IN VITRO MUTAGENESIS AND EVALUATION OF SOMATIC EMBRYO DERIVED PLANTLETS IN CASSAVA (*Manihot esculenta* Crantz.)

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"*In vitro* mutagenesis and evaluation of somatic embryo derived plantlets 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.

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24-09-2016

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CERTIFICATE

Certified that this thesis entitled **"*In vitro* mutagenesis and evaluation of somatic embryo derived plantlets in cassava (*Manihot esculenta* Crantz.)"** is a record of research work done independently by Riya Antony (2014-11-117) 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 her.

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LIST OF ABBREVIATIONS

2,4-D	-	2,4-Dichlorophenoxyacetic acid
BA	-	Benzyl Amino purine
$CaCl_2$. $2H_2O$	-	Calcium chloride dehydrate
CG	-	Cyanogenic Glucocides
CLS	-	*Cercospora* Leaf Spot
CMD	-	Cassava Mosaic Disease
cm	-	Centimeter
Co_{60}	-	Cobalt 60
$CoCl_2$. $6H_2O$	-	Cobalt chloride hexa chloride
CRD	-	Completely randomized design
CSS	-	Cumulative Sensory Score
CTCRI	-	Central Tuber Crops Research Institute
$CuSO_4$	-	Copper sulphate
DM	-	Dry Matter
EDTA	-	Ethylene Diammene Tetra Acetic Acid
EMS	-	Ethyl Methane Sulphonate
FAO-IAEA	-	Food and Agricultural Organization- International Atomic Energy Association
$FeSO_4$	-	Iron sulphate
FEC	-	Friable embryogenic callus
FFEC	-	Fresh Friable embryogenic callus
Gy	-	Gray
Ha	-	Hectare
H_3BO_3	-	Boric acid
HCl	-	Hydrochloric acid
HCN	-	Hydrogen cyanide

HI	-	Harvest Index
IBA	-	Indole-3-Butyric Acid
IITA	-	International Institute of Tropical Agriculture
J	-	Joule
Kg	-	Kilogram
KI	-	Potassium iodide
KH_2PO_4	-	Potassium hydrogen phosphate
KNO_3	-	Pottasium nitrate
LAF	-	Laminar Air Flow
LD_{50}	-	Lethal Dose 50
µM	-	Micromolar
$\mu Mm^{-1}s^{-1}$	-	Micromolar meter per second
MAP	-	Months after planting
mg	-	Milligram
$MnSO_4.4H_2O$	-	Magnisum sulphate tetra hydrate
$MgSO_4.\ 7H_2O$	-	Magnisum sulphate hepta hydrate
Ml	-	Milliliter
$mg\ L^{-1}$		Milligrams per liter
MS media	-	Murashige and Skoog media
NAA	-	Naphthalene acetic acid
Na_2EDTA	-	Di-sodium ethylene diammene tetra acetic acid
NaOH	-	Sodium hydroxide
Na_2MoO_4	-	Sodium molybedate
NH_4NO_3	-	Ammonium nitrate
No.	-	Number
PAR	-	Photosynthetically Active Radiation

RH	-	Relative humidity
Sl no.	-	Serial number
SA	-	Sodium Azide
SE	-	Somatic embryo
i.e.	-	That is
TDZ	-	Thidiazuron
t ha^{-1}	-	Tonne per hectare
TG	-	Tuber girth
V2, V4	-	Vegetative stage 1, Vegetative stage 2
viz.	-	Namely
WP	-	Wettable Powder
$ZnSO_4$. $7H_2O$	-	Zinc sulphate hepta hydrate

Dedication

Dedicated to my Father, Mother, brother, Teachers with humble reverence to Almighty

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Introduction

1. INTRODUCTION

Cassava (*Manihot esculanta* Crantz.) is a nutritionally important root crop grown in tropics and is the most widely grown staple crop in the world after maize. Every year the global production is expanding, on an average, by 1.2 per cent (Ford, 2015). However its production has been hampered by low yield due to biotic and abiotic stresses, poor agronomic practices and lack of new improved clones. A number of biological constraints including slow multiplication rate, rapid drying of stakes, requirement of bulky planting material for cultivation and incidence of cassava mosaic disease (CMD) and root rot hinder the yield improvement. Hence, it is important to address these issues through properly oriented research programmes to sustain high productivity.

