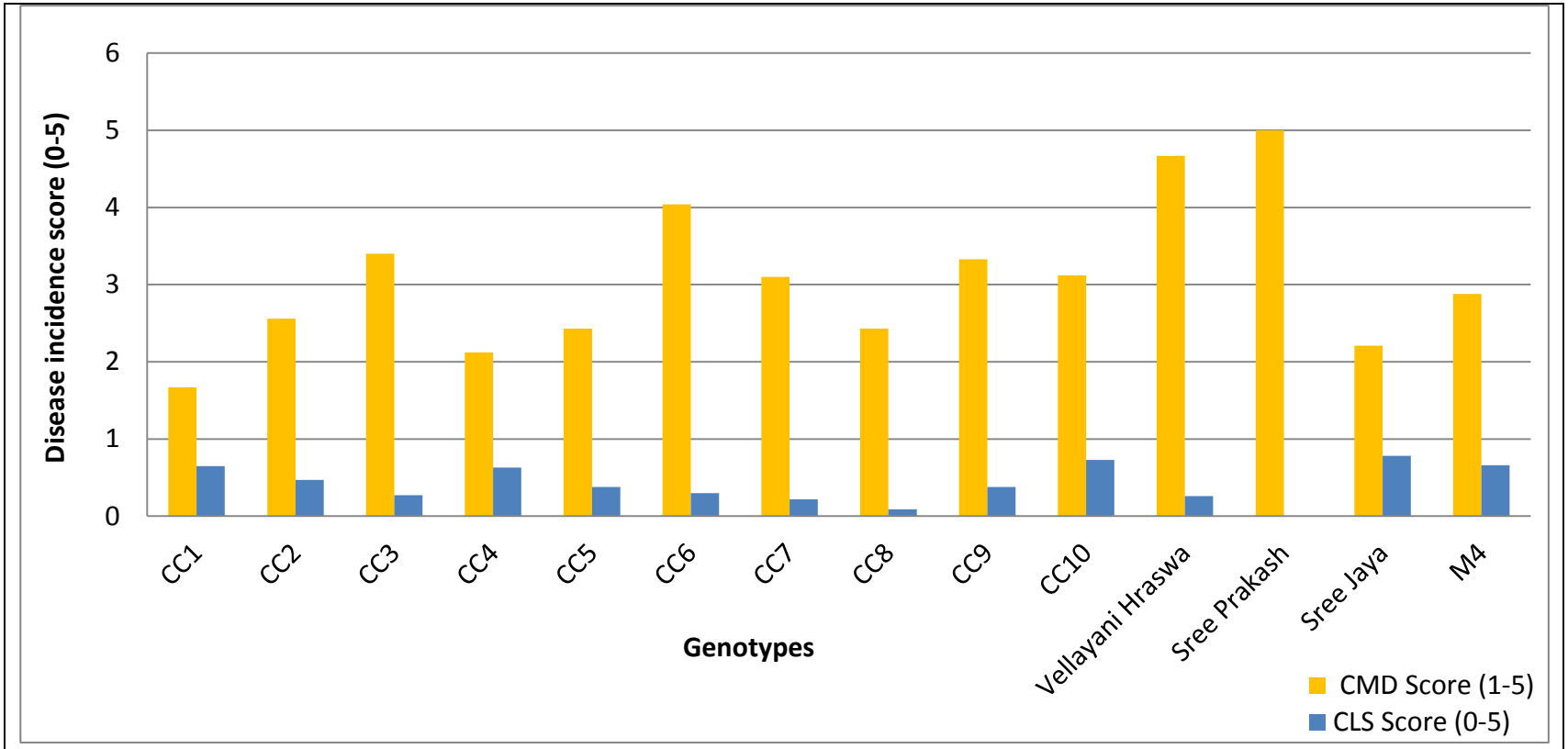
Cassava genotypes can be grouped as highly resistant - HR (score ≤ 1.00), moderately resistant – MR (score: 1.00 to 2.00), susceptible – S (score: 3.00 to 4.00), and highly susceptible – HS (score≥ 4.00) based on their susceptibility to Cassava Mosaic Disease (CMD), (Asare *et al*., 2014). Only one genotype CC1 had less score value of less than two for CMD which can be classified as moderately resistant, while genotypes Sree Jaya, M4, CC2, CC3, CC5, and CC8 had score value less than three but more than two which can be considered as moderately susceptible (Figure 6). However, the genotypes Sree Prakash, Vellayani Hraswa and CC6 were found to be highly susceptible (Plate 4).

As indicated by CTCRI (2006) and KAU (2011), the tuber fresh yield potential of Sree Prakash and Vellayani Hraswa were about 35.00 and 45.00 t/ha, respectively. However, in this study only about half of this yield potential was attained, *i.e.* 15 t/ha. This can be due to the higher susceptibility of these genotypes to CMD. This finding is in conformity with the results of Jose *et al*. (2011), which indicated that the major negative influence on tuber fresh yield in Kerala is exerted by mosaic disease of susceptible cassava genotypes. Thus, resistance/tolerance to CMD should always be emphasised in cassava breeding programmes. However, in contrast to CMD, occurrence of Cercospora Leaf Spot (CLS) had less negative impact on tuber yield.



Legend: 1 - CC1; 2 - CC2; 3 - CC3; 4 - CC4; 5 - CC5; 6 - CC6; 7 - CC7; 8 - CC8; 9 - CC9; 10 - CC10; 11 - Vellayani Hraswa; 12 - Sree Prakash; 13 - Sree Jaya; 14 - M4

Figure 5: Dendrogram of genetic divergence in cassava genotypes - using Average Linkage Between Groups



CMD – Cassava Mosaic Disease; CLS – Cercospora Leaf Spot

Figure 6: Biotic stress incidence of short duration cassava genotypes

Plate 4: Incidence of Cassava Mosaic Disease on Sree Prakash cassava genotype

5.1.4 Selection index

The concept of selection index (SI) can be used for identification of the best genotypes in breeding programmes. In the present study SI was calculated as suggested by Ojulong *et al* 2010 based on fresh root yield, dry matter content, harvest index, score for CMD, CLS and tuber girth are given in the section 3.1.3.5. An SI value of 120 and above was found to be preferential.

Genotypes CC1, Sree Jaya and CC2 had an SI of 143.08, 126.58 and 120.52, respectively (Figure 7). Based on the present study, the CC1 which is a farmers' cultivar from Thavannur of Malappuram district was found to the best genotype with respect to yield and resistance to CMD. CC1 had the thickest tuber, highest harvest index, highest tuber numbers and lowest CMD score, and high CLS score. CC1 was found to be superior to the released varieties.

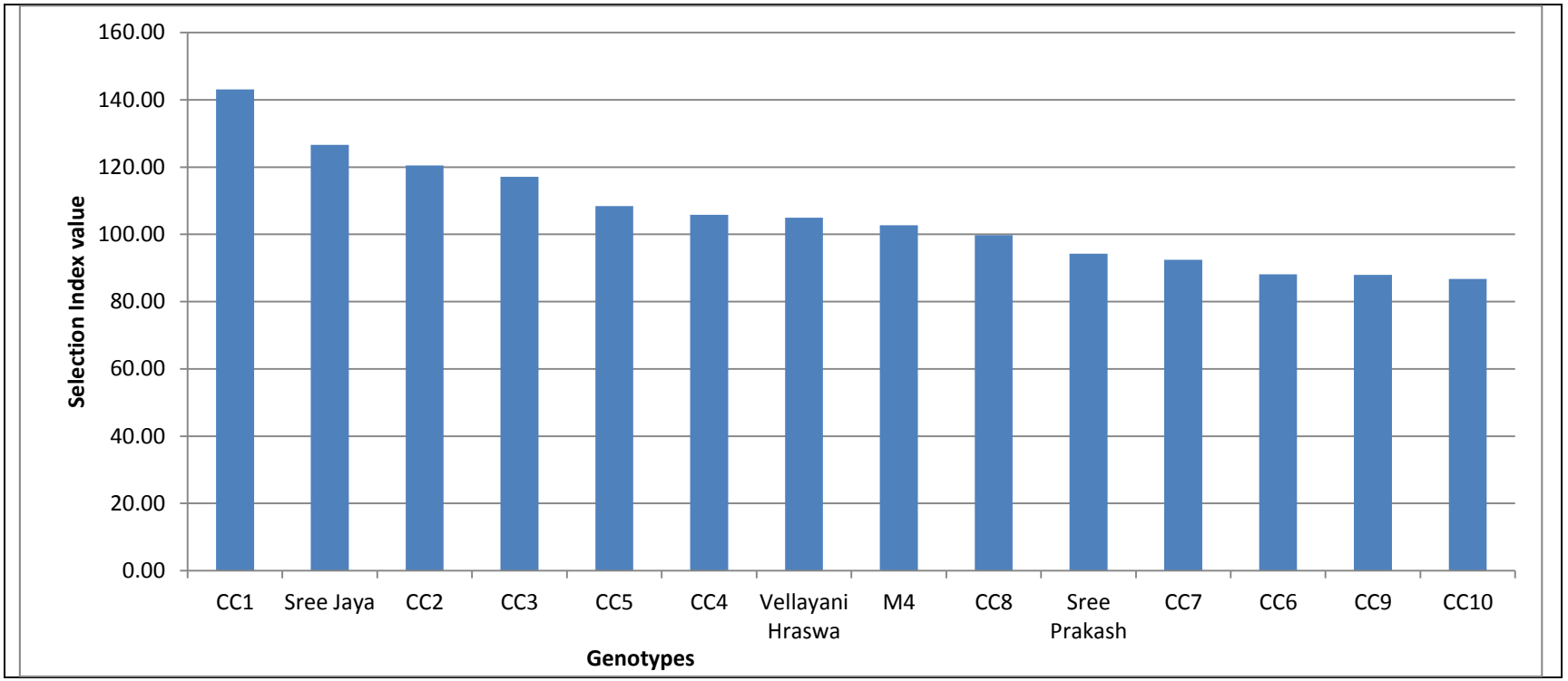


Figure 7: Selection index (SI) of the evaluated short duration cassava genotypes

According to Suja *et al*. (2010), short-duration varieties of cassava provides opportunities to smallholder farmers for effective utilisation of resources such as land, moisture and nutrients as well as diversification of enterprise and income. Hence, the accession CC1 could be used for direct cultivation after further evaluation. Based on the evaluation of genotypes by applying the concept of SI the best two genotypes CC1 and Sree Jaya (Plate 5) were chosen for mutation studies to induce further variability.

5.1.5 Biochemical traits

The biochemical traits were evaluated on dry weight basis, using peeled tuber sample dried in oven. The values of biochemical traits are illustrated in Figure 8. The study indicated the occurrence of high starch genotypes (Sree Jaya, CC10 and CC7 with (87.86 per cent, 84.24 per cent and 87.72 per cent, respectively) in short duration cassava genotypes. According to Alves (2002), varieties with more than 80 per cent of starch can be recommended for industrial starch production. Variety Vellayani Hraswa with lower starch content (56.51 per cent) is more suited for food and feeding purposes.

The varieties having amylose higher than 21.00 per cent are commonly of good cooking quality and highly recommended for food purposes (Jennings and Iglesias, 2002). The amylose content of most genotypes except CC4, CC6, CC7, CC8, Vellayani Hraswa was above 23.00 per cent. The genotypes, Sree Jaya, CC7 and CC4 having amylose content of around 18 per cent, are found suitable for industry, food and feeding purposes. Genotypes having less than 15.00 per cent amylose is considered as waxy variety (Jennings and Iglesias, 2002). Vellayani Hraswa with low starch (56.51per cent) and low amylose content of 12.85 per cent can be recommended for food and feed as well as for starch extraction industries. Because the lower the amylose content better is its acceptability in food industry as “thickner”.

Emerging leaf	Purplish green	Central lobe shape	Lanceolate
Petiole colour	Pink	Root external	Light brown
Stem exterior	Golden	Root pulp colour	Cream
Stipule colour	Light Green	Root cortex colour	Pink

CC1

Emerging leaf	Purplish green	Central lobe shape	Lanceolate
Petiole colour	Purple	Root external	Dark brown
Stem exterior	Light Brown	Root pulp colour	White
Stipule colour	Light green	Root cortex colour	Purple

Sree Jaya

Plate 5: Plant and tuber traits of the best cassava genotypes

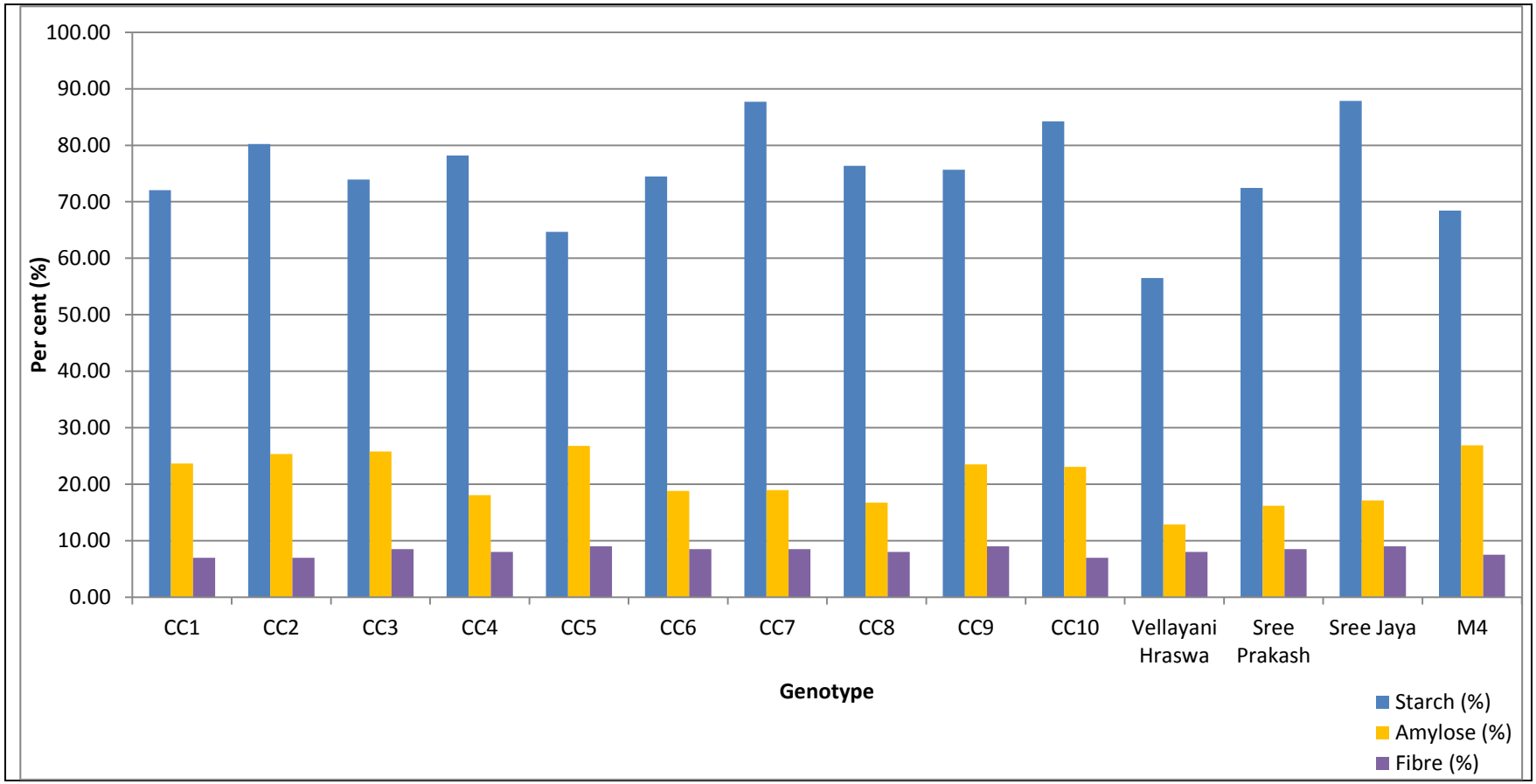


Figure 8: Starch, amylose and fibre content on dry weight basis in evaluated short duration cassava genotypes

The fibre content for all the genotypes was low (below 9.00 per cent). Higher values of crude fibre interfere with digestibility, hence is undesirable for both food and feed purposes (Raji *et al*., 2007; Aro and Aletor, 2012). Considering the above, all the evaluated genotypes are found to be suitable for multipurpose uses.

HCN content of CC3, CC8, CC9, and Sree Jaya was below 10.00 mg kg^{-1} on dry weight basis, and hence safe for human consumption as fresh tuber, without further processing or cooking (FAO/WHO, 1991). Genotypes CC1, CC7, and CC10 had high HCN content of more than 30 mg kg^{-1} which is unsafe for direct consumption (Figure 9). Therefore, the tubers of these genotypes can be recommended for consumption only on cooking or processing. Kizito *et al*. (2007) had observed genotypes with HCN content as high as of 1000.00 mg kg^{-1} on dry weight basis. The low HCN content of the genotypes observed in the present study can be attributed to the selection undertaken by the farmers for genotypes with low HCN content.

The most variable biochemical trait was starch content and the least variable was the amylose content. The heritability estimates of all the biochemical traits were high, indicating the high variability as well the amenability for selection for improvement.

The importance of amylose/amylopectin ratio, when is less than one, it means that there is less amylose and therefore is more acceptable in food industry. The higher proportion of long chain (amylopectin) forms longer helical structures with high gelatinization. This confers better shear resistance and higher gel firmness, making it suitable for application of low amylose/amylopectin genotype starches in diverse food industry. This makes cassava starches in par with cereal starches for this peculiar starch quality (Charles *et al.*, 2005; Nuwamanya *et al.*, 2011).

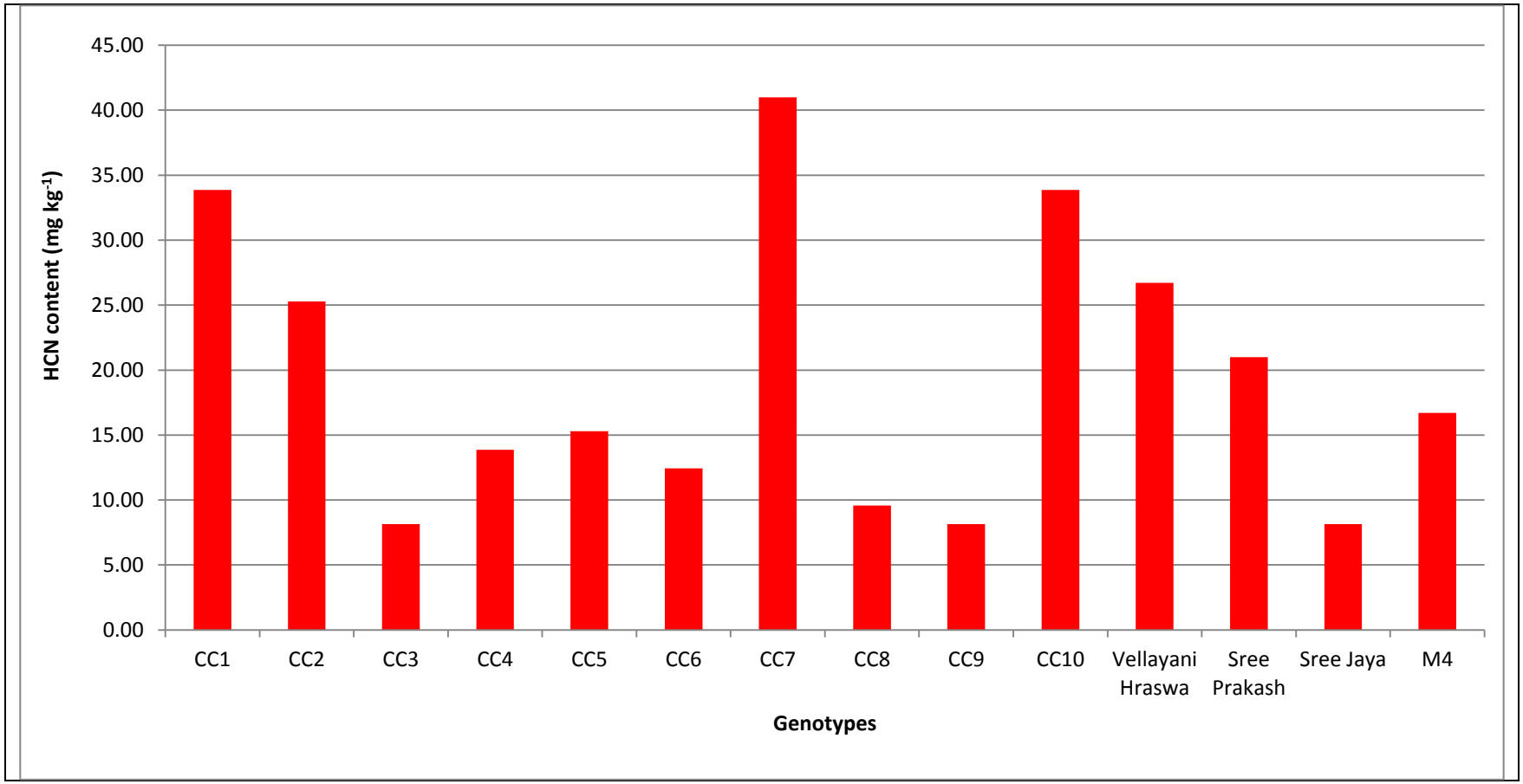


Figure 9: Cyanide content on dry weight basis in evaluated short duration cassava genotypes

From this study, the genotypes Sree Jaya, Sree Prakash, Vellayani Hraswa, CC7 and CC8 showed the lowest amylose/amylopectin ratio (between 0.24 and 0.29). Therefore, are recommended for starch food industry.

5.1.6 Organoleptic traits

The early maturing cassava genotypes were evaluated for organoleptic and chemical properties at six months after planting. Organoleptic evaluation indicated that genotype CC10 collected from farmers fields at Vellanikkara, Trichur district, was the best in terms of easiness in peeling, taste after cooking and cookability with mean scores of 4.56, 4.44 and 4.00, respectively. Sree Jaya, (from CTCRI), was the best with respect to sweetness chewing. It also recorded the highest starch content and the lowest HCN content. According to Frank *et al.* (2011), sweetness on chewing before cooking or taste after cooking is an indication to categorize cassava tubers into sweet or bitter cassava, based on high HCN concentration. However, CC10 the genotype having good score for sweetness on chewing of fresh tubers and good taste after cooking had HCN content as high as 33.86 ppm. Hence, the results are in contrast with the findings of Frank *et al.* (2011) and also suggested that HCN content is not the only factor causing bitterness of cassava tuber.

Considering the organoleptic traits *in toto*, genotype CC10 with a total score of 33.13 was adjudged the best (Figure 10). CC8 and Sree Jaya were the next best indicating the availability of early cassava genotypes with good organoleptic properties in the farmers' collections.

The analysis suggests that the most important quality traits for cassava consumers' preference before cooking are fresh tuber colour (0.53) and chewing sweetness (0.53). The quality traits, after cooking are taste (0.71), texture (0.72) and fibre content (0.62). Those traits are highly correlated with cumulative ranking. Similar results were obtained by Gonzalez & Johnson (2009).

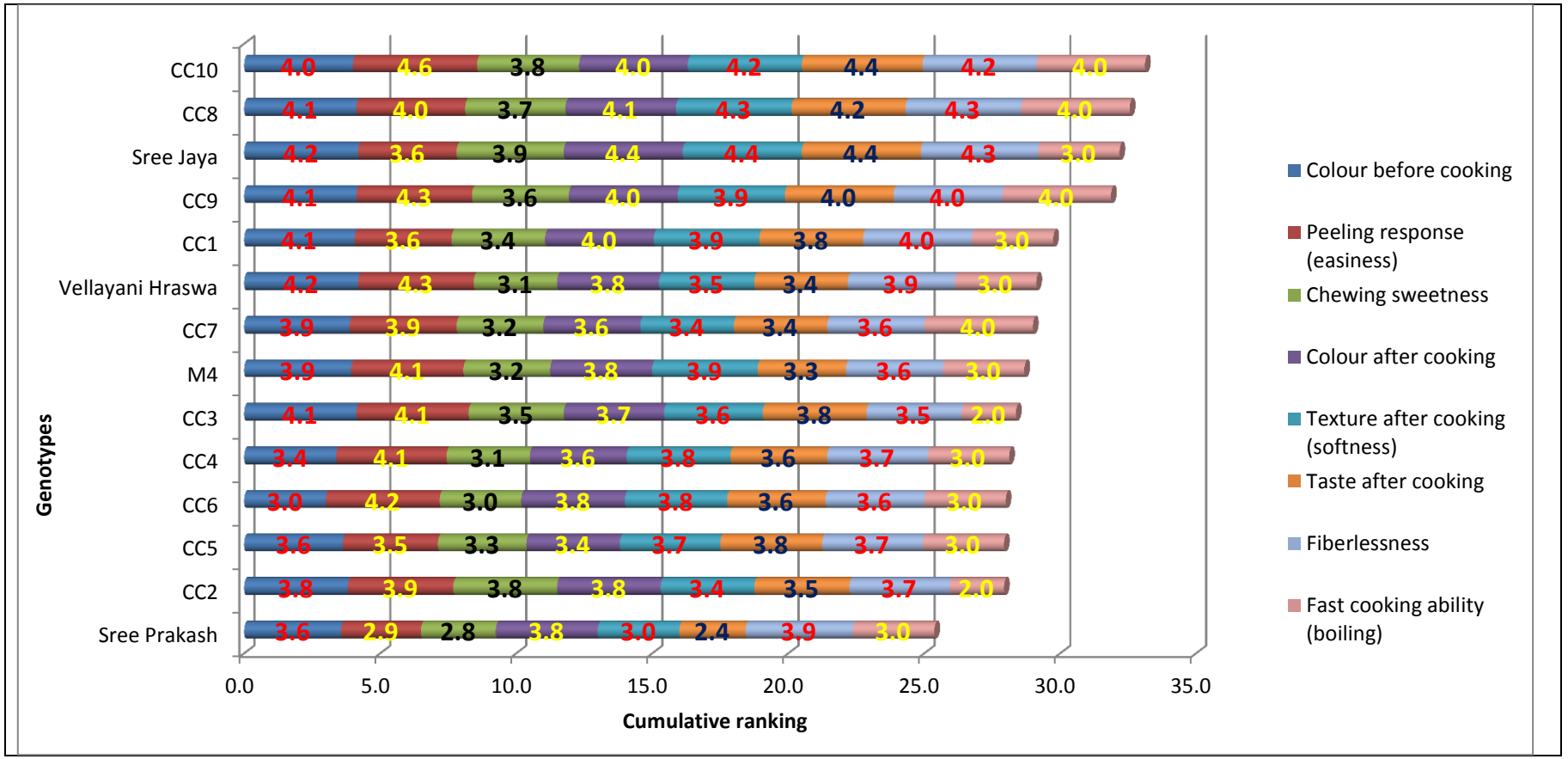


Figure 10: Organoleptic evaluation for short duration cassava genotypes

Additionally, the chewing sweetness before cooking or taste after cooking can be an indication of tuber categorization into sweet or bitter cassava, corresponding to low and high cyanogenic (HCN) concentration, respectively (Frank *et al*., 2011). In the present study, although the chewing sweetness is not significantly correlated to HCN content, the taste after cooking is significant and negatively correlated to HCN (-0.11), supporting similar results from literature (Bayoko *et al*., 2009). However, considering the case of CC10 genotype, one of the tastiest with score of 4.44, its HCN content is high as 33.86 ppm, and then this suggests that HCN content is not the only causing factor of cassava tuber bitterness (King and Bradbury, 1995).

In turn, the crude fibre content is negatively correlated to the chewing sweetness (-0.12), while the organoleptic fibrelessness is positively correlated to it (0.20). This suggests that the organoleptic evaluators esteem the absence of fibre in the tuber flesh to consider its taste (Padonou *et al.*, 2005). Furthermore, starch content is positively genetic correlated to cookability (0.24) and amylose content negatively correlated to it (-0.19). This suggests that high amylose content interferes with cassava tuber cookability (Kouadio *et al*., 2013).

5.2 Standardization of *in vitro* propagation methods in cassava

5.2.1 Propagation through somatic embryogenesis from leaf explants

5.2.1.1 Sterilization of leaf explant

The sterilisation treatment consisting of a pre-sterilization with five per cent Teepol solution for two minutes, sterilization with ethanol (75.00 per cent) for either one or two minutes, followed by treatment of 0.1 per cent mercuric chloride for one minute and chopping of leaf explant after sterilization resulted in survival of all the cultures without contamination or mortality. The result is in confirmation with that of RuiMei *et al*. (2009) with respect to mercuric chloride. Maturity of leaf explants exerted no influence on the effect of sterilizastion.

5.2.1.2 Effect of 2,4-D on callusing of leaf explants in cassava

Effectiveness of 2,4-D on cassava embryogenesis in immature leaf lobes in cassava was reported by Stamp and Henshaw (1986), Szabados *et al*. (1987) and Berhanu (2011). However, in the present study 2,4-D induced callusing response only observed. The concentration of 6.00 to 10.00 mg l^{-1} yielded a mixture of friable embryogenic callus and hard callus. Thus results indicated that 2,4-D at 8 mg l^{-1} (Figure 11) was only useful in producing friable embryogenic callus and the highest callusing response (Plate 6).

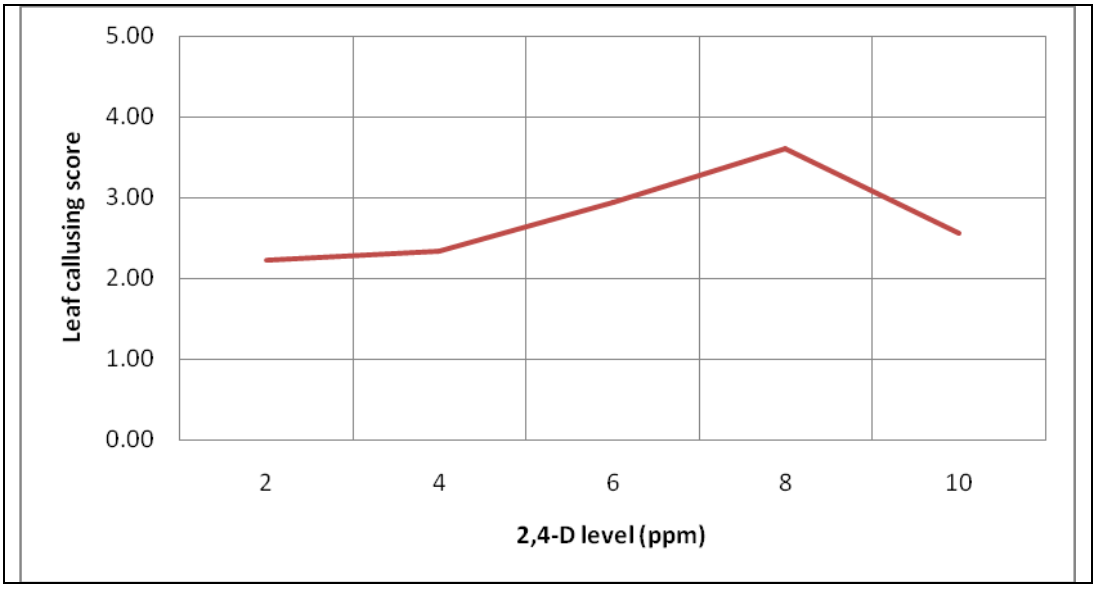


Figure 11: Effect of 2,4-D on leaf callusing of cassava genotypes

5.2.1.3 Effect of light on callusing of leaf explants in cassava

Apio *et al*. (2007) reported the effect of darkness in inducing somatic embryogenesis in cassava. Hence, the treatment effect of complete darkness on callusing was tested against the treatment under light exposure of nine hours per day. However, no significant difference in callusing of leaf explants was observed. Therefore, cultures were maintained under normal tissue culture conditions.

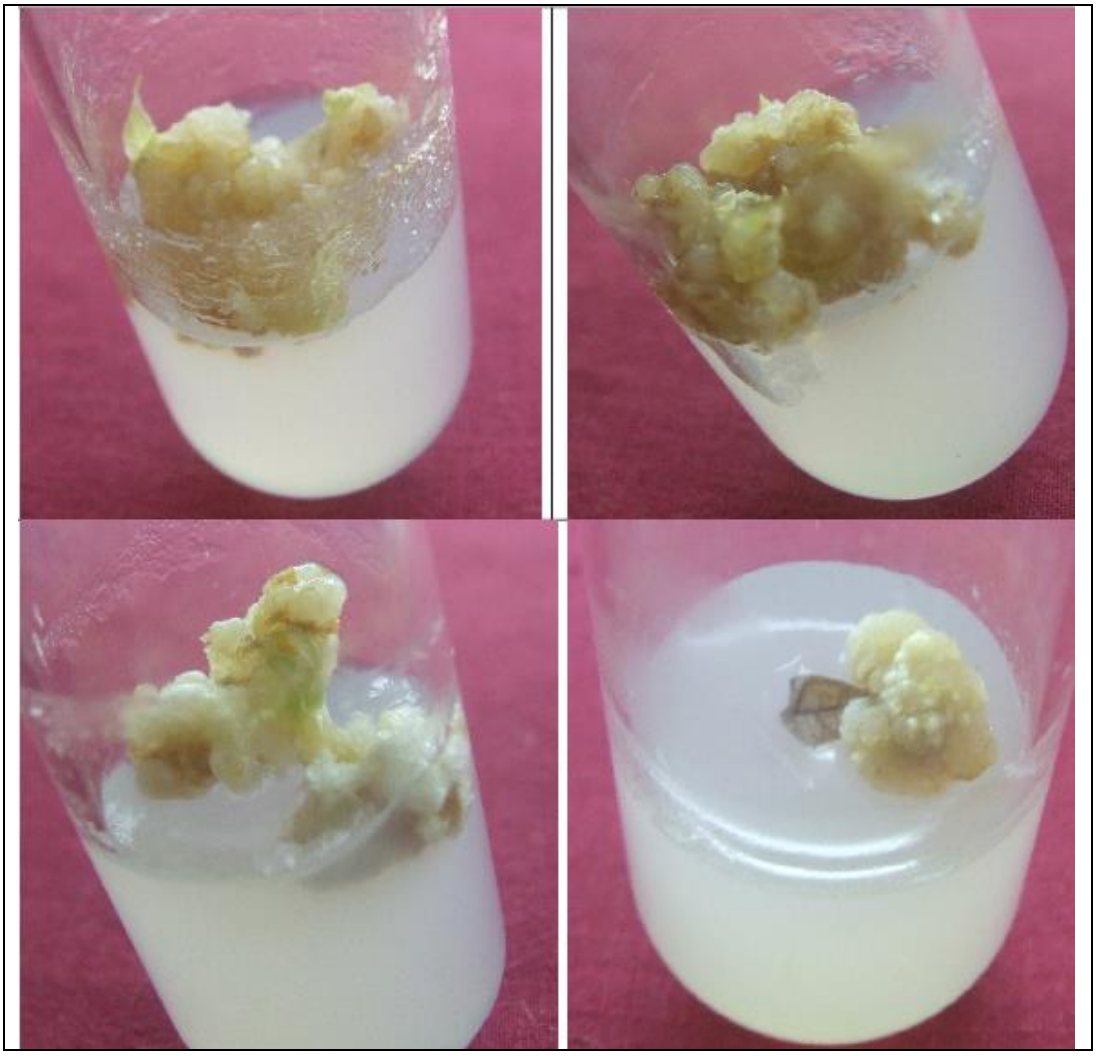


Plate 6: Callus induced in cassava leaf explants through 2,4-D

5.2.1.4 Effect of BA and NAA on somatic embryogenesis in cassava

Sofiari *et al.* (1998), Apio *et al.* (2007), Ibrahim *et al.* (2008), and Berhanu (2011) had reported the beneficial effect of BA and NAA on somatic embryogenesis in cassava. However, no formation of somatic embryo was observed in the 16 combinations of BA and NAA tried in the present study. Conversely, the BA at 1.0 mg l^{-1} level combined with NAA, *i.e.*, 0.10, 0.20, 0.30 or 0.50 mg l^{-1}, resulted in friable embryogenic callus (Figure 12).

5.2.1.5 Effects of Picloram and CuSO4 on somatic embryognesis in cassava

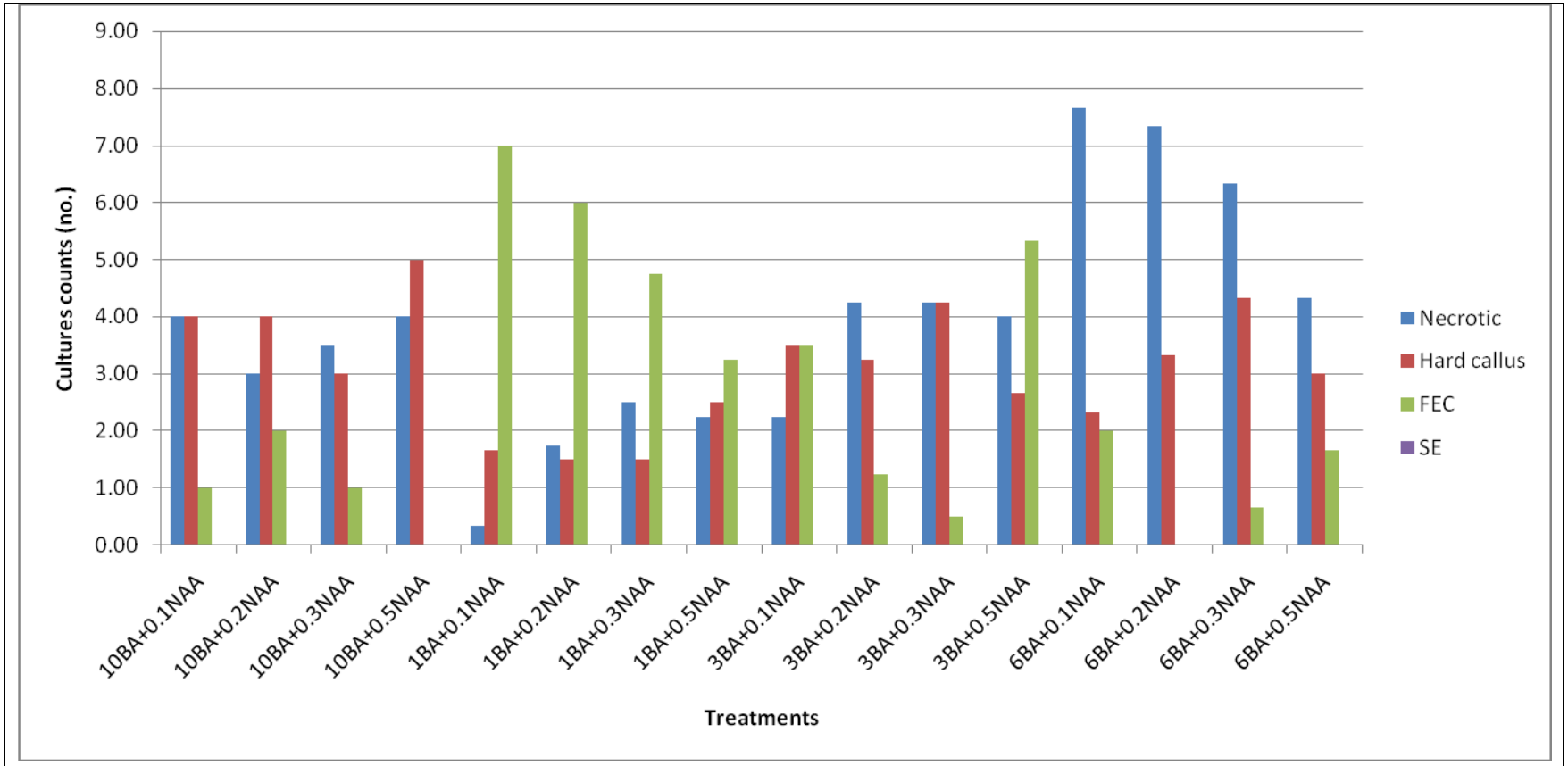
Groll *et al*. (2001), Zhang *et al.* (2001) and Feitosa *et al*. (2007) reported the effect of $CuSO_4$ on induction of both primary and secondary embryogenesis in cassava either on leaf or nodal explants. However, in the study 0.30 mg l^{-1} $CuSO_4$ in combination with 10.00 mg l^{-1} Picloram in MS3 media resulted only in friable embryogenic callus.