Majority of cassava cultivars mature in ten months. This restricts cassava as a monocrop in Kerala, especially in the uplands. However, at present, more and more cultivation is being undertaken in low lying areas in rotation with short duration rice varieties (Abraham *et al*., 2002). Development of early maturing varieties with qualities like stress resistance and good cooking quality, enable their effective utilisation in cassava-rice double cropping system practised in Kerala (Nair *et al.*, 1996 and Nair and Unnikrishnan, 2007).

The conventional propagation method of cassava is by stem cuttings. This propagation method leads to the accumulation of viral and bacterial diseases which reduce productivity of the crop and may cause loss of superior genotypes (Nassar and Ortiz, 2007). The major breeding objectives in cassava are to generate clones with high yield, low HCN content, early maturity and resistance to cassava mosaic disease. Crop improvement programmes rely mainly on genetic variation available in crops. Cassava seeds are normally dormant and germinate very slowly. As a result stem cuttings are used for propagation. Being a clonally propagated exotic plant, the variability in cassava natural populations is expected to be low. Hence, creation of variability is the prime step for further crop improvement programmes.

Hybridisation programmes in cassava is however, severely constrained by reproductive and breeding barriers, such as low fertility, low hybrid seed set and poor germination rate (Nassar, 2001). Hence the breeder has to adopt alternative ways to increase genetic variability.

One of the alternate strategies is the use of induced mutation techniques. Induced mutagenesis holds promise for the subtle manipulation of traits of interest in crop plants. The frequency of obtaining desired variability is more when mutation is done *in vitro.* As *in vitro* techniques have been standardized in cassava, genetic improvement adopting this methodology seems more desirable. Cassava breeding through *in vitro* mutagenesis will speed up the time needed to produce new clones compared to currently used approaches.

Mutagenesis in cassava was attempted by several researchers to improve the dry matter content, cooking quality, starch yield and to reduce HCN content. In addition, there are a few reports on variation in several morphological characters between the mutagen treated genotypes of cassava.

Giving due consideration to the role of *in vitro* mutagenesis in inducing genetic variability in cassava, the present investigation entitled '*In vitro* mutagenesis and evaluation of somatic embryo derived plantlets in cassava (*Manihot esculanta* Crantz.)' was taken up with the following objectives:

- To evaluate the plantlets already derived from mutagen treated callus through somatic embryogenesis
- To undertake *in vitro* mutagenesis to create further variability

Review of literature

2. REVIEW OF LITERATURE

Cassava (*Manihot esculanta* Crantz.) is an energy efficient crop which produces more starch/ energy per unit area. It is regarded as a poor man's crop and considered as a crop with immense potential to alleviate world hunger. In countries where malnutrition is widely spread, it is a rich source of energy and acts as valuable source of calories, after rice, sugarcane and maize (Scott *et al*., 2000; Ceballos *et al.,* 2004). Variability is limited in cassava as it is a vegetatively propagated crop with slow and tedious multiplication rate (Santana *et al.,* 2009; Taye, 1998). High disease susceptibility and inadequate high yielding varieties of the crop are the key challenges to the expansion of cultivation. The bulkiness of planting material, its low propagation rates, high distribution costs, and high perishability magnifies the challenges (Mahngu *et al.,* 1983; Escobar *et al.,* 2006) of cassava production. Therefore, efforts geared towards reducing or removing these limitations and genetic improvement are inevitable.

In vitro techniques provide a back up support to plant breeding and have created a new paradigm in the use of mutations in crop improvement. The determination of radiosensitivity tests followed by irradiation with optimal doses and multiplication of irradiated material through tissue culture has assumed a new dimension in crop improvement, especially in vegetatively propagated crops (Ahloowalia and Maluszynski, 2001). Hence, mutation breeding has immense scope in the improvement of cassava. The brief review of literature available on the above mentioned factors is detailed under the following heads:

2.1. Genetics and propagation

Cassava is a heterozygous, cross pollinated crop (2n = 2x = 36) which is propagated by vegetative means. Erect, non-branching types, are frequently preferred by farmers. Cassava can either be propagated by stem cuttings; the most common practice used by farmers for multiplication and planting purposes, or by sexual seed (Alves, 2002). The only significant contribution of true cassava seeds is towards plant breeding works (Allem, 2002). However, as starting point for the

generation of useful genetic diversity, propagation from true seed occurs occasionally in farmers' fields (Alves, 2002). Most breeding programmes generate seed through crossing as a means of creating new genetic variation. Rarely botanical seed is used in commercial propagation schemes in cassava (Rajendran *et al.,* 2000).