5.2.1.6 Effect of Picloram treatments on somatic embryogenesis

Picloram at 8.00 to 10.00 mg l^{-1} incorporated into MS3 resulted in production of somatic embryos from the friable embryogenic callus produced on 2,4-D combination with BA and NAA (Plate 7). Effectiveness of picloram in induction of somatic embryoids in cassava has been reported by earlier researchers (Taylor *et al*., 1996; Raemakers *et al*., 1997; Groll *et al*., 2001; Magaia, 2003 and Kordestani and Karami, 2008).

5.2.1.7 Effect of TDZ and BA on germination of somatic embryos in cassava

Both BA at 4.0 mg l^{-1} and TDZ at 0.25 mg l^{-1} TDZ in MS3 resulted in germination of somatic embryos. In both cases, induction of somatic embryos were successful and on par. The effectiveness of BA either alone or in combination with other plant growth regulators in germination of somatic embryos were reported by Szabados *et al.* (1987), Raemakers *et al.* (1997), Li *et al.* (1998) and Munyikwa *et al.* (1998) and Berhanu (2011).



FEC – friable embryogenic callus; SE – somatic embryos

Figure 12 : Effect of BA and NAA on somatic embryogenesis in cassava

Plate 7: Cassava somatic embryos obtained in media containing picloram

Effectiveness of TDZ for regeneration of somatic embryos into plantlets has not been reported earlier. Since there was no significant difference among the two plant growth regulators, TDZ and BA, for standardization procedure the media MS3 + 0.25 mg l^{-1} TDZ was adopted for embryo regeneration.

For induction of embryoids in cassava genotypes, CC1 and Sree Jaya callusing was obtained in MS3 + 6 - 8 mg l^{-1} 2,4-D or in MS3 +1 mg l^{-1} BA +0.2 to 0.5 mg l^{-1} of NAA. The friable embryogenic callus (FEC) obtained in this medium was transferred to media containing MS 3 + 8-10 mg l^{-1} picloram for induction of somatic embryos after one month. After one month of culturing in this medium the FEC yielded somatic embryos. The somatic embryos were then sub-cultured in media containing MS3 + 0.25 mg l^{-1} TDZ or MS 3 + 4 mg l^{-1} BA for germination (Plate 8).

5.2.2 Propagation through morphogenesis from nodal explants

Pre-sterilization of nodal explants was done with with Teepol 5.00 per cent solution for two minutes. These were then, sterilised under laminar air flow chamber, by washing with ethanol (75.00 per cent) solution for one minute, and followed by washing with mercuric chloride (0.05 per cent) solution for one minute. This resulted in lesser contamination and good culture establishment in nodal explants of cassava.

Hai Wang *et al*. (2010) reported that disinfection with 0.1 per cent solution of mercuric chloride on the axillary explants of cassava for 6-10 minutes showed survival rate of explants ranging from 32.3 to 37.9 per cent. However, in the present study lower concentration of mercuric chloride for lesser time gave a better culture establishment, i.e., 0.05 per cent mercuric chloride for one minute and yielding 8 to 23 per cent of culture establishment.

Plate 8: Somatic embryo development and germination in cassava

5.2.2.1 Effect of BAP and kinetin on morphogenesis from nodal explants

Results on the effect of BAP and kinetin on morphogenesis from nodal explants indicated that MS3 + 2.0 mg l^{-1} BA was the best medium. Genotype M4 multiplied faster than Hraswa (Figure 13). The MS3 + BA media gave the highest response for morphogenesis with success (22 per cent), compared to basal MS3 media in M4 (6.0 per cent).

5.2.2.2 Effect of TDZ and BA on morphogenesis from nodal explants

Results on the effect of TDZ and BAP on morphogenesis from nodal explants indicated that MS3 + 0.25 mg l^{-1} TDZ media resulted in higher morphogenesis (Figure 14 and Plate 9). Similar result was obtained by Berhanu (2011). Hraswa followed by Sree Jaya and CC1 had higher morphogenesis rate. M4 exhibited lower morphogenesis in the same media indicating the role of genotype under tissue culture system. Escobar *et al.*, 1997 and Feitosa *et al.*, 2007 similarly reported variation in response of cassava genotypes to *in vitro* regeneration.

5.2.2.3 Effect of solid or liquid media on *in vitro* morphogenesis from nodal explants

Results indicated that there was no difference in the effect of solid or liquid media (MS3 + 0.25 mg l^{-1} TDZ) on *in vitro* morphogenesis from nodal explants. For convenience and simplicity, solid media was adopted.

5.2.2.4 Effect of genotype on morphogenesis through nodal explant

Results indicated that genotype CC1 responded better than Sree Jaya with respect to node morphogenesis in media MS3 + 0.25 TDZ. In a period of four weeks time CC1 has added 26 new nodes out of 26 subcultured, while Sree Jaya add none out of 26 (but three got lost by necrosis). Escobar *et al.*, 1997 and Feitosa *et al.*, 2007 reported variation in response of cassava genotypes to *in vitro* node regeneration**.**

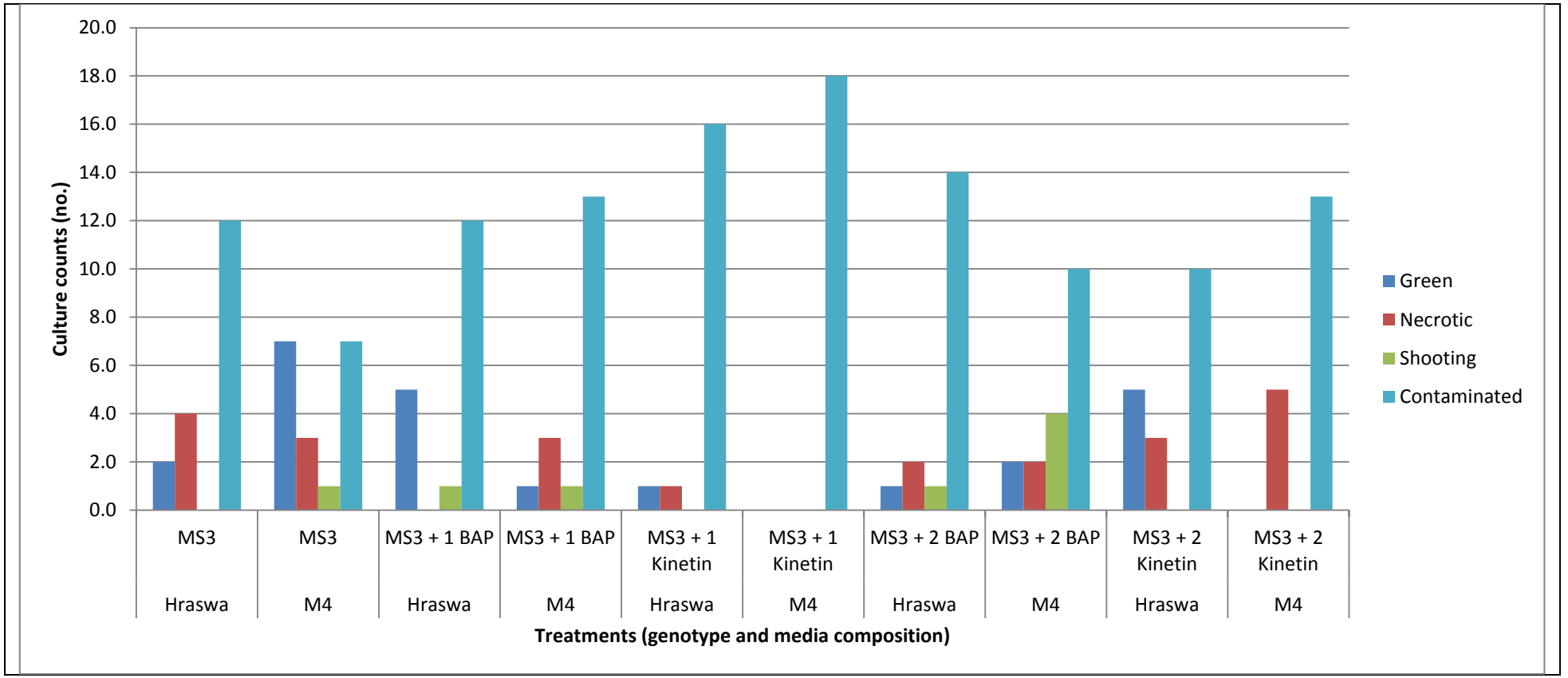


Figure 13 : Effect of BA and kinetin on morphogenesis from nodal explants in cassava

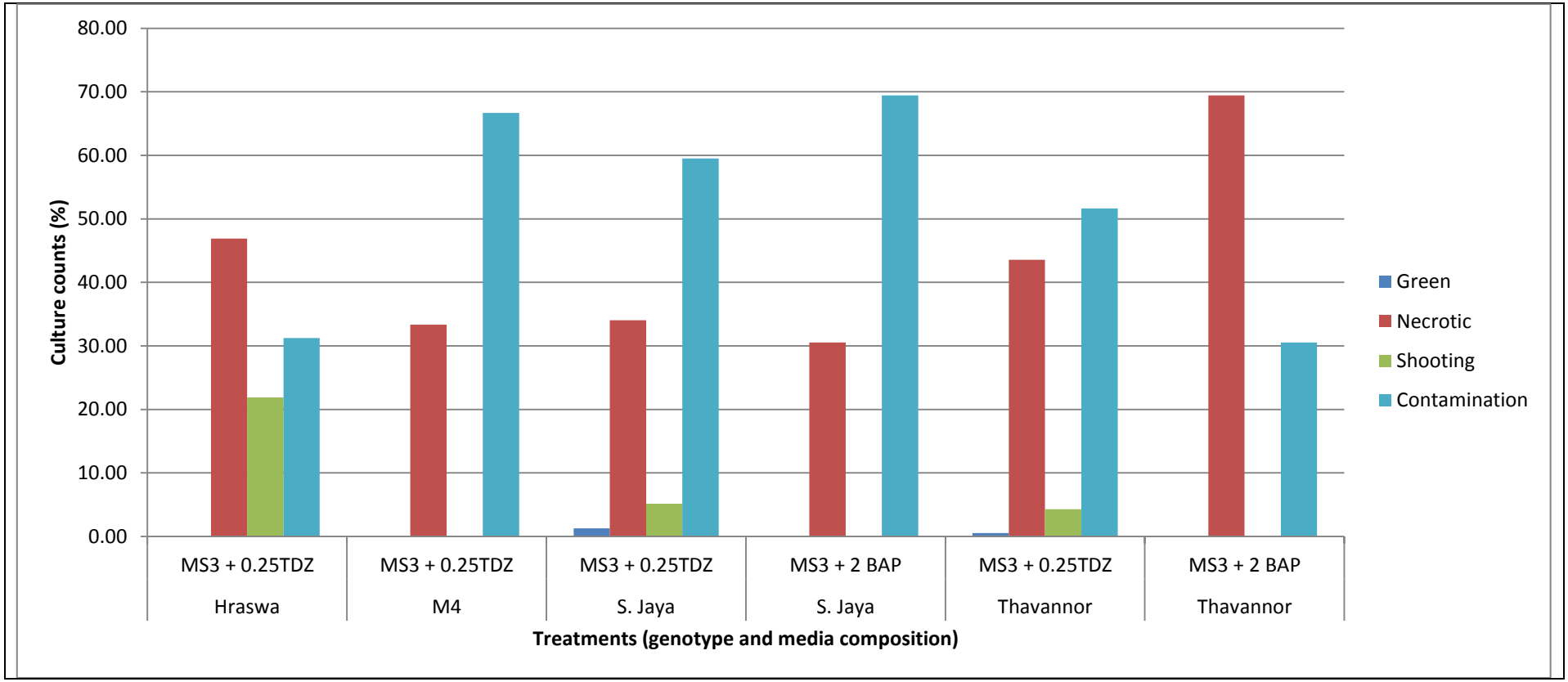


Figure 14: Effect of TDZ and BA on morphogenesis from nodal explants

Plate 9: Plantlet regeneration from nodal explants in cassava

5.2.2.5 Effect of reduction in MS salt strength on *in vitro* cultures in cassava

According to Ziv, 1986, hardening is gradual strengthening the delicate, soft and fragile tissue of *in vitro* plants, in order to achieve maximum survival when transferred to *ex vitro* conditions. It is commonly performed under lab conditions by reducing the media strength and sugars and avoiding plant growth regulators. The effective reduction of MS media strength on CC1 indicated that there is an improvement of plantlet height, number of shoots per culture and length and size of the roots. In Sree Jaya there was an improvement of plantlet height but the number of shoots and leaves was affected. Here also the difference in the response of genotypes is observed.

5.2.2.6 Effect of rooting media on *in vitro* plantlets in cassava

Rooting media improved plant height in *in vitro* plantlets of CC1. However, the number of shoots and leaves as well as the number, length and size of the root was drastically reduced. A similar trend was also observed in Sree Jaya. Dipping of lower portion of plantlet on 1.0 mg l^{-1} NAA for one minute, before sub culturing in the propagation media, resulted in decreased shoot and leaf numbers and also decrease the number, length and size of root (Plate10). Hence, it can be concluded that the medium MS3 + 0.25 mg l^{-1} TDZ was suitable for rooting.

5.3 Standardization of *in vitro* mutagenesis in cassava

Mutation is the most promising breeding techniques in the genetic improvement of vegetatively propagated plants. The distinct advantage of breeding through induced mutations is the possibility to bring about a positive change in one or few characters of an otherwise superior genotype with no detriment to its desirable traits. Isolated attempts have been made in cassava to explore possibilities of inducing mutations with the aid of physical mutagens (Vasudevan and Jos, 1990). Gamma rays have been successfully used to develop mutants in ornaments like chrysanthemum (Bowen *et al*., 1962), gladioli (Buatti *et al*. 1965), rose (Chan, 1966; Gupta and Shukla, 1971; and Lata and Gupta, 1971) and in many other vegetatively propagated crops (Broertjes and Vanharten, 1978).

A: MS3 + 0.25TDZ	B: Dip in 1% NAA for 1 minute then MS3 + 0.25TDZ	C: MS3 + 0.25TDZ + 0.5 NAA

Plate 10: Effect of *in vitro* rooting media in cassava

Though chemical mutagens are not widely used to induce mutations in vegetatively propagated crops, the results obtained so far are promising. The present investigation was undertaken to induce genetic variability using gamma radiation and EMS in cassava. Results obtained in the present study are discussed in the following sections.

5.3.1 Effect of mutagen treatment on *in vitro* cultures in cassava and LD50 determination

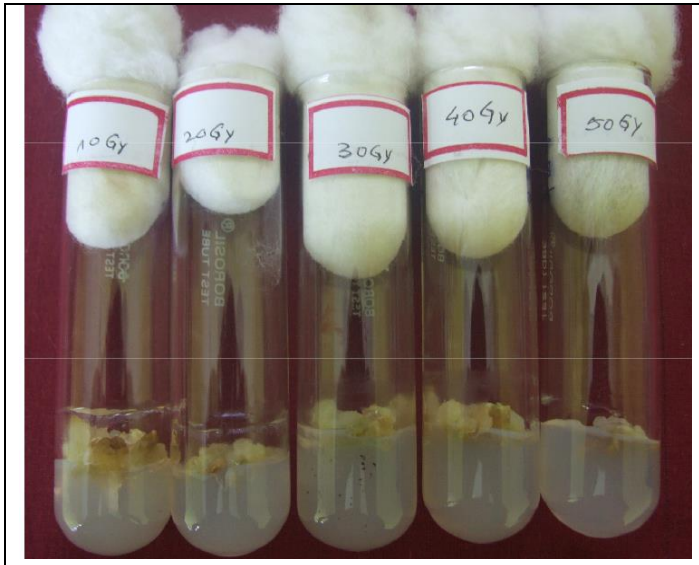
Information on the sensitivity of the plant material to mutagens is essential to arrive at the optimum dose of the mutagen. The unit of absorbed dose of radiation energy is the Gy (Gray) (equivalent to 1.00 J kg^{-1} or equivalent to 100.00 rad) the routine procedure in assessing the most appropriate dosage is based on radiosensitivity, which is estimated through the physiological response of the irradiated material (Neville *et al*., 1998). It involves the determination of dose that

causes a 50.00 per cent reduction of vegetative growth of the treated material (LD 50) when compared with the control (Gaul, 1977).

The choice of the dose to apply for the highest probability for the rescue of useful mutant is then left to the breeders experience with specific material, its genetics and its physiology. Several parameters have been used to determine the sensitivity of crop plants to different mutagens. In this study, survival of plantl*et al*one is used for assessing the sensitivity to gamma rays and EMS. However, Sambandamurthi (1983) and Jayachandran and Mohanakumaran (1992) considered sprouting of the buds along with survival of the plantlets as the parameters for assessing the sensitivity to gamma rays and EMS in vegetatively propagated plants like tuberous ones.

In the present study increasing doses of mutagen in the range of 10.0 to 110.00 Gy of gamma rays and 0.30 to 1.50 per cent of EMS manifested declining trend in survival of the treated material *in vitro* (Plate 11). Based on the results, LD50 of gamma rays was fixed as 30 - 50.00 Gy and that of EMS mutagen as 0.9 - 1.20 per cent. The established LD_{50} for gamma rays is comparable with the results obtained by Castillo *et al*. (1997) and Love *et al*. (1996) in potato and Jayachandran and Mohanakumaran (1992) in ginger.

LD50 value for gamma irradiation for somatic embryos and plantlets was fixed as 30.00 Gy and 50 Gy, respectively (Figure15), while, the LD50 for callus was fixed as 40.00 Gy. The results suggested that somatic embryos were more sensitive to the intensity of gamma rays than callus tissue. On the other hand, the LD50 value of EMS for plantlets (0.90 per cent) was lower than the value of both somatic embryos and callus (1.2 per cent) (Figure 16). For callus the LD 50 value was taken as 1.2 per cent because there is no statistical difference in terms of cultures survival rate among the 0.3, 0.6 and 0.9 per cent treatments. Hence, results indicated that for the effectiveness of mutagenesis work, care should be taken to subject the plantlets to lower doses than both somatic embryos and callus in terms of EMS.



A – Gamma irradiated callus

B – Gamma irradiated plantlets

C –Gamma irradiated somatic embryos

Plate 11: *In vitro* gamma irradiated cultures of cassava

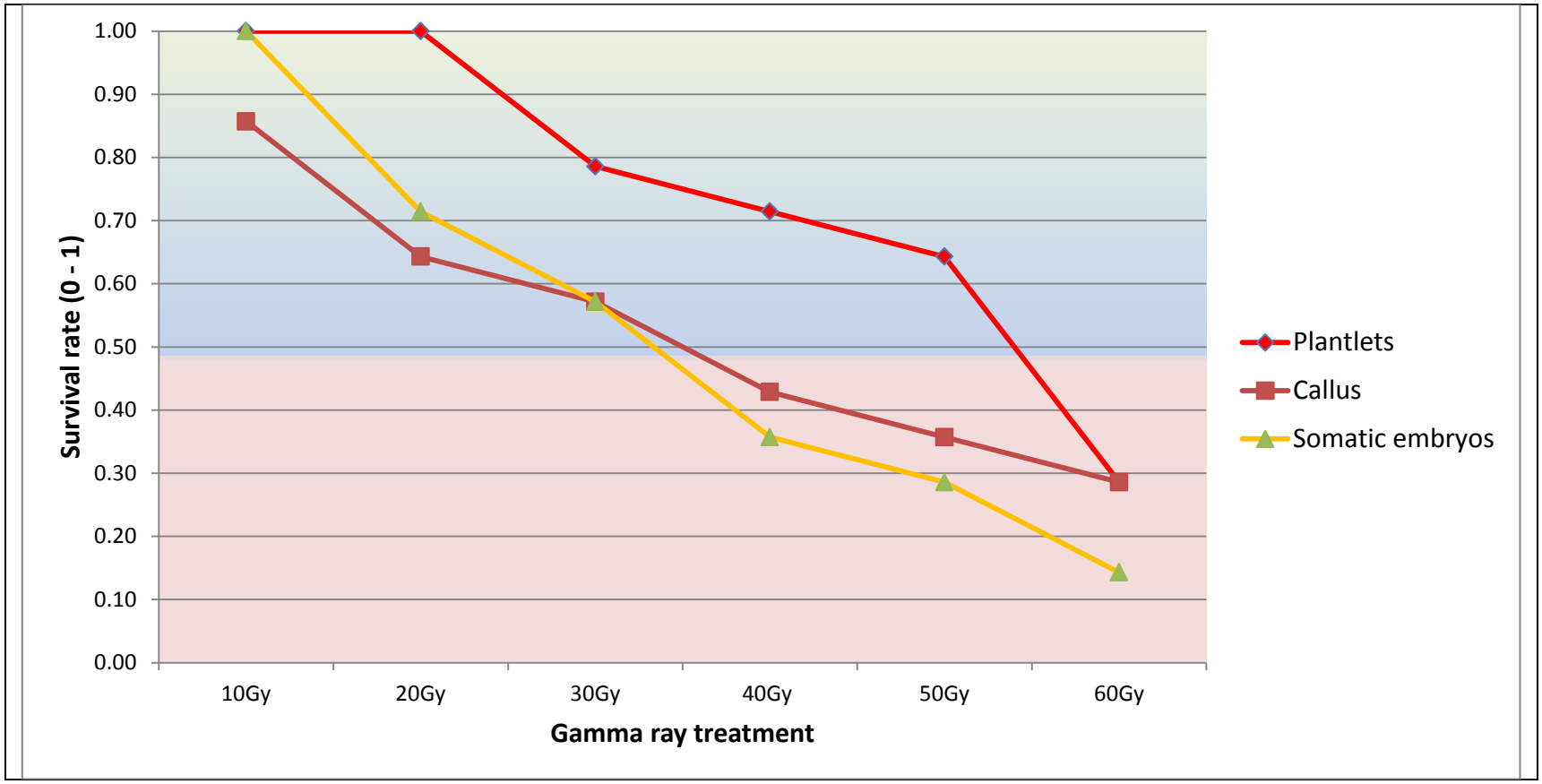


Figure 15: Sensibility of cultures to gamma rays in cassava

5.3.2 Evaluation of *in vitro* mutants of cassava

In vitro techniques are becoming increasingly important in mutation breeding as it helps to develop desirable mutants, restricting the chance of chimera formation (Broertjes, 1976; Roest *et al*., 1980; Ahloowalia, 1995). These variations can be amplified using mutagens. Successful somaclonal variants were obtained in crops like cane sugar, potato and banana. Das *et al*. (2000) could produce heat tolerant mutants in potato. Sunnino *et al.* (1984, 1986) reported a procedure for *in vitro* mutation breeding in potato. Aparna *et al.* (1999) produced late blight resistant mutants in potato using gamma rays. Chemical and irradiation induced mutation in *in vitro* culture has been successfully used to improve banana (*Musa sapientum*) and plantain (*Musa paradisiaca*). This has been reported by Novak *et al.* (1986) and Novak and Micke (1988). Visual examination of tissue culture plants *in vitro* is suggested as a simple and preliminary option to monitor somaclonal variation in cassava. Subsequently green house and field evaluation are performed for either confirmation or identification of additional mutated traits (Reed *et al.*, 2004).

In the present study, an attempt was made to amplify somaclonal variation to increase the genetic variability in cassava. The evaluation of the respective treatments is discussed hereunder.

The treatment of EMS resulted in more variability at morpho-physiological level of *in vitro* sub-cultured cassava somatic embryos and plantlets when compared to gamma irradiation. Also it was observed that higher doses of any of the mutagen, clearly suppressed the root development. Tuber being the economical part, suppression of roots can adversely affect the cassava yield

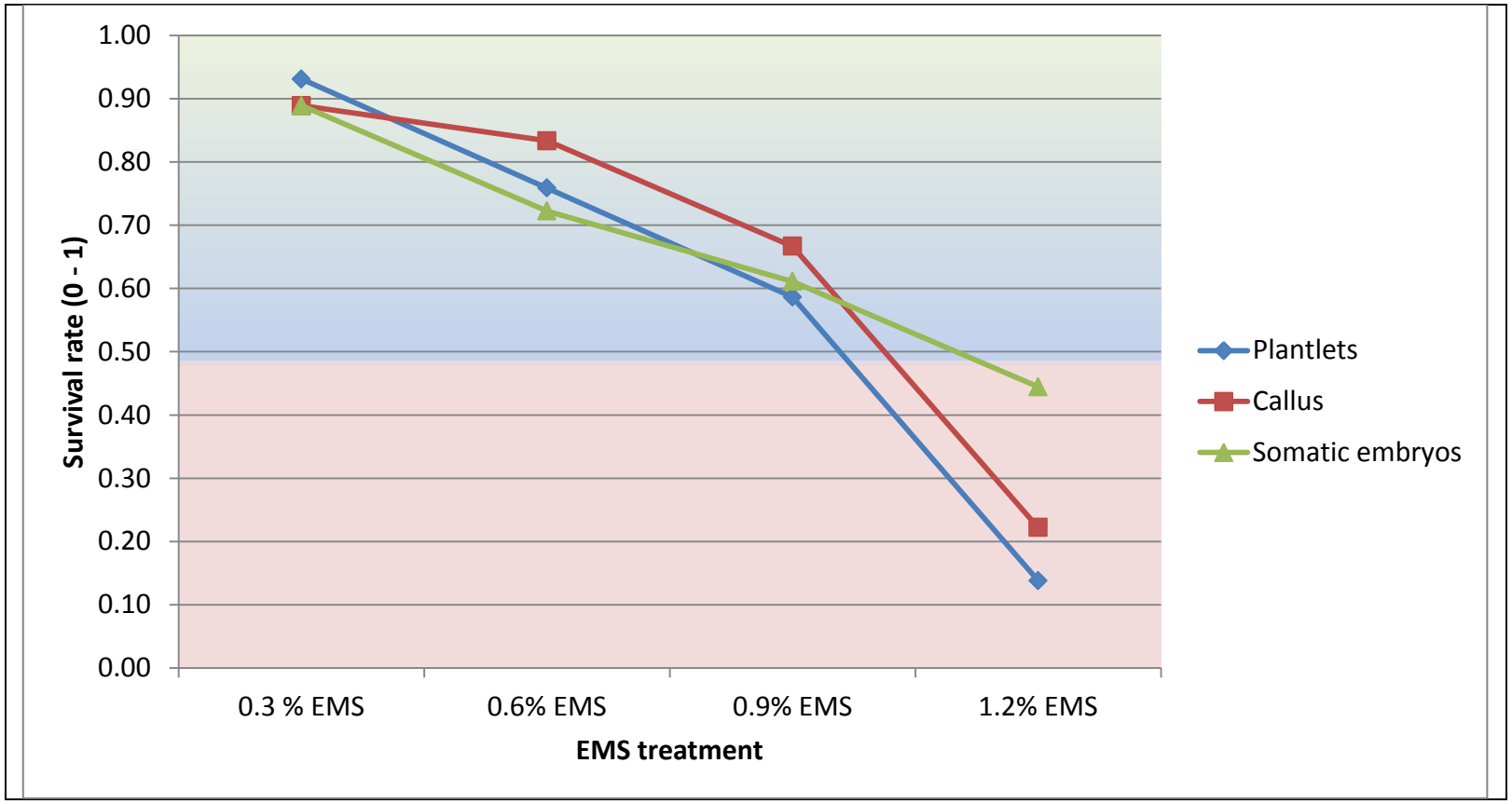


Figure 16: Sensibility of cultures to EMS treatment in cassava

5.3.2.1 Evaluation of somatic embryos of Sree Jaya treated with EMS

The effect of EMS on the somatic embryos of the Sree Jaya genotype indicates that there is a significant reduction in the plantlet height along with reduction in the number of shoots, number of leaves and the length of the roots (Figure 17). However, there was no effect on the number and size of the roots. Thus, the effect of EMS as a mutagen is evident on the tissue development, resulting in measurable physiological and morphological changes (Plate 12).

5.3.2.2 Evaluation of somatic embryos derived plantlets of Sree Jaya treated with mutagens

The EMS treatment resulted in reduction in the number of shoots along with reduction in the number of leaves in somatic embryo derived plantlets of Sree Jaya (Plate 13). When treated with both the mutagens significant variation was observed for number of shoots, leaves and roots (Figure 18).

5.3.2.3 Evaluation of nodal plantlets of CC1 treated with mutagens

The treatment of mutagen EMS caused reduction on plantlet height along with reduction on the number of shoots and the number of leaves in nodal plantlets of CC1 (Plate 14). Plantlet height, shoot number, number of leaves and roots and length of the roots showed variation with the treatment of gamma radiation and Ems at different levels (Figure 19).

5.3.2.4 Evaluation of somatic embryos of Sree Jaya treated with Gamma rays

The irradiation of somatic embryos of the Sree Jaya with gamma rays caused a reduction of plantlet height. No effect was observed on the number of shoots and leaves, as well as, on the number, length and size of the roots.

5.3.2.5 Effect of gamma irradiation on plantlets of CC1

Gamma irradiation in the plantlets of the CC1 resulted in reduction in the plantlet height along with reduction on the number of shoots. There was also reduction in the number of leaves and in the number of roots.