Flowering in cassava depends on the genotype and the environmental conditions. Cassava is monoecious, with female flowers opening 10-14 days before the male ones on the same branch. But self pollination can also occur because of simultaneous opening of male and female flowers on different branches or on different plants of the same genotypes (Jennings and Iglesias, 2002). Being a cross pollinated species, repeated selfing can result in inbreeding depression (Ceballos *et al.,* 2004).

There are several constraints that make conventional breeding in cassava difficult. Scarcity of flowers, lack of synchrony in flowering, and the long time taken for seed maturation (it takes generally not less than a year to obtain seeds of a planned cross) are some of the constraints in producing hybrids. The trilocular fruit formed, possesses only about one to two seeds per pollination. Seeds often have a few months of dormancy and they require relatively high temperatures (30-35 °C) for optimum germination (Ellis *et al.,* 1982).

2.2. Cassava breeding: Opportunities

Cassava roots are not true tubers and, therefore, cannot be used for reproductive purpose (Beeching *et al.,* 1998). Besides tubers, every part of the plant can be utilized. But, except for cassava seed every tissue possesses the antinutritional cyanogenic glucosides (CG). So cassava breeding can be oriented to reduce the presence of CG. In addition to reduction in the cyanogen content breeding in cassava aims to improve dry matter content, percentage of amylose in the starch composition, protein and carotenoid contents which determines the root quality.

Another major constrain is the rapid initiation of post harvest physiological deterioration (PPD) leading to low shelf life (Beeching *et al.,* 1998). Till date, little useful genetic variation to delay or reduce PPD has been found and is one of the most important goals for cassava research.

Cassava was mainly grown as an upland crop till 1970s, but later it gained importance in low land areas by replacing paddy. At present, cassava shows a declining trend in upland cultivation as a result of competition from more remunerative crops like rubber and coconut (Abraham *et al.,* 2002). With gradual reduction in the monoculture in uplands (Vanitha *et al.,* 2013) and progressing cultivation in low lying areas after main crop, there is a need for improved short duration varieties.

Traditional breeding resulted in genetic improvements in this crop through the introgression of bacterial and virus resistance into the cassava germplasm (Hahn *et al.,* 1980; Okogbenin *et al,*. 2007) along with other useful traits (Chávez *et al.,* 2005; Ceballos *et al.,* 2007; Morante *et al.,* 2010; Rudi *et al.,* 2010). Further variability can be expanded by using modern breeding methods like mutation breeding.

2.3. *In vitro* propagation in cassava

Plant breeding is based on creating variation, selection, evaluation and multiplication of desired genotypes. Genetic make-up of cassava can be improved through genetic manipulation by *in vitro* propagation. Tissue culture has been exploited for rapid clonal multiplication, transformation and conservation of elite clones of cassava with higher yields (Garcia *et al.,* 1993; Jorge *et al.,* 2000; Onuoch and Onwubiku, 2007; Staden *et al.,* 2008).

Micropropagation techniques have been employed to produce high yielding, disease-free and resistant planting materials, even from infected mother plants (Acedo, 2006). In addition to this, *in vitro* techniques can produce a cultivar that will retain its distinguishing characteristics. Besides being a faster and

convenient system, *in vitro* propagation facilitates the storage as well as international exchange of cassava germplasm (Smith *et al.,* 1986; Onuch and Onwabikur, 2007). *In vitro* propagation can be carried out regardless of growing season. Hence, this propagation method enables the year round availability of planting materials and thus ensures the expansion of production.

In vitro propagation helps to overcome the constraints of environmental conditions, planting season and phytosanitory conditions of propagation (Santana *et al*., 2009). Hence, under optimum conditions, micropropagation acts as a cheap and simplified laboratory technique for cassava production.

According to Mapayi *et al.* (2013), for *in vitro* cultures the use of full strength MS resulted in better performance of cassava *in vitro* cultures for all the traits measured than those with half strength. Semi-solid media enhanced the regeneration of recalcitrant cassava better than liquid medium. In addition, the semi solid media showed no degeneration in culture compared to the liquid medium (Mapayi *et al.*, 2013).