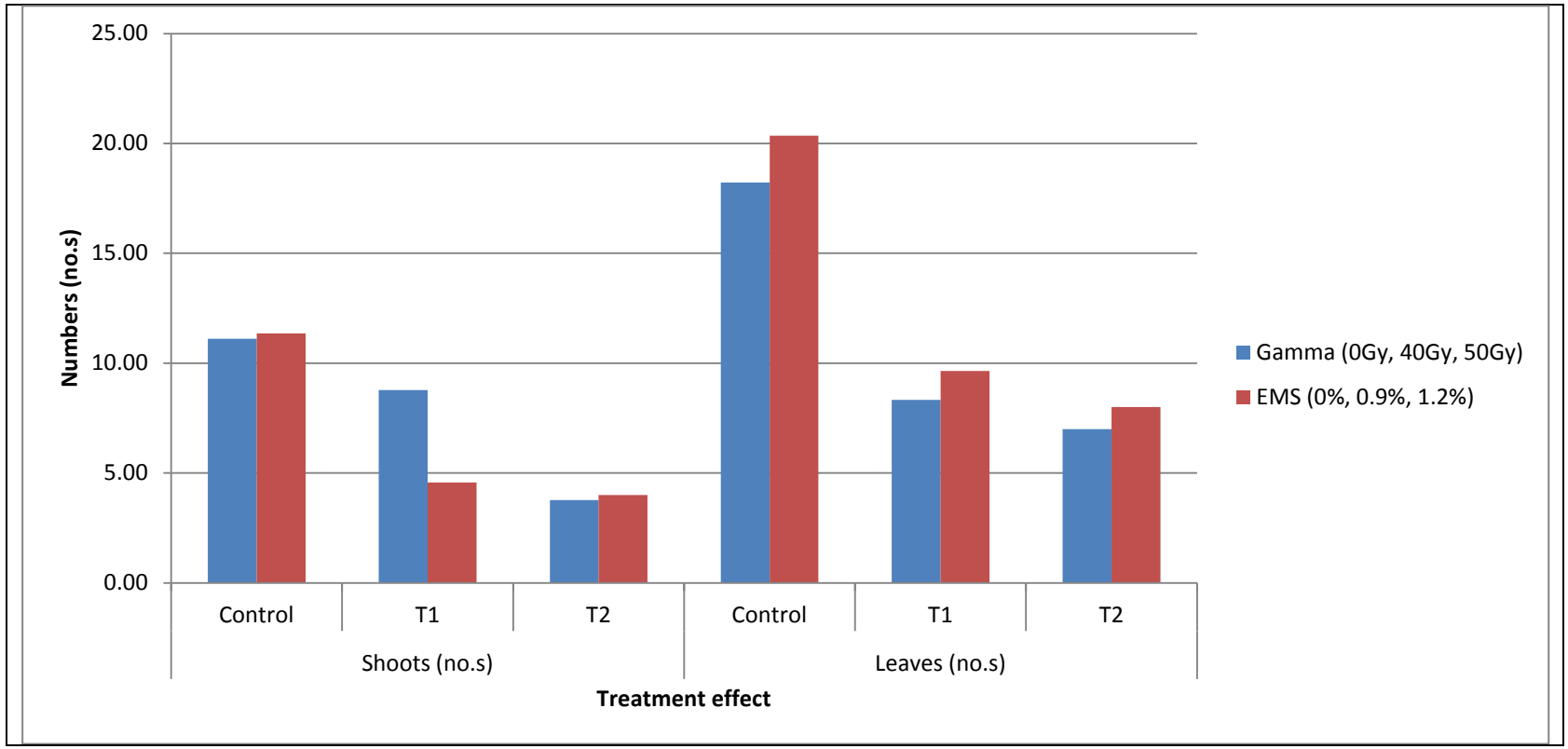


Figure 17: Evaluation of somatic embryos of Sree Jaya treated with Mutagens

Plate 12: Effect of EMS on somatic embryos of cassava

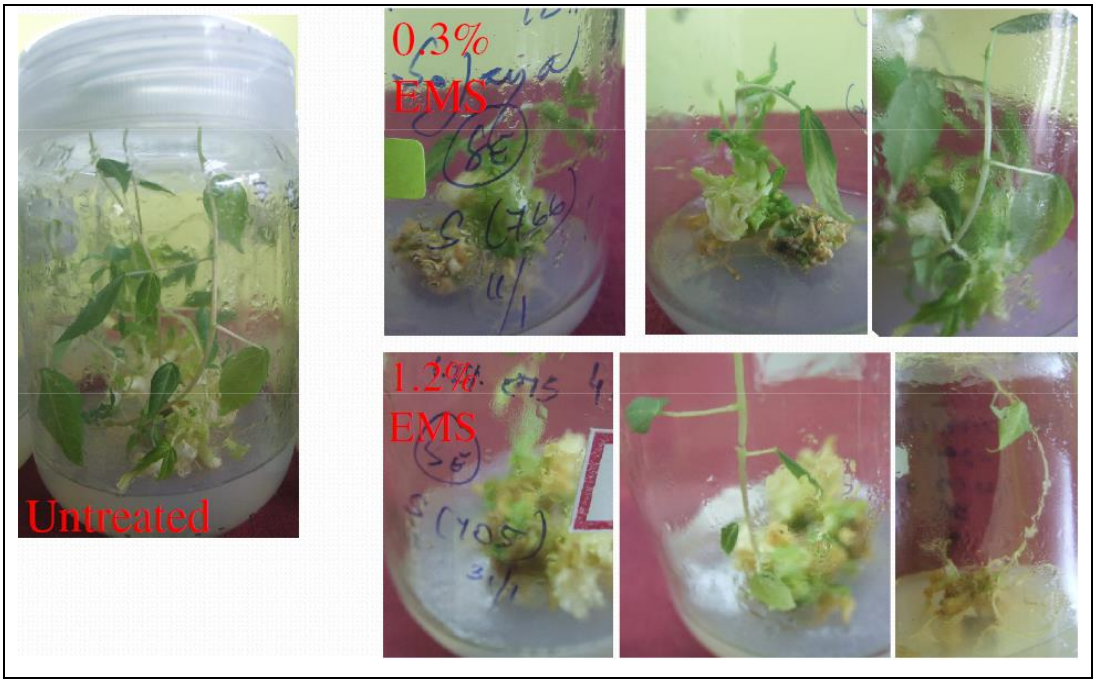


Plate 13: Effect of EMS on somatic embryo derived plantlets of Sree Jaya

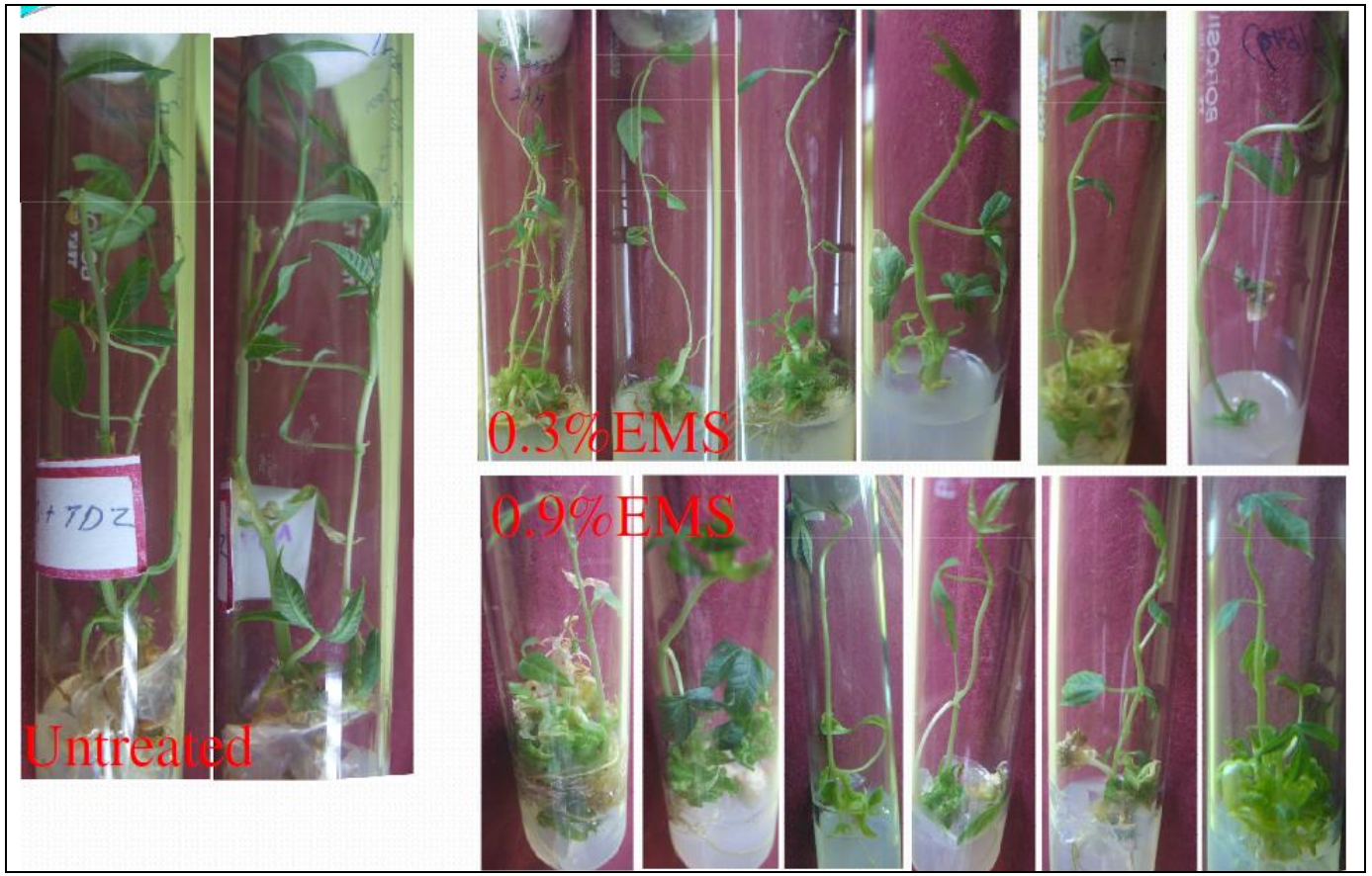


Plate 14: Effect of EMS treatment on nodal derived plantlets of CC1

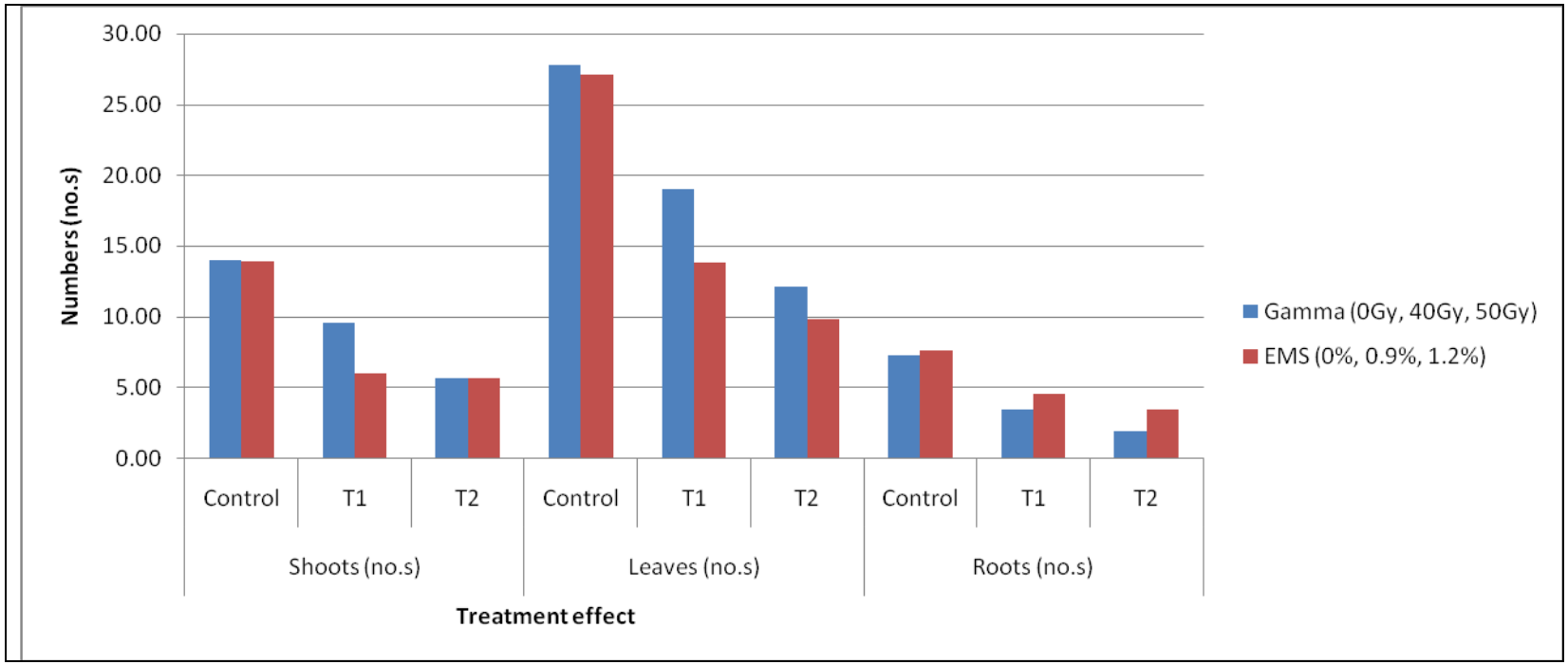
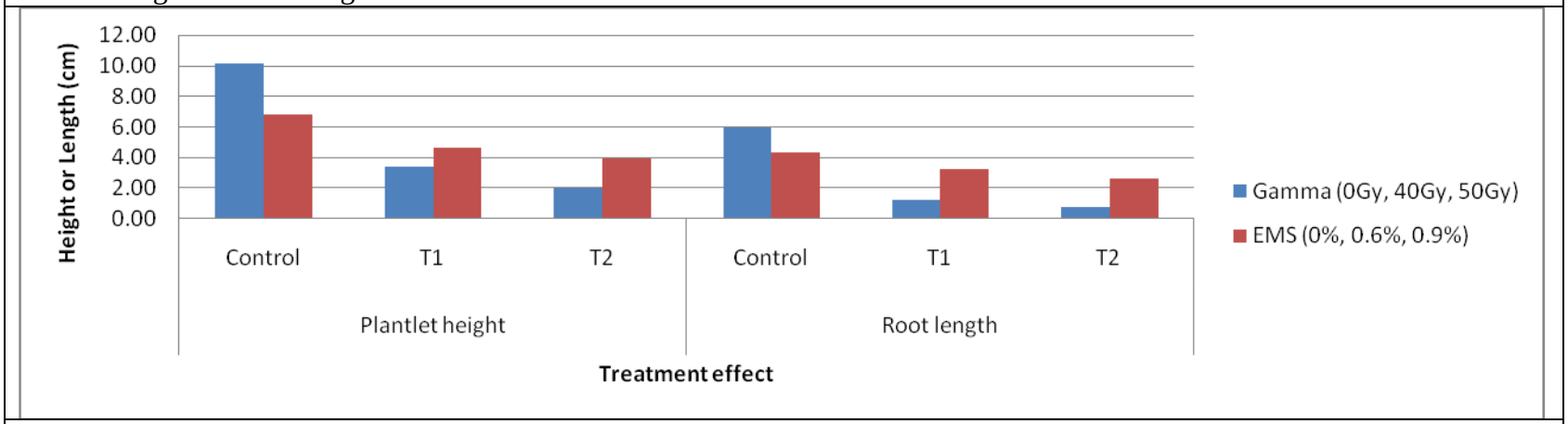


Figure 18: Evaluation of somatic embryo derived plantlets of Sree Jaya treated with mutagens

Plantlet height and root length



Number of shoots, leaves and roots

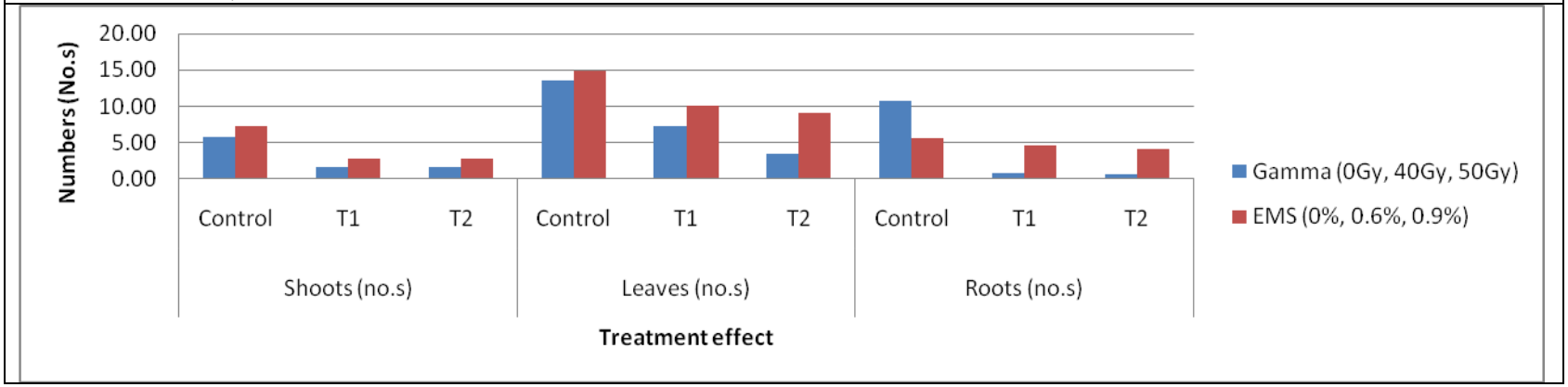


Figure 19: Evaluation of nodal derived plantlets of CC1 genotype treated with mutagens

5.4 Acclimatization of *in vitro* plantlets for field conditions

5.4.1 Acclimatization in tissue culture laboratory

Plantlets transferred to acclimatization media from tissue culture system in tissue culture laboratory did not survive. This may be due difficulty in maintaining the relative humidity which was always less than 50 per cent.

5.4.2 Acclimatization in the net house conditions

The soil substrate was standardized in order to improve the transplanting success. Soil substrate mix consisting of soilrite (a commercial product) and pure sand in the proportion of 1:1, was found the best (Plate 15). The acclimatization success rate of CC1 nodal plantlets multiplied *in vitro* on MS3+0.25TDZ mg l^{-1} media was 33.33 per cent. Only a single plantlet that originated from untreated group (control) was successful. The reason for such poor survival rates under net house acclimatization may be attributed to difficulties in maintaining the humidity. Many scientists have reported the difficulties in hardening. Significant loss of cassava *in vitro* plantlets occur during hardening in semi-arid climatic zones (George, 1996) as well as in culture rooms where the environment is less critical than greenhouses (Jorge, 2002).

Plate 15: Soil substrate standardization for transplanting of *in vitro* plantlets

5.4.3 Acclimatization in the pad and fan Green House

The humidity and temperature conditions in the fan and pad green house system structure were kept constant throughout and are not affected by the fluctuation of daily weather conditions. High humidity is maintained at above 85 per cent and temperatures as low as about 27 ºC. Although its maintenance costs are higher than conventional green house conditions, it has a fundamental advantage of granting higher survival rates for acclimatization of *in vitro* plantlets of cassava (Plate 16). The results achieved under such acclimatization structure are discussed below.

Plate 16: *In vitro* cassava plantlets acclimatization in pad and fan green house

5.4.4 Evaluation of the mutagen treated *in vitro* derived plantlets under pad and fan green house

As per the studies by Jorge *et al*., 2000; Mapayi *et al*. 2013 under green house conditions mutants can be assessed for survival rate, plant height and thickness, number of nodes, number of leaves, leaf length, root number and or length and thickness of roots.

Once the mutants are established in field conditions the IITA descriptors for cassava genotypes might be used for both characterization and performance evaluation against the original genotype. Since, each mutant plant has potential to become a new clone the data should be recorded at individual basis. The qualitative characters to be considered are stipule colour, petiole colour, emerging

leaf colour, leaf shape, internodal length, leaf scar and stem colour. Plant biometrical traits to be recorded are plant height, height of 1st branch, stem girth, number of branches per plant and incidence of pests and diseases. Harvesting data should consist of tuber colour rind colour, tuber length, length of neck, tuber weight, tuber girth and number of commercial tubers (IITA, 1990; Mapayi *et al*., 2013).

The mutagen treated *in vitro* derived plantlets for quantitative traits showed that there are variations in the in plant height, number of branches and leaf number at the age of two to six months after transplanting. All the plants are still in the establishment stage. In Sree Jaya the mutated plants showed plant height varying between 10 cm to 58cm. The shoot numbers were varied between one and four and leaf number between two and fourteen (Plates 17, 18 and 19). In case of CC1, the plant height ranged between 20 cm to 54 cm, number of shoots between one and three and the number of leaves between four and 12 (Plates 20 and 21).

In Sree Jaya, the wild type was having petiole colour purple while in the mutated plants there were light red, yellowish green, pink and red coloured petiole. Stipule colour changed from light green of the wild type to purplish green in few mutated plants while others had light green stipule colour. Emerging leaf colour in the wild type was purplish green, while, the mutated plants showed variations as green and light green. In wild type stem colour was light brown, while the mutated plants showed variations as orange, greenish yellow, green, light brown, yellow and green (Plates 22 and 23).

In case of CC1, petiole colour in the wild type was pink, while the tissue culture derived plants showed variations as reddish green and red. Stipule colour showed variations as green and purplish green, where the wild type had light green stipule. Emerging leaf colour in the wild type was purplish green, while the tissue culture derived plants had green and purplish green emerging leaf colour. Lancolate leaf shape of the wild type was showed variation in one plant showing

ovoid shape. Golden stem colour of the wild type was changed to orange, brown and greenish brown (Plate 24).

The results show that the mutagen treated plants are showing variability with respect to quantitative and qualitative traits which has to be established under field condition. Mutants even at lower levels than the LD 50 shows variation which can be exploited as the crop can be propagated vegetatively. Also in case of CC1 plantlets derived from non treated callus showed some variation indicating the presence of somaclonal variation. Hence, all the plantlets coming out of tissue culture system has to be evaluated individually to screen useful variants under field conditions. The Appendix 3 shows cassava plants derived from this *in vitro* mutagenesis study by five to ten months after transplanting from tissue culture lab to greenhouse conditions.

Petiole colour	Light red
Stipule colour	Light green
Emerging leaf	Green
Leaf shape	Lanceolate
Stem colour	Orange

Petiole colour	Purple
Stipule colour	Light green
Emerging leaf	Purplish green
Leaf shape	Lanceolate
Stem colour	Light Brown

Mutant	Mother plant

Petiole colour	Yellowish-green
Stipule colour	Light green
Emerging leaf	Green
Leaf shape	Lanceolate
Stem colour	Green-yellowish

Petiole colour	Purple
Stipule colour	Light green
Emerging leaf	Purplish green
Leaf shape	Lanceolate
Stem colour	Light Brown

Mutant	Mother plant

Plate 17: Comparison of Sree Jaya mutants and wild type

Petiole colour	Yellowish-green
Stipule colour	Light green
Emerging leaf	Green
Leaf shape	Lanceolate
Stem colour	Green

Petiole colour	Purple
Stipule colour	Light green
Emerging leaf	Purplish green
Leaf shape	Lanceolate
Stem colour	Light Brown

Mutant | Mother plant

Petiole colour	Red
Stipule colour	Light green
Emerging leaf	Light green
Leaf shape	Lanceolate
Stem colour	Light brown

Petiole colour	Purple
Stipule colour	Light green
Emerging leaf	Purplish green
Leaf shape	Lanceolate
Stem colour	Light Brown

Mutant | Mother plant

Plate 18: Comparison of Sree Jaya mutants and wild type

Petiole colour	Reddish green
Stipule colour	Purplish green
Emerging leaf	Green
Leaf shape	Lanceolate
Stem colour	Yellow

Petiole colour	Purple
Stipule colour	Light green
Emerging leaf	Purplish green
Leaf shape	Lanceolate
Stem colour	Light Brown

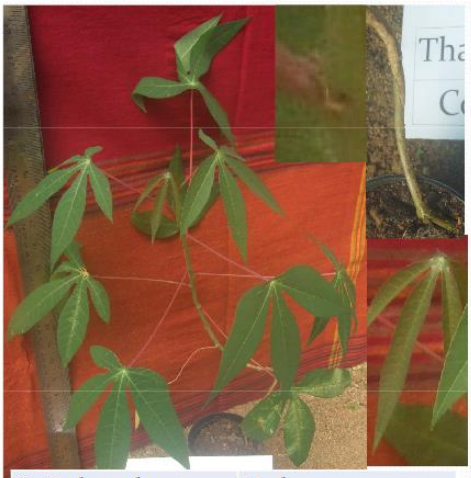
Mutant | Mother plant

Petiole colour	Red
Stipule colour	Purplish green
Emerging leaf	Purplish green
Leaf shape	Lanceolate
Stem colour	Light brown

Petiole colour	Purple
Stipule colour	Light green
Emerging leaf	Purplish green
Leaf shape	Lanceolate
Stem colour	Light Brown

Mutant | Mother plant

Plate 19: Comparison of Sree Jaya mutants and wild type

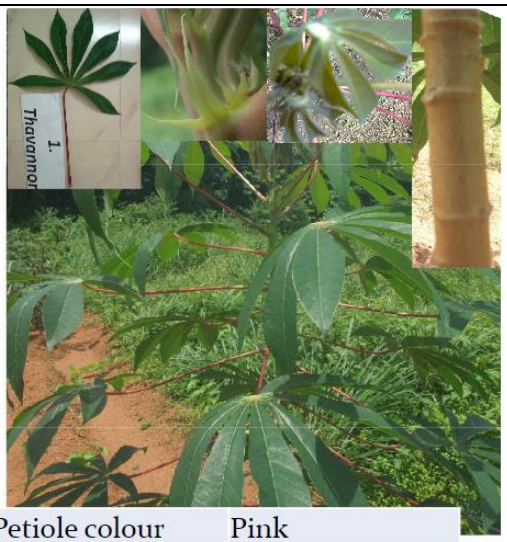


Petiole colour	Red
Stipule colour	Purplish green
Emerging leaf	Purplish green
Leaf shape	Lanceolate
Stem colour	Green yellowish

Petiole colour	Pink
Stipule colour	Light green
Emerging leaf	Purplish green
Leaf shape	Lanceolate
Stem colour	Golden

Mutant	Mother plant

Petiole colour	Reddish green
Stipule colour	Green
Emerging leaf	Green
Leaf shape	Lanceolate
Stem colour	Orange



Petiole colour	Pink
Stipule colour	Light green
Emerging leaf	Purplish green
Leaf shape	Lanceolate
Stem colour	Golden

Mutant	Mother plant

Plate 20: Comparison of CC1 variant and wild type

Petiole colour	Reddish green
Stipule colour	Light green
Emerging leaf	Green
Leaf shape	Ovoid
Stem colour	Greenish brown

Petiole colour	Pink
Stipule colour	Light green
Emerging leaf	Purplish green
Leaf shape	Lanceolate
Stem colour	Golden

Mutant	Mother plant

Plate 21: Comparison of CC1mutant and mother plant

Plate 22: Variation in petiole colour of Sree Jaya mutants

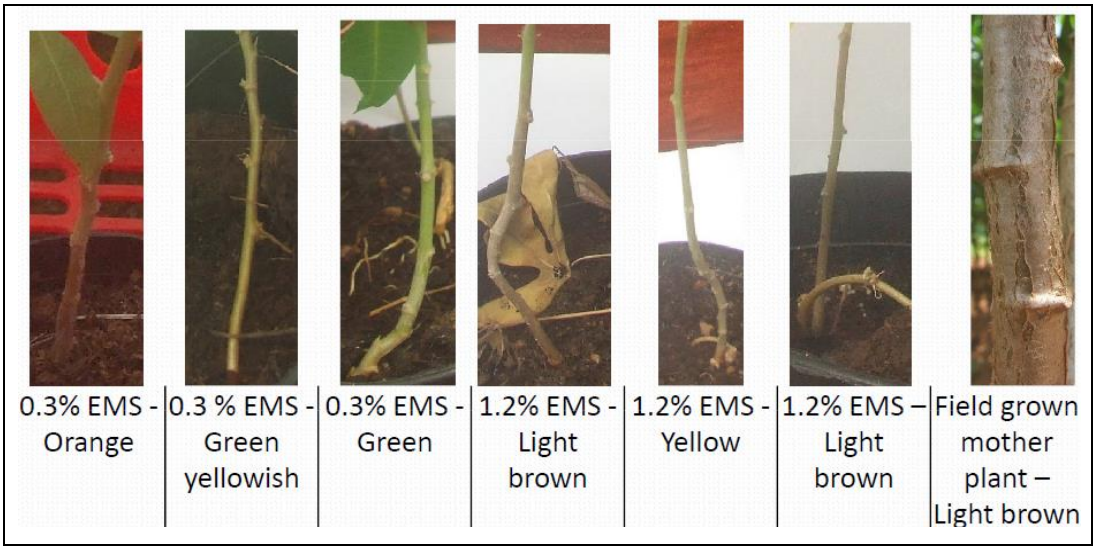


Plate 23: Variation in stem colour of Sree Jaya mutants

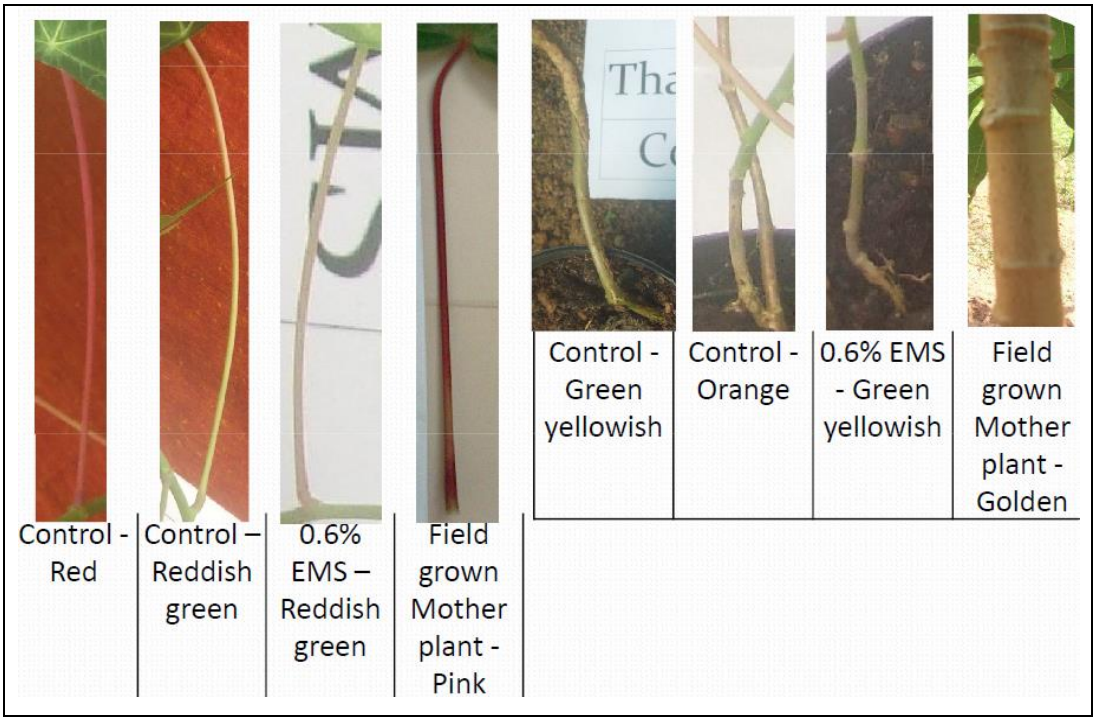


Plate 24: Variation in petiole and stem colour of CC1 mutants

Summary

6 SUMMARY

A study was conducted in the Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University Vellanikkara, during 2012-2014 to assess and induce variability through *in vitro* mutagenesis in cassava (*Manihot esculenta*, Crantz). The salient findings are summarized below:

1. Fourteen short duration cassava genotypes were collected from different places in Kerala.

2. Wide range of variability was observed for qualitative, quantitative, biochemical and organoleptic characters indicating substantial genetic variability among the genotypes.

3. Most variable qualitative trait for upper ground plant portion was petiole colour and for root it was cortex colour. These traits can be used to assess the extent of diversity present among the genotypes.

4. High magnitude of phenotypic and genotypic coefficient of variation along with high heritability and high genetic gain was observed for branch number, branch height, tuber fresh weight, and cassava mosaic disease suggesting scope for genetic improvement of these traits through selection.

5. Plant height and stem girth showed low PCV and GCV but high heritability and low genetic gain, indicating that these traits are governed by non additive gene action and therefore selection is not appropriate.

6. Tuber length and harvest index showed low values for PCV, GCV, heritability and genetic gain indicating the influence of environment.

7. All the traits studied were correlated with fresh tuber yield, except tuber neck, branch numbers, scar numbers and internode length. The traits except CMD had positive correlation with tuber yield.

8. High direct contribution towards tuber yield was exerted by shoot biomass, tuber dry matter content and harvest index.

9. The cassava genotypes were grouped into five and two clusters at 5 and 15 rescaled distance, respectively. The grouping indicated that there is no parallelism between geographic distribution and genetic diversity.

10. A high heritability and high genetic gain was noticed for the biochemical traits namely, HCN content, amylose content and starch content. Hence, selection can improve these biochemical traits.

11. Tuber taste and texture after cooking were the most important traits for organoleptic evaluation and hence for consumers' preference. The genotype CC10 was adjuged the best followed by CC8 and Sree Jaya.

12. A selection index was constructed as SI = [10 * Fresh Root Weight] + [8 * Root Dry Matter Content] + [5 * Harvest Index] – [3 * Cassava Mosaic Disease score] – [2 * Cercospora Leaf Spot score] + [2 * Tuber Girth].

13. The genotypes CC1 and Sree Jaya were selected based on selection index. These genotypes were subsequently subjected to *in vitro* mutagenesis.

14. Sterilization for both cassava nodal and leaf explants was accomplished by washing with 5 per cent Teepol solution for two minutes, followed by washing for one minute with 75 per cent ethanol and washing for one minute with 0.05 per cent solution of mercuric chloride.

15. Friable embryogenic callus (FEC) for both CC1 and Sree Jaya genotypes was obtained from immature leaf explants cultured in Murashige and Skoog media with 3.0 per cent sucrose (MS3), either with 6.0 to 8.0 mg l^{-1} of 2,4-D, or with 1.0 mg l^{-1} BAP + 0.2 to 0.5 mg l^{-1} NAA.

16. Somatic embryos for both genotypes were obtained from FEC cultured in media MS3 with 8.0 to 10.0 mg l^{-1} picloram and germinated into the plantlets on MS3 media either with 4.0 mg l^{-1} BA or 0.25 mg l^{-1} TDZ.

17. *In vitro* regeneration and multiplication from nodal explants for both genotypes were obtained in MS3 media containing either 0.25 mg l^{-1} TDZ or 2.0 mg l^{-1} BAP.

18. Genotypes responded differently to the same dose of mutagen with respect to the growth of plantlets. Reduction of the number of shoots and leaves were more in CC1 compared to Sree Jaya in response to both the mutagens.

19. There was significant difference in the characteristics of the *in vitro* cultures treated with mutagen in both the genotypes.

20. LD 50 value for gamma radiation was 40 Gy, 30 Gy and 50 Gy for FEC, embryoids and plantlets, respectively.

21. LD 50 value for EMS was 1.20, 1.20 and 0.90 per cent for FEC, embryoids and plantlets.

22. There was a gradual reduction in plant height, number of plantlets, number of leaves and length of root in the *in vitro* cultures derived from gamma irradiated and EMS treated callus, embryoids as well as plantlets in both the genotypes with increase in concentration or dose of mutagen.

23. Successful *in vitro* rooting of the plantlets was obtained in culture media MS3 combined with 0.25 mg l^{-1} TDZ or 1/2MS1.

24. A combination of Soilrite^{TM} with pure sand at 1:1 proportion was the best substrate for acclimatization of the plantlets outside the tissue culture system.

25. Pad and fan green house was the best structure for acclimatization of *in vitro* plantlets resulting in about 50 per cent of success rate, when compared against only 3 per cent success from the net house.

26. In the mutated, hardened plants of Sree Jaya cassava genotype, at the age of two to six months after transplanting, the plant height varied from 10.00 to 58.00 cm, shoots from 1 to 4 and leaves from 2 to 14.

27. In the mutated, hardened plants of Sree Jaya petiole colour showed variations as light red, yellowish green, pink and red instead of the wild type purple. Stipule colour was light green and purplish green, emerging leaf colour purplish green, green, and light green. Stem colour varied from orange of wild type to green yellowish, green, light brown and yellow.

28. In the mutated, hardened plants of CC1 cassava genotype, at the age of three to six months after planting, the plant height varied from 20.00 to 54.00 cm, shoots from 1 to 3 and leaves from 2 to 12.

29. In the mutated, hardened plants of CC1 petiole colour showed variation to red from reddish green, stipule colour from light green to purplish green and green, emerging leaf colour from purplish green to green, leaf shape from lanceolate to ovoid and stem colour from golden to orange, brown and greenish brown.

References

7 REFERENCES

Abraham, K., Nair, S., and Naskar, S. 2002. *Cassava breeding and varietal dissemination in India - Major achievements during the past 25-30 years*. CTCRI. Trivandrum. pp. 174-184

Abraham, M. 2002. *Assessment and induction of variability for higher yield and photoinsensitivity in coleus (Coleus parviflorus Benth)*. Ph.D. thesis. Thrissur: KAU, College of Horticulture, Vellanikkara. 162p.

Acanda, Y., Martínez, Ó., Prado, M., González, M., and Rey, M. 2014. EMS mutagenesis and qPCR-HRM pre screening for point mutations in an embryogenic cell suspension of grapevine. *Plant Cell Rep.* 33: 471-481.

Acquaah, G. 2009. *Principles of Plant Genetics and Breeding*. Blackwell Publishing Ltd. Oxford. 569p.

Ahiabu, R., Lokko, Y., Danso, K., and Klu, G. 1997. Mutagenesis for ACMV resistance in Ghanaian cassava cultivar 'Bosomnsia'. IAEA-TECDOC, 951:9-18.

Ahloowalia, B. 1995. In vitro mutagenesis for the improvement of vegetatively propagated crops. *Induced Mutations and Molecular Techniques for Crop Improvement (Proceedings FAO/IAEA Symposium, Vienna)* pp. 531-541. IAEA. Vienna.

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

Alam, S., Narzary, B., and Deka, B. 1998. Variablity, character association and path analysis in sweet potato. *J. Agric. Sci.* 11:177-181.

Allem, A. C. 2002. The Origins and Taxonomy of Cassava. In: Hillocks, R., Thresh and, J. and Bellotti, A. (eds), *Cassava: Biology, production and utilization*. CAB International. London. pp 1 – 16.

Alves, A. A. 2002. Cassava Botany and Physiology. In Hillocks, R., J. Thresh, and Bellotti, A. (eds). *Cassava: Biology, Production and Utilization*. London: CAB International. pp. 67-89

Angadi, S. 1976. *Multivariate analysis in cowpea*. MSc. (Ag.) thesis. Tamil Nadu Agricultural University. Coimbatore. 92p.

Aparna, D., Menocha, J., Thend, T., Dhaliwal, H., and Das, A. 1999. In vitro induction and selection for late blight resistance in potato. *Indian Phytopath.* 52: 169-171.

Apio, H., Alicai, T., Mukasa, S. B., Baguma, Y., Bua, A., and Taylor, N. 2007. Friable *embyogenic callus production and regeneration of Ugandian farmer preferrd cassava genotypes*. NARO-NaCRRI. Kampala.

Aro, S., and Aletor, V. 2012. Proximate composition and amino acid profile of differently fermented cassava tuber wastes collected from a cassava starch producing factory in Nigeria. *Livestock Research for Rural Development.* 24(3).

Asante, I. and Dixon, G. 2002. Heritability studies of some cassava genotypes. *West Afr. J. Appl. Ecol*. 3: 49-53.

Asare, P., Galyuon, I., Asare-Bediako, E., Sarfo, J., and Tetteh, J. 2014. Phenotypic and molecular screening of cassava genotypes for resistance to CMD. *J. Gen. Mol. Virol.* 6(2): 6-18.

Bakayoko, S., Soro, D., Nindjin, C., Dao, D., Tschannen, A., Girardin, O. and Assa, A. 2009. Evaluation of cyanogenic potential and organoleptic properties in cassava

(*Manihot esculenta* Crantz.) roots of improved varieties in Côte d'Ivoire. *Afr. J. Food Sci.* 311: 328-333.

Benesi, I., Labuschagne, M., Dixon, A., and Muhangu, N. 2004. Genotype x environment interaction effects on native cassava starch quality and potential for starch use in the commercial Sector. *Afr. Crop Sci. J.* 3: 205-216.

Berhanu, R. 2011. *Factors influencing micropropagation and somatic embryogenesis of two, Kello and Qulle, cassava varieties*. MSc. Thesis. Addis Ababa University. Addis Ababa. 50p.

Bhagwat, B., Vieira, G., and Erickson, L. R. 1996. Stimulation of in vitro shoot proliferation from nodal explants of cassava by TDZ, BA and GA. *Plant Cell, Tissue Organ Cult.* 46: 1-7.

Bhatt, G. 1970. Multivariate analysis approach to selection of parents for hybridization aiming at yield in self pollinated crops. *Aust. J. Agric. Res.* 21: 1-7.

Biradar, R., Rajendran, P.and Hrishi, N. 1978. Genetic variability and correlation studies in cassava. *J. Root Crops* 4: 7-10.

Blench, R. 1997. Neglected species, livelihood and biodiversity in different areas. *Pakist. J. Sci. Ind. Res.* 40: 236-241.

Bokanga, M. 1994. The cyanogenic potential of cassava. In: Akaroda, M.O. (ed.), *Root Crops and Food Security in Africa*: Proceedings of the fifth triennial symposium of the International Society for Tropical Root Crops-Africa Branch, Kampala, Uganda. International Institute of Tropical Agriculture, Nigeria.

Bowen, H., Cawse, P., and Dick, M. 1962. The induction of sports in chrysanthemum by gamma radiation. *Radiat. Bot.* 2: 297-303.

Broertjes, C. 1976. Mutation breeding of auto-tetraploid Achimenes cultivars. *Euphytica* 25: 297- 304.

Broertjes, C., and Vanharten, A. 1978. *Application of mutation breeding methods in the improvement of vegetatively propagated crops*. Elsevier Scientific Publishing Co. New York. 313 p.

Brown, J., and Caligari, P. P. 2008. *An Introduction to Plant Breeding*. Blackwell Publishing. Oxford. 209 p.

Buatti, M., Ragazzini, R., and D'Amato, F. 1965. Somatic mutation in carnation induced by gamma radiation. *Radiat. Bot.* 5: 719-723.

Burton, G., and DeVane, E. 1953. Estmating heritability in tall fesque (Festuca arundinacea) from replicated clonal material. *Agron. J.* 45: 478-481.

Cacaï, G., Ahanhanzo, C., Adjanohoun, A., Houédjissin, S., Azokpota, P., and Agbangla, C. 2013. Hormonal influence on the in vitro bud burst of some cassava varieties and accessions from Benin. *Afr. J. Biotechnol.* 12 (13): 1475-1481.

Castillo, J., Estevez, A., Gonzalez, M., Castillo, E., and Romero, M. 1997. Radio-sensitivity of two potato cultivars to 60Co gamma rays. *Cultivos-Tropicales.* 18: 62-65.

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

Chan, A. 1966. Chrysanthemum and rose mutations by X-rays. *J. Am. Soc. Hort. Sci.* 88: 613-620.

Charles, A.L., Chang, Y.H., Ko, W.C., Sriroth, K. and Huang, T.C. 2005. Influence of amylopectin structure and amylose content on the gelling properties of five cultivars of cassava Starches. *J. Agric. Food Industries: 1-9*.

Chawla. H.S. 2010. *Introduction to Plant Biotechnology*. 3rd Edition. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi. 699p.

Chaudhary, S., Harshkumar, Verma, V. and Nasar, S. 1999. Path analysis of yield components in a few sweet potato cultivars. *J. Appl. Biol.* 146-148.

Chavez, A. L., Bedoya, J. M., Sanchez, T., Iglesias, C., Ceballos, H. and Roca, W. 2000. Iron, Carotene, and Ascorbic acid in Cassava roots and leaves. *Food Nutr. Bull.* 21(**4**): 410-413.

Chioma, N. J. 2009. Cluster analysis of improved cassava varieties cultivated at Onne, Nigeria by IITA. *J. Mod. Mat. Stat.* 3(3): 51-55.

Chishimba, W., and Lingumbwanga, E. 1997. In vitro propagation of six Zambian clones of cassava (Manihot esculenta Crantz). Proceedings of the 3rd International Scierntific Meeting of Cassava Biotechnology Network (pp. 2 (1/2): 212-213). Edited by A.M. Thro and M.O. Akoroda: *Afr. J. Root Tuber Crop*.

Cock, J. H. 1985. *Cassava new potential for a neglected crop.* West View Press. London. 192p.

CTCRI [Central Tuber Crops Research Institute]. 2006. *Agro-Techniques of Tuber Crops*. CTCRI. Trivandrum. 172p.

CTCRI [Central Tuber Crops Research Institute]. 2013. *CTCRI Vision 2050*. CTCRI. Trivandrum. 53p.

Danso, K., and Ford-Lloyd, B. 2002. Induction of high-frequency somatic embryos in cassava for cryopreservation. *Plant Cell. Rep.* 21:226–232.

da Silva, A., Pasqual, M., Ishida, J., and Antunes, L. 1995. Aclimatação de plantas provenientes da cultura in vitro. *Pesqui. Agropecu. Bras.* 30:49-53.

Das, A., Gosal, S.S., Sidhu, J.S. and Dhaliwal, H.S. 2000. Induction of mutations for heat tolerance in potato by using *in vitro* culture and radiation. *Euphytica* 49: 205-209

de Melo, N. F. 2002. Somatic embryogenesis and ploidy stability in cassava regenerated by in vitro culture of young leaves. *Cytologia* 67(4): 337-341.

Dumet, D., Adeyemi, A., and Ojuederie, O. 2007. Cassava in vitro processing and gene banking. IITA. Ibadan.

Duraisamy, R., Rathinasamy, S. A., Natesan, S., Muthurajan, R., Ramineni, J. J., Karuppusamy, N., Lakshmanan, P., Chokkappan, M. and Gandhi, K. 2011. Starch content and Cassava Mosaic Disease genetic diversity with relation to yield in South Indian cassava (*Manihot esculenta* Crantz) germplasm. *J. Crop Sci. Biotechnol.* 14(3): 179-189.

Edison, S., Anantharaman, M. and Srinivas, T. 2006. Status of cassava in India - an overall view: *Technical Bulletin Series: 46*. Indian Council of Agricultural Research. CTCRI - Central Tuber Crops Research Institute. Thiruvananthapuram. 172p.

Escobar-Perez, R., Munoz, L., Dorado, C., and Tohme, J. 2008. Towards the development of a chimera-free in vitro induced mutagenesis system in cassava (Manihot esculenta Crantz). *IAEA-CN*-167-369P.

Escobar, R. H., Mafla, G, and Roca ,W.M. 1997. A methodology for recovering cassava plants from shoot tips maintained in liquid nitrogen. *Plant Cell Reports* 16: 474–478A.

Falconer, D. 1981. *Introduction to Quantitative Genetics*. Long Man Inc. New York. 340p.

Falconer, D. and Mackay, T. F. 1996. *Introduction to Quantitative Genetics*. Longman. New York. 476p.

FAO and IFAD. 2006. *The world cassava economy*. IFAD - International Fund for Agricultural Development. Rome. 74p.

FAO. 2013. FAOSTAT. Retrieved on December 16, 2013, from *FAO Statistical Agricultural Data*: faostat.fao.org/site/567/

FAO/WHO. 1991. Joint FAO/WHO food standards programme. *Codex Alimentarius Commission XII (Suppl. 4)*. FAO. Rome.

Feitosa, T., Bastos, J. L., Ponte, L. F., Juca, T. L., and Campos, F. 2007. Somatic embryogenesis in Cassava genotypes from the Northeast of Brazil. *Braz. Arch. Biol. Technol.* 50(2): 201-206.

Frank, H., Chritian, M., Noel, A., Brigitte, P., Joseph, H., Cornet, D. and Mathurin, N. 2011. Effects of cultivar and harvesting conditions age, season on the texture and taste. *Food Chem.* 126: 127-133.

Fregene, M., Ospina, J., and Roca, W. 1999. Recovery of cassava (Manihot esculenta Crantz) plants from culture of immature zygotic embyos. *Plant Cell, Tissue Organ Cult,* 55: 39-43.

Fukuda, W., Guevara, C., Kawuki, R. and Ferguson, M. 2010. *Selected morphological and agronomic descriptors for the characterization of Cassava*. IITA. Ibadan. 19p.

Gaul, H. 1977. *Induced Mutations in Vegetatively Propagated Plants*. IAEA. Vienna 236p.

George, E. 1996. *Plant propagation by tissue culture-part 2 in Practice*. 2nd Ed. 1993/1996. Exenics Limited. 1361p.

Gonzalez, C. and Johnson, N. 2009. Consumer preferences for table cassava characteristics in Pernambuco, Brazil. *Rev. Econ. e Agroneg*. 7(**3**): 363-384.

Groll, J., Gray, V.M., Mycock, D.J., Thro, A.M. and Akoroda, M.O. 1997. Friable embryogenic callus formation from thin layer explants of cassava. International Society for Tropical Root Crops – Africa Branch (ISTRC-AB). Ibadan, Nigeria. *Afr. J. Root Tuber Crops*. 2(1/2): 154-158.

Groll, J., Mycock, D., and Gray, V. 2002. Effect of medium salt concentration on differentiation and maturation of somatic embryos of cassava (Manihot esculenta). *Ann. Bot.* 89: 645-648.

Groll, J., Mycock, D., Gray, V., and Laminski, S. 2001. Secondary somatic embryogenesis of cassava on picloram supplemented media. *Plant Cell, Tissue Organ Cult.* 65: 201-210.

Gupta, M., and Shukla, R. 1971. Mutation breeding of garden roses. *J. Pl. Breed.*, 21: 129-136.

Hahn, S., Isoba, J. and Ikotun, T. 1989. Resistance breeding in root and tubercrops at IITA, Ibadan, Nigeria. *Crop Prot.* 8(3): 147-168.

Hahn, S., Terry, E., and Leuschner, K. 1980. Breeding cassava for resistance to Cassava Mosaic Disease. *Euphytica* 29: 673-683.

HaiWang, H., LongFei, H., Ke, M., and ChuangZhen, L. 2010. Rapid propagation technology for new cassava variety Xinxuan 048 by microcuttage. *Guangxi Agri. Sci.* 41(4): 307.

Henry, G., Westby, A. and Collinson, C. 1998. *Global Cassava End-uses and Markets: Current situation and recommendation for further studies*. FAO. Ontario.

Hillocks, R. J. and Wydra, K. 2002. Bacterial, Fungal and Nematode Diseases. In Hillocks, R., Thresh, J. and Bellotti, A. (eds.). *Cassava: Biology, Production and Utilization.* CAB International. London. pp. 261-280.

Hoffmann, N., Raja, R., Nelson, R. and Korban, S. 2004. Mutagenesis of embryogenic cultures of soybean and detecting polymorphisms using RAPD markers. *Biol. Plant.* 48: 173-177.

Hogarth, D. 1971. Quantitative inheritance studies in sugarcane_correlations and predicted response to selection. *Crop Sci.* 21: 21-25.

IAEA. 2006. *Plant Breeding Unit - Activities Report 2006*. FAO/IAEA. Seibersdorf

Ibrahim, A., Heredia, F., Pinheiro, C., Aragao, F., and Campos, F. 2008. Optimization of somatic embryogenesis and selection regimes for particle bombardment of friable embryogenic callus and somatic cotyledons of cassava. *Afr. J. Biotechnol.* 7(16): 2790-2797.

IITA [International Institute of Tropical Agriculture]. 1990. *Annual Report 1990*, IITA. Nigeria. Ibadan.

Jayachandran, B. and Mohanakumaran, N. 1992. Effect of gamma ray irradiation on ginger. *South Indian Hort.* 40: 283-288.

Jennings, D. L. and Iglesias, C. 2002. Breeding for crop improvement. In: Hillocks, R. J., Threshand, J. M. and Bellotti, A. C. (eds.). *Cassava: Biology, Production and Utilization*. CAB international. London. pp. 149-166.

Johnson, H., Robinson, H. and Comstock, R. 1955. Estimates of genetic and environmental variability in soybean. *Agron. J.* 314-318.

Jorge, M. 1996. Cassava acclimatization in Mozambique. *Afr. J. Root Tuber Crop.* 2: 216-219.

Jorge, M. A. 2002. *Factors affecting the hardening and acclimatization of tissue cultured cassava plantlets*. Ph.D. Thesis. University of Zimbabwe. Harare.

Jorge, M., Robertson, A., Mashingaidze, A. and Keogh, E. 2000. How *in vitro* light affects growth and survival of ex vitro cassava. *Ann. Appl. Biol.* 137: 311-319.

Jose, A., Makeshkumar, T. and Edison, S. 2011. Survey of Cassava Mosaic Disease in Kerala. *J. Root Crop.* 37(1): 41-47

Joseph, R., Yeoh, H.-H. and Loh, C.-S. 2004. Induced mutations in cassava using somatic embryos and the identification of mutant plants with altered starch yield and composition. *Plant Cell Rep.* 23: 91–98.

Joseph, T., Yeoh, H.-H. and Loh, C. 2001. Linamarin content and genetic stability of cassava plants derived by somatic embryogenesis. *Euphytica* 120: 7-13.

KAU [Kerala Agricultural University]. 2011. *Package of Practices recommendations: Crops*. 14th Ed. KAU press. Thrissur. 360p.

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

Kawano, K., Amaya, A., Daza, P. and Rios, M. 1978. Factors affecting hybridization and selection in cassava. *Crop Sci*. 18: 373-376.

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

Kawuki, R., Ferguson, M., Labuschagne, M., Herselman, L., Orone, J., Ralimanana, I., Bidiaka, M., Lukombo, S., Kanyange, M.C., Gashaka, G., Nkamilo, G., Gethi, J. and Obiero, H. 2011. Variation in qualitative and quantitative traits of cassava germplasm from selected national breeding programmes in Sub-Saharian Africa. *Field Crops Res.* 122: 151-156.

King, N. L.and Bradbury, J. H. 1995. Bitterness of Cassava: Identification of a New Apiosyl Glucoside and other Compounds that Affect its Bitter Taste. J. *Sci. Food Agric.* 68(2): 223-230.

Kizito, E. B., Ronnberg-Wastljung, A.-C., Egwang, T., Gullberg, U., Fregene, M. and Westerbergh, A. 2007. Quantitativ trait loci controlling cyanogenic glucoside and dry matter content in cassava roots. *Hereditas.* 144: 129-136.

Konan, N., Sangwan, R., and Sangwan-Norreel, B. 1994. Efficient in vitro shootregeneration systems in cassava (Manihot esculenta Crantz). *Plant Breed.* 113: 227–236.

Konan, N., Schiipke, C., Carcamo, R., Beachy, R. and Fauquet, C. 1997. An efficient mass propagation system for cassava (Manihot esculenta Crantz) based on nodal explants and axillary bud-derived meristems. *Plant Cell Rep.* 16: 444-449.

Konzak, C. 1984. *Role of Induced Mutation in Crop Breeding*. Pergamon Press. New York. 292p.

Kordestani, G. and Karami, O. 2008. Picloram-induced somatic embryogenesis in leaves of strawberry. *Acta Biol. Crac. Ser. Bot.* 50(1): 69-72.

Kouadio, O.K., N'dri, D.Y., Nindjin, C., Marti, A., Casiraghi, M.C., Faoro, F., Erba, D., Bonfoh, B. and Amani, N.G. 2013. Effect of resistant starch on the cooking quality of yam (Discorea spp.) and cassava (Manihot esculenta) based paste products. *Int. J. Food Nutr*. 64(4): 484-493.

Kruskal-Wallis. (1952). Use of ranks in one-criterion variance analysis. *J. Am. Stat. Assoc.* 47 (260): 583–62.

Kumar, R., Jain, P., Ganguli, D., Kumar, R. and Kurup, G. 1996. Tropical Tuber Crops: Problems, Prospects and Future Strategies. In V. Potti, K. Padmajah, S. Kabeerathumma, and S. Pillai (eds). New Hampshire: Science Publishers Inc., Lebanon. 203p.

Kyujung, V., Jang, H. J., Jang, Y.-E. and Lee, S.-H. 2008. Regeneration of Plants from EMS treated Immature Embryo Cultures in Soybean [Glycine max (L.) Merr.]. *J. Crop Sci. Biotech.* 11(2): 119-126.

Lata, P., and Gupta, M. 1971. Effects of gamma rays on stem cuttings of essential oil of Rosa spp. *Flavour Indian.* 2: 421-425.

Lebot, V. 2009. Tropical root and tuber crops: cassava, sweet potato, yams, aroids. In: Atherton, J. and Rees, A. (eds.). Crop *Production Science in Horticulture 17*. CAB International. London. pp. 1-413.

Lee, K., Van Duren, M. and Morpurgo, R. 1997. Somatic embryogenesis in cassava: a tool for mutation breeding. In B. Ahloowalia (ed.). *Improvement of basic food crops in Africa through plant breeding, including the use of induced mutations.* IAEA-TECDOC-951: International Atomic Energy Agency. Vienna. pp.55–60.

Lekha, S. S., da Silva, J. A. and Pillai, S. V. 2011. Genetic variability studies between released varieties of cassava and central Kerala cassava collections using SSR markers. *J. Stored Prod. Postharvest Res.* 2(4): 79-92.

Lenka, D. and B. Mishra (1973). Path coefficient analysis of yield in rice varieties. *Indian J. Agric. Sci.* 43: 376-379.

Li, H.-Q., Guo, J.-Y., Huang, Y.-W., Liang, C.-Y., Liu, H.-X. and Potrykus, I., 1998. Regeneration of cassava plants via shoot organogenesis. *Plant Cell Rep.* 17: 410–414.

Love, S., Thompson, J., and Werner, B. 1996. Mutation breeding for improvd internal quality and appearance in Russet Burbank. *Am. Potato J.* 73: 155-165.

Luan-YuShi, Zhang-Juan, Gao-XiaoRong and An-LiJia. 2007. Mutation induced by ethyl methanesulfonate (EMS), in vitro screening for salt tolerance and plant regeneration of sweet potato (Ipomoea batatas L.). *Plant Cell Tissue Organ Cult.* 88(1): 77-81.

Lush, J.L. 1940. Intersize correlation regression of offspring on dams as a method of estimating heritability of characters. *Anim. Sci.* 33: 293-301.

Ma, G. and Xu, Q. 2002. Induction of somatic embryogenesis and adventitious shoots from immature leaves of cassava. *Plant Cell, Tissue Organ Cult.* 70: 281–288.

Magaia, H. E. 2003. *Starch Quality Improvement on Cultivated Cassava - Selection of transgenic genotypes*. MSc. Thesis. WAU [Wageningen University and Research Center]. Wageningen. 63p.

Mahangu, N., Cheda, H., Hahn, S. and Fatokin, C. 1983. Genetic parameters of cassava. In: Fatokin, C. (eds.). *Tropical Root Crops: Production and uses in Africa*. Tonabu Publishing House. Kentucky. pp. 5-10

Mahalanobis, P.C. (1978). A statistical study at Chinese head measurement. *J. Asiatic Soc. Bengal*. 25: 301-77.

Mannan, M., Saleh, A., Reshid, M., Bhuiyan, M. and Gomes, R. 1993. Genetic diversity of Colocasia esculenta (L). Scott. in Bangladesh. *J. Root Crop.* 19: 95-99.

Manu-Aduening, J. A., Peprah, B. B. and Agyeman, A. 2013. Genetic variability of Cassava progenies developed through introgression of Cassava Mosaic Disease resistance into Ghanaian landraces. *J. Crop Sci. Biotech*. 16(1): 23-28.

Mapayi, E., Ojo, D. K., Oduwaye, O. A. and Porbeni, J. B. 2013. Optimization of in vitro propagation of cassava (Manihot esculenta Crantz) genotypes. *J. Agric. Sci.* 5(3): 261-269.

Mba, C., Afza, R., Bado, S. and Jain, S. M. 2010. Induced Mutagenesis in Plants Using Physical and Chemical Agents. In M. R. Davey, and P. Anthony (eds.). *Plant Cell Culture: Essential Methods*. John Wiley and Sons. London. pp. 111-130.

Mezette, T. F., Blumer, C. G. and Veasey, E. A. 2013. Morphological and molecular diversity among cassava genotypes. *Pesq. Agropec. Bras.* 48(5): 510-518.

Miller, P. and Marani, A. 1963. Heterosis and combining ability in diallel crosses of upland cotton (Gossypium hirsutum L.). *Crop Sci*. 3: 441-444.

Mohankumar, C., Saraswathy, P. and Sadanandan, N. 1990. Correlation and path coefficient analysis on yield and yield components n taro. *J. Root Crop.* 16: 140-141.

Moll, R., Salhuoma, W. and Robinson, H. 1969. Heterosis and diversity in variety crosses of maize. *Crop Sci.* 2: 197-199.

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.

Mulualem, T. and Dagne, Y. 2013. Studies on correlation and path analysis for root yield and related traits of Cassava Manihot esculenta Crantz in South Ethiopia. *J. Plant Sci*. 13: 33-38.

Munyikwa, T. R., Raemakers, K. C., Schreuder, M., Kok, R., Schippers, M. and Jacobsen, E. 1998. Pinpointing towards improved transformation and regeneration of cassava. *Plant Sci.* 135: 87-101.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15(3): 473-497.

Murthy, B. and Anand, I. 1966. Combinig ability and genetic diversity in some varieties of Linum usitatissimum. *Indian J. Genet.* 26: 21-36.

Murthy, B.R. and Arunachalam, V. 1966. The nature of divergence in relation to breeding system in some crop plants. *Indian J. Genet*. 26: 188-198.

Mussio, I., Chaput, M., Serraf, I., Ducreux, G. and Sihachakr, D. 1998. Adventitious shoot regeneration from leaf explants of an African clone of cassava (Manihot esculenta Crantz) and analysis of the conformity of regenerated plants. *Plant Cell Tissue Organ Cult.* 53: 205–211.

Nair, S. G. and Unnikrishnan, M. 2007. Recent Trends in Cassava Breeding in India. In: Ortizand, R. and Nassar, N. (eds.). *Proceedings First International Meeting on Cassava Breeding, Biotechnology and Ecology*. Thesaurus Editora. Brasilia. pp. 37-53

Nair, V., Seow, C. K. and Sulebele, G. A. 1996. Effects of frying parameters on physical changes of tapioca chips during deep-fat frying. *Int. J. Food Sci. Technol.* 31: 249–256.

Namitha. 2010. *Mashed tapioca aka Cassava*. Retrieved May 25, 2014, from Kerala Sadya Recipes: 2010, January 4.

Naskar, S., Singh, D. and Lakshmi, K. 1991. Variability and correlations in F1 populations of cassava. *J. Root Crops*. 17: 139-141.

Neville, P., Nayana, N. and Tanguay, M. 1998. Mutagenic effects of acute gamma irradiation on miniature roses: Target theory approach. *Hort Science,* 33: 127-129.

Ng, N., and Ng, S. 2002. Genetic Resources and Conservation. In Hillocks, R., Thresh, J. and Bellotti, A. (eds.). *Cassava: Biology, Production and Utilization*. CAB International. London. pp. 167-177.

Novak, F., Hermelin, T., Brunner, H. and Afza, R. 1986. In vitro mutation breeding of banana and plantain. *Hort Science,* 21: 684.

Novak, F. and Micke, A. 1988. Induced mutations and in vitro techniques for plant breeding. *J. Pl. Breed. Genet. Engg.* 90: 63-86.

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

Nuwamanya, E., Baguma, Y., Emmambux, N., Taylor, J. and Patrick, R. 2010. Physicochemical and functional characteristics of cassava starch in Ugandan varieties and their progenies. *J. Plant Breed. Crop Sci.* 2: 001-011.

Nuwamanya, E., Baguma, Y., Wembabazi, E. and Rubaihayo, P. 2011. A comparative study of the physicochemical properties of starches from root, tuber and cereal crops. *Afr. J. Biotechnol. 10(56): 12018-12030*

Nybom, N. and Koch, A. 1965. Induced mutations and breeding methods in vegetatively propagated plants. *Radiat. Bot.* 5: 24-35.

O'Brien, G., Mbome, L., Taylor, A. and Poulter, N. 1992. Variations in cyanogen content of cassava during village processing in Cameroon. *Food Chem.* 44(2): 131–136.

Ogburia, M. 2002. An in-vitro study of callogenesis in recalcitrant plant organs of cassava (Manihot esculenta). *Niger Delta Biol.* 4(1): 1-5.

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

Onuoch, C. and Onwubiko, N. 2007. Micropropagation of Cassava (Manihot esculentum, Crantz) using different concentartions of BA. *Nat. Sci*. 5(2): 37-40.

Onwueme, I. and Sinha, T. 1991. *Field Crop Production in Tropical Africa*. CTA. Ede. 319p.

Ospina, B., Segovia, R. and Bedoya, A. 2007. Micro-propagation of cassava plants through the temporary immersion system and hardening of massive numbers of cassava vitroplants. CIAT (CLAYUCA). Cali.

Owoseni, O., Okwaro, H., Afza, R., Bado, S., Dixon, A. and Mba, C. 2006. Radiosensitivity and in vitro mutagenesis in African accessions of cassava, Manihot esculenta Crantz. *Plant Mutat. Rep.* 1(2): 32-36.

Padma, S. V., Kameswari, P. and Ramana, K. V. 2009. Correlation studies and path analysis in edible cassava germplasm in the tribal zone of Andhra Pradesh. *Indian J. Hort.* 663: 319-322.

Padonou, W., Mestres, C., and Nago, M. C. 2004. The quality of boiled cassava roots – instrumental characterization and relationship with physicochemical properties and sensorial properties. *Food Chem.* 89: 261–270.

Panse, V. and Sukhatme, P. 1954. Statistical Methods for Agricultural Workers. IACR [Indian Council of Agricultural Research]. New Delhi.

Pillai, S. V. 2002. Variability and genetic diversity in exotic germplasm of cassava (Manihot esculenta Crantz). *Indian J. Genet.*, 62(3): 242-244.

Pillai, S. V., Nair, R. R., Palaniswami, M. S., Ravindran, C. S., Moorthy, S. N., Ravi, V. and Sree Lakha, S. 2006. Evaluation and utilization of biodiversity in Cassava (Manihot esculenta, Crantz.). *Second National Plant Breeding Congress 2006*. CTCRI. Trivandrum. pp. 78-82

Prabhakar, K. and Raguchander, T. 2001. Field reaction of cassava varieties to brown leaf spot and cassava mosaic. *Madras Agric. J*. 87(7/9): 523-525.

Purseglove, J. W. 1982. *Tropical Crops: Dicotyledons*. John Willey. London. 719p.

Radhakrishnan, V. and Gopakumar, K. 1984. Correlation between yield and its components in tapioca. *Indian J. Agric. Sci.* 54: 975-978.

Raemakers, C., Amati, M., Staritsky, G., Jacobsen, E. and Visser, R. 1993. Cyclic somatic embryogenesis and plant regeneration in cassava. *Ann. Bot.* 71: 289-294.

Raemakers, C., Jacobsen, E. and Visser, R. 1995. Seconday somatic embryogenesis and applications in plant breeding. *Euphytica* 81: 93-107.

Raemakers, C., Rozenboom, M., Danso, K., Jacobsen, E., Visser, R. and Thro, A. 1997. Regeneration of plants from somatic embryos and friable embryogenic callus of cassava (Manihot esculenta Crantz). *Afr. J. Root Tuber Crop.* 2(1-2): 238-242.

Rahman, A. M. and Akter, M. 2013. Taxonomy and Medicinal Uses of Euphorbiaceae Spurge Family of Rajshahi, Bangladesh. *Res. Plant Sci.* 1(3): 74-80.

Rai, C., Bapu, J. and Nanda, H. 1986. Correlation and path studies in cassava. *J. Root Crop*. *12: 202-205.*

Raji, A., Ladeinde, T. and Dixon, A. 2007. Agronomic traits and tuber quality attributes of farmer grown cassava landraces in Nigeria. *J. Trop. Agric.* 45 (1-2): 9–13.

Rao, C.R. 1948. The utilization of multiple measurement in problems of biological classification. *J. Roy. Stat. Soc.* 10-B: 159-203.

Rao, C.R. 1952. *Advanced Statistical Methods in Biometrical Research*. John Wiley and Sons. Inc., New York.

Raut, S., Berkel, V., Bokelmann, G. and Broertjes. 1980. The use of an in vitro adventitious bud technique for mutation breeding of begonia hiemais. *Euphytica* 30: 381-388.

Rekha, V., Nair, P., Sreekumar, S., Asan, B. and Pillai, M. 1991. Path analysis of yield components in a few cassava cultivars. *J. Root Crop.* 17: 35-38.

Reed, B.M., Engelmann, F., Daloo, M.E. and Engels, J.M.M. 2004. *Technical guidelines for the management of field and in vitro germplasm collections*. IPGRI Handbooks for Genebanks No.7. Rome. 106p.

Roca, W. 1984. Cassava. In Sharp, W., Evans, D., Ammirato, P. and Yamada, Y. (eds.) *Handbook of Plant Cell Culture, Vol. 2. Crop Species*. MacMillan. New York. pp. 269–301.

Roest, S., Van Berkel, M., Bokelmann, G. and Broertjes, C. 1980. The use of in vitro adventitious bud technique for mutation breeding of Begonia hiemalis. *Hawaiian Plant. Rec.* 58: 293-314.

Rossin, C. and Rey, M. 2011. Effect of explant source and auxins on somatic embryogenesis of selected cassava (Manihot esculenta Crantz) cultivars. *South Afr. J. Bot.* 77: 59–65.

Roy, A. and Panwar, D. 1993. Genetic divergence in rice. *Oryza,* 30: 197-201.

Roychowdhury, R. and Tah, J. 2013. Mutagenesis—A Potential Approach. In Hakeem, K. R. (ed.). *Crop Improvement*: Springer Science and Business Media. West Bengal. pp. 149-187

Ruimei, L., Xinwen, H., and Jianchun, G. 2009. Simple and Efficient Methods of Cassava Explant Disinfection. *Genom. Appl. Biol.* 04.

Saborio, F., Torres, S. and Gomez, L. 1998. Development of a clean planting material production system on tropical root and tuber crops, using in vitro propagated plants. *Acta Hort.* 461: 495-501.

Sadasivam, S. and Manickam, A. 1997. *Biochemical Methods*. New Age International Ltd. New Delhi.

Saelim, L., Phansir, S., Netrphan, S., Suksangpanomrung, M. and Narangajavana, J. 2006. Optimization of in vitro cyclic somatic embryogenesis and regeneration of the Asian cultivars of casava (Manihot esculenta crantz) for genetic manipulation system. *Global J. Biotechnol. Biochem.* 1: 7-15.

Safo-Kantanka, O., Owusu-Nipah, J., Acquistucci, R., Osei-Minta, M., Aubyn, A. and Quansah, C. 1997. Contribution to the understanding of the cooking quality of cassava. *Improvement of basic food crops in Africa through Plant Breeding, including the use of induced mutations*. IAEA. Vienna. pp. 121-129.

Sambandamurthi, S. 1983. *Studies on induced mutations in tuberose (Polianthes tuberosa L.*). Ph.D. thesis. Tamil Nadu Agricultural University. Coimbatore. 348p.

Santana, M. A., Romay, G., Matehus, J., Vicente-Villardón, J. L., and Demey, J. R. 2009. A simple and low-cost strategy for micropropagation of cassava (Manihot esculenta Crantz). *Afr. J. Biotechnol.* 8(16): 3789-3897.

Sheela, M., Taylor, M., Kechavachandran, R., Nazeen, P., Girija, D. and John, P. 2007. development of transgenic cassava: optimization of protocol for Agrobacterium mediated gene transfer. *ICAR National Symposium on Biotechnological inventions for Improvement of Horticultural Crops*. New India Publishing Agency. Vellanikkara. pp. 739-745

Sinclair, R. 1998. Historical changes in harvest index and crop nitrogen accumulation. *Crop Sci*. 38: 638-643.

Singh, R.K. and Chaudhary, B.D. 1985. *Biometrical Methods in Quantitative Genetic Analysis* (3rd Ed., Reprint, 2012). Kalyani Publishers. New Delhi. 318p.

Sivasubramanian, S. and Madhava Menon, P. 1973. Genotypic and phenotypic variabilty in rice. *Madras Agric. J.* 60: 1093-1096.

Smith, M., Biggs, B. and Scott, K. 1986. In vitro propagation of cassava (Manihot esculenta Crantz). *Plant Cell Tissue Organ Cult.* 6: 221–228.

Sofiari, E., Raemakers, C., Bergervoet, J., Jacobsen, E. and Visser, R. 1998. Plant regeneration from protplasts isolated from friable embryogenic callus of cassava. *Plant Cell Rep.* 18: 159-165.

Sreekumari, M. and Abraham, K. 1985. Variation and correlation studies in Chinese potato (Coleus parvifloris, Benth). In Ramanujam, T., Rajendran, P., Thankappan, M. Balagopal, C. and Nair, K. (eds.). *Tropical Tuber Crops: Production and Utilization*. Indian Society for Root Crops. Sreekaryam. pp. 77-82.

Stamp, J. and Henshaw, G. 1982. Somatic embryogenesis in cassava. *Z Pflanzenphys.* 105:183-187.

Stamp, J. and Henshaw, G. 1986. Adventitious Regeneration in Cassava. In Alderson, P. and Withers, L. (eds). *Plant Tissue Culture and Its Agricultural Applications*. Butterworths. London. pp. 149-157.

Suja, G., John, K., Sreekumar, J. and Srinivas, T. 2010. Short-duration cassava genotypes for crop diversification in the humid tropics: growth dynamics, biomass, yield and quality. *J. Sci. Food Agric.* 90(2): 188-98.

Sunnino, A., Ancora, G. and Locardi, C. 1986. *In vitro* mutation breeding of potato: The use of propagation by micro cuttings and in vitro cultures for plant improvement. *Mutation Breed. Newsl.* 26: 18-22.

Sunnino, A., Ancora, G. and Locardi, L. 1984. *In vitro* mutation breeding of potato. *Mutation Breed. Newsl.,* 24: 9-10.

Suthanthirapandian, I., Jeeva, S. and Thamilarassi, R. 1994. Genetic variability for metric traits. *J. Root Crops*. 20: 12-14.

Szabados, L., Hoyos, R. and Roca, W. 1987. In vitro somatic embryogenesis and plant regeneration of cassava. *Plant Cell Rep.* 6: 248-251.

Takahashi, E., Kobayashi, A. and Vieira, L. 2000. Induction of cassava somatic embryogenesis in liquid medium associated to floating membrane rafts. *Braz. Arch. Biol. Technol*. 43: 35-39.

Taylor, N. J., Edwards, M., Kiernan, R. J., Davey, C. D., Blakesley, D. and Henshaw, G. G. 1996. Development of friable embryogenic callus and embryogenic suspension culture systems in cassava (*Manihot esculenta* Crantz). *Nat. Biotechnol.* 14: 726-730.

Taylor, N., Masona, M., Carcamo, R., Ho, T., Schopke, C. and Fauquet, C. 2001. Production of embryogenic tissues and regeneration of transgenic plants in cassava. *Euphytica,* 120: 25-34.

Tejaswini, S. C., Paramesh, H. and Tripathi, N. 2006. In vitro approaches for chemical mutagenesis in carnation (Dianthus caryophyllus). *Indian J. Genet.* 66(1): 71-72.

Toffiño, A., Cabal, D., Sánchez, T. and Ceballos, H. 2011. Identification of radiation induced mutants of cassava (Manihot esculenta Crantz) using morphological and physicochemical descriptors. *Agron. Colomb.* 29(2): 361-371.

Vanitha, S., Chaurasia, S., Singh, P. and Naik, P. S. 2013. Vegetable Statistics. *Technical Bulletin No. 51*. Indian Institute of Vegetable Research. IIVR. Varanasi. 251p.

Vasudevan, K. and Jos, J. 1990. Mutation breeding of several root and tuber crops. *Mutation Breed. Newsl.* 35: 105-112.

Vieira, A. E., Fialho, J., Faleiro, F., Bellon, G., da Fonseca, K., Carvalho, L., Silva, M.S., de Paula-Maraes, S.V., de Oliveira, C.M. and Denke, M.L. 2011. Characterization of sweet cassava accessions based on molecular, quantitaive and qualitative data. *Crop Breed. Appl. Biotechnol.* 11: 232-240.

Westby, A. 2002. Cassava Utilization, Storage and Small-scale Processing. In Hillocks, R., Thresh, J. and Bellotti, A. (eds.). *Cassava: Biology, Production and Utilization*. CAB International. London. pp. 281-300.

Woodward, B., and Puonti-Kaerlas, J. 2001. Somatic embryo from floral tissues of cassava (Manihot esculenta Crantz). *Euphytica*, 120:1–6.

Wright, S. 1921. Correlation and causation. *J. Agric. Res.* 200: 557-587.

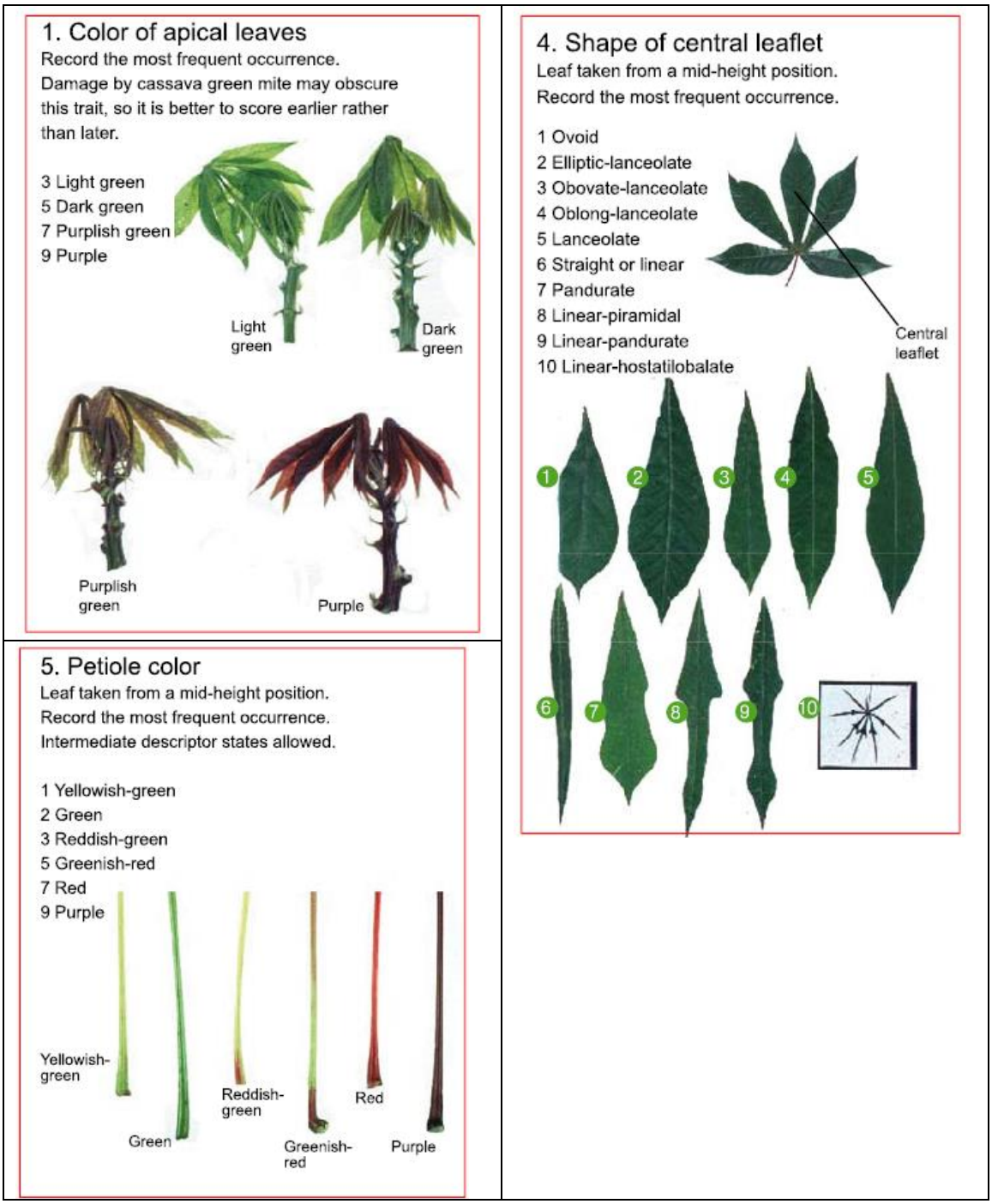
Zhang, P., Phansiri, S. and Puonti-Kaerlas, J. 2001. Improvement of cassava organogenesis by the use of silver nitrate in vitro. *Plant Cell Tissue Organ Cult.* 67: 47-54.

Ziv, M. 1986. In vitro hardening and acclimatization of tissue culture plants. In Withers, L. and Alderson, P. (eds.). *Plant Tissue Culture and its Agricultural Applications*. Butterworths. London. pp. 187- 196

Appendices

APPENDICES

Appendix 1: IITA cassava descriptor



IITA cassava descriptor scores - continued

20. Color of stem exterior

Observe on the middle third of the plant.

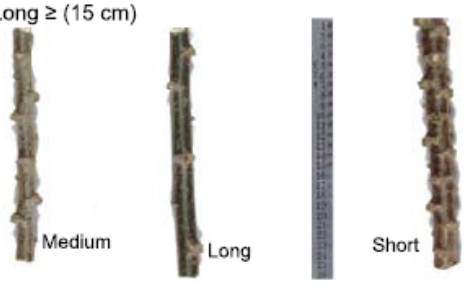
3 Orange
4 Greeny-yellowish
5 Golden
6 Light brown
7 Silver
8 Gray
9 Dark brown

21. Distance between leaf scars

Measure from the middle of stem on the middle third of the plant, where the scars are not flat. Make a measurement along the stem then divide the distance by the number of nodes in the measured part. Avoid taking measurements on mealy bug infected plants.

For conversion to qualitative data:

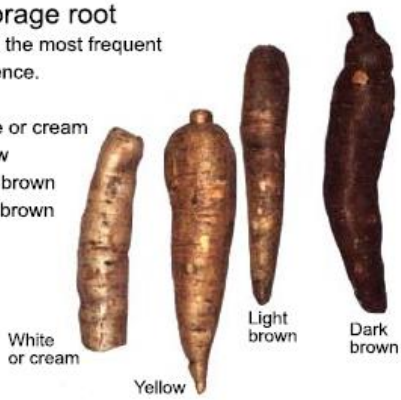
3 Short ≤ (8 cm)
5 Medium (8–15 cm)
7 Long ≥ (15 cm)



39. External color of storage root

Record the most frequent occurrence.

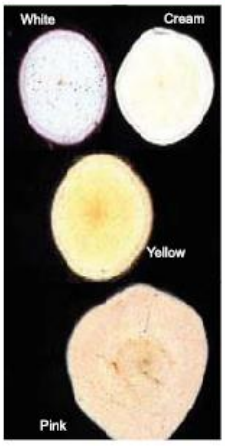
1 White or cream
2 Yellow
3 Light brown
4 Dark brown



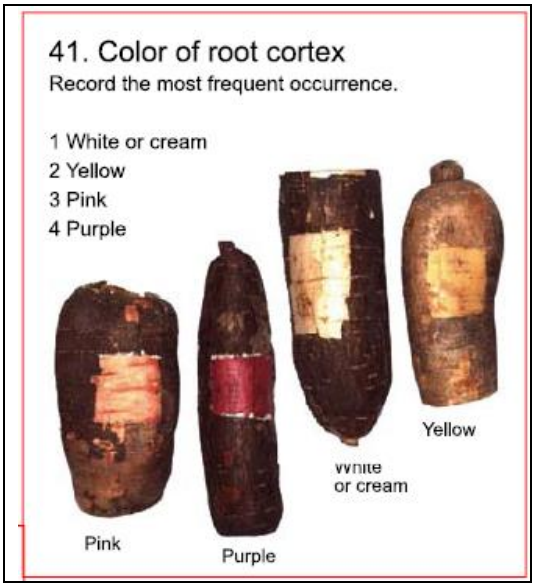
40. Color of root pulp (parenchyma)

Record the most frequent occurrence.

1 White
2 Cream
3 Yellow
4 Orange (no photo)
5 Pink



IITA cassava descriptor scores - continued



Appendix 2: Panel members or judges for organoleptic analysis

	Name	Occupation	Nationality
1	Dr. C.R. Elsy	Professor and Head of Department Plant Breeding	India
2	Dr. Gayathri G.	Assistant lecturer – Plant breeding	India
3	Mr. Abdu Ibrahim	MSc. student – Soil science	Eritreia
4	Mr. Abdul Razzaq	MSc. student – Floriculture	Afganistan
5	Mr. Anoop	Lab assistant - Agricultural meteorology	India
6	Mr. Emmanuel Zivenge	Ph.D student – Agricultural economics	Zimbabwe
7	Mr. Msgna Sium	MSc. student – Veterenary	Eritreia
8	Mr. Ramnarace Sukhna	MSc. student – Plantations	West Indies
9	Mr. Sahle Asghede	MBA student	Eritreia
10	Mr. Shiferaw Tebeka	Ph.D Rural Marketing	Ethiopia
11	Mrs. Gadha	Lab assistant – Plant breeding	India
12	Mrs. Mithu Thomas	Lab assistant – Molecular biology	India
13	Mrs. Shina	Lab assistant – Plant physiology	India
14	Mrs. Smitha P.S.	Lab assistant – Plant breeding	India
15	Mrs. Yaha Nakaande	MSc. Horticulture	Namibia
16	Ms. Lalitha M.V.	Lab assistant – Plant breeding	India

Appendix 3: Cassava plants derived from *in vitro* mutagenesis in greenhouse 5 to 10 months after transplanting

	
Genotype: CC1; Genesis of culture: Node Mutagen treatment: Control	Genotype: Sree Jaya; Genesis of culture: Somatic embryo Mutagen treatment: 1.2% EMS

Appendix 3 - continued

	
Genotype: Sree Jaya Genesis of culture: Somatic embryo Mutagen treatment: 0.3% EMS	Genotype: Sree Jaya Genesis of culture: Somatic embryo Mutagen treatment: 0.3% EMS

Appendix 3 - continued

	
Genotype: CC1 Genesis of culture: Node Mutagen treatment: Control	Genotype: Sree Jaya Genesis of culture: Somatic embryo Mutagen treatment: 0.3% EMS

Appendix 3 - continued

	
Genotype: Sree Jaya Genesis of culture: Somatic embryo Mutagen treatment: 1.2% EMS	Genotype: Sree Jaya Genesis of culture: Somatic embryo Mutagen treatment: 1.2% EMS

Appendix 3 – continued

Genotype: CC1

Genesis of culture: Node

Mutagen treatment: 0.6% EMS

ASSESSMENT AND INDUCTION OF VARIABILITY THROUGH *IN VITRO* MUTAGENESIS IN CASSAVA (*Manihot esculenta*, Crantz)

By
HILÁRIO ERNESTO MAGAIA
(2011-21-118)

THESIS

Submitted in partial fulfillment of the requirement for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture
Kerala Agricultural University

Department of Plant Breeding and Genetics
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA
2015

ABSTRACT

The study entitled "Assessment and induction of variability through *in vitro* mutagenesis in cassava (*Manihot esculenta*, Crantz.)" was carried out between 2012 and 2014 in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara. The objective of the study was to assess the genetic variability in the short duration cassava germplasm and induction of variability through *in vitro* mutagenesis in selected genotypes. Field evaluation, standardization of protocols for *in vitro* regeneration, *in vitro* mutagenesis and assessment of variability of *in vitro* mutated plants were done.

Wide genetic variability existed among the collected short duration cassava genotypes. The colour of petiole and root cortex was found to be the most variable qualitative trait for above ground and tuber portions respectively. High magnitude of phenotypic and genotypic coefficient of variation along with high heritability and high genetic gain was observed for branch number, branch height, tuber fresh weight, and Cassava Mosaic Disease. All traits except tuber neck, branch number, scar number and internode length were positively correlated with fresh tuber yield. High direct contribution towards tuber yield was exerted by shoot biomass, tuber dry matter content and harvest index indicating that these are reliable predictor variables for increased yield.

Among the biochemical traits, high heritability and high genetic gain was observed for HCN content, amylose content and starch content. Biochemical analysis indicated the occurrence of high starch genotypes (Sree Jaya, CC10 and CC7) suitable for industrial starch production. Vellayani Hraswa with lower starch content was more suited for food or feed purposes, than industrial purposes. The genotype CC10 with easiness in peeling, good taste after cooking and less cooking time scored maximum in organoleptic evaluation. Sree Jaya was sweet on chewing, had highest starch content, lowest HCN content and less fibre content.

Diversity analysis indicated that the cassava genotypes grouped into five clusters. No parallelism was found to occur between geographic distribution and genetic diversity. Selection index constructed for the identification of the best genotypes indicated that CC1 and Sree Jaya were the most promising genotypes. CC1, a farmers' variety from Malappuram district was found to be the best genotype with respect to yield and resistance to CMD, but with a comparatively high HCN content. *In vitro* mutagenesis in cassava was done using the genotypes CC1 and Sree Jaya.

Sterilization of cassava nodal and leaf explants was accomplished by washing with 5 per cent Teepol solution for two minutes, followed by washing for one minute with 75 per cent ethanol and washing for one minute with 0.05 per cent solution of mercuric chloride. Friable embryogenic callus (FEC) for both CC1 and Sree Jaya genotypes was obtained from immature leaf explants cultured in MS media with 3.0 per cent sucrose (MS3), either with 6.0 to 8.0 mg l^{-1} of 2,4-D, or with 1.0 mg l^{-1} BAP + 0.2 to 0.5 mg l^{-1} NAA. Somatic embryos for both genotypes were obtained from FEC cultured in media MS3 with 8.0 to 10.0 mg l^{-1} picloram and germinated into the plantlets on MS3 media with 4.0 mg l^{-1} BA or 0.25 mg l^{-1} TDZ. *In vitro* regeneration and multiplication from nodal explants were obtained in MS3 media containing either 0.25 mg l^{-1} TDZ or 2.0 mg l^{-1} BAP. Rooting of the *in vitro* plantlets was obtained in MS3 + 0.25 mg l^{-1} TDZ or 1/2MS1.

The LD 50 value varied with the cultures used for *in vitro* mutagenesis. The LD 50 value for gamma radiation was 40 Gy, 30 Gy and 50 Gy for FEC, somatic embryoids and plantlets, respectively. LD 50 value for EMS was 1.20 per cent for FEC and somatic embryoids and 0.90 per cent for plantlets. Variation in response to mutagenesis was also observed between the two genotypes subjected to *in vitro* mutagenesis. There was significant difference in the growth characteristics of the mutagen treated *in vitro* cultures in both genotypes. Reduction of the number of shoots and leaves were more in CC1 compared to Sree Jaya.

A combination of SoilriteTM with pure sand at 1:1 proportion was the best substrate for acclimatization of the plantlets outside the tissue culture lab. Fan and pad green house was the best structure for in vitro acclimatization of plantlets resulting in 47 per cent of success rate. Variability with respect to quantitative traits like height, number of shoots and number of leaves was observed in vitro plantlets in the hardening stage. The qualitative traits like colour of the petiole, stipule, emerging leaf and of the stem and the shape of central lobe of leaves varied between the mutated plants.