2.4. Establishment of *in vitro* cultures

Callus induction is a complicated biological process regulated by genetic and epigenetic mechanisms of cell differentiation and dedifferentiation (Ikeuchi *et al.*, 2013). Any part of the plant can be used as the explants. In general, maximum callus induction in cassava was recorded for leaf followed by stem and root explants (Neama *et al.,* 2013). The induction of adventitious shoots (Guohua and Qiusheng, 2002) and primary somatic embryos from immature leaves (Mathews *et al.,* 1993; Raemakers *et al.,* 1993a; Stamp and Henshaw, 1987a; Szabados *et al.,* 1987) or zygotic embryos (Konan *et al.,* 1994; Stamp and Henshaw, 1982) has been also described in cassava. Similarly, in a culture medium supplemented with an auxin like herbicide such as Picloram or 2,4-D, primary and secondary somatic embryos (SEs) are easily achieved by incubating young leaf lobes or isolated shoot apices of cassava (Taylor *et al.,* 1996; Bull *et al.,* 2009).

The first attempt to initiate *in vitro* callus in tuber crops was achieved by Chapman (1955) in potato. Under a suitable condition, friable embryogenic callus (FEC) clusters of cassava can be induced from secondary SEs by continuous incubation on a growth and development medium supplemented with 50µM Picloram (Taylor *et al.,* 1996; Bull *et al.,* 2009). The process includes several steps *viz.,* primary SE induction, secondary SE multiplication, fresh FEC (FFEC) induction, and FEC subculturing and multiplication (Zhang, 2000). No change in ploidy levels of FEC was observed during subculture (Ma *et al.,* 2015). Further, primary somatic embryos can be used as explants to initiate secondary somatic embryos (Mathews *et al.,* 1993; Raemakers *et al.,* 1993b; Stamp and Henshaw, 1987b). Primary somatic embryos can be induced to produce secondary somatic embryos by further subculturing on auxin containing medium (Saelim *et al.,* 2006).

While attempting cassava somatic embryo induction, Joseph *et al.,* (2004) callus formation was observed on the adaxial surface of most of the leaf lobes after 7 days in culture. Successful long-term embryogenic cultures (Woodward and Puonti-Kaerlas, 2001) with high regeneration potential and cyclic secondary somatic embryogenic systems (Joseph *et al.,* 1999a; Groll *et al.,* 2001; Danso and Ford-Lloyd, 2002) have been established in several varieties of cassava.

In vitro callus induction was not only dependent on plant species but also on type of explants, light, temperature and explants age (Salehi *et al.,* 2008; Mohebodini *et al.,* 2011; Shirin *et al.,* 2007).

MS medium supplemented with different concentrations of growth regulators was used in various tuber crops for callus shoot and SE induction, SE regeneration and plantlet regeneration in various tuber crops are presented in Table 1.

Table 1. Tissue culture media for callus, shoot and SE induction, SE regeneration and plantlet regeneration in tuber crops

***In vitro* response**	**Medium**	**Crop**	**Author**
Callus	MS + 15 mg L^{-1} 2,4-D	Cassava	Neama *et al.*, 2013
Callus	MS + 2.0-5.0 mg L^{-1} 2,4-D	Cassava	Khalafalla *et al.*, 2010
Callus	2.5 mg L^{-1} NAA + 2 mg L^{-1} BAP.	Cassava	Yasmin *et al*,.2003
Callus Shoot	MS + 3.0 mg L^{-1} 2,4-D MS + 2.0 mg L^{-1} 2,4-D + 2.0 mg L^{-1} BA. MS + 5 mg L^{-1} TDZ	Cassava	Abd-Elaleem *et al.* 2009
Shoot	MS + 0.25 mg L^{-1} TDZ	Cassava	Magaia, 2015
Shoot	2.46 µM NAA + 1.0 µM TDZ	Potato	Sajid, 2010
Shoot	MS + 0.5-1.0 mg L^{-1} NAA	White yam	Nwachukwu *et al.*, 1997
Callus and SE	MS + 50 µM Picloram	Cassava	Taylor *et al.*, 1996 Bull *et al.*, 2009
SE	MS + 8 mg L^{-1} 2, 4-D	Cassava	Guohua *et al.*, 1998
SE	MS + 8 mg L^{-1} Picloram MS + 11 mg L^{-1} Picloram	Cassava	Guohua *et al.*, 1998 Li *et al.*, 1995 Taylor *et al.*, 2001 Zhang and Puonti-Kaerlas, 2005 Feitosa *et al.*, 2007 Li *et al.*, 1998 Saelim *et al.*, 2006
SE germination	MS + 5 mg L^{-1} BA + 0.1 mg L^{-1} NAA	Cassava	Le *et al.*, 2007
SE germination	MS + 0.25 mg L^{-1} TDZ MS + 4 mg L^{-1} BA	Cassava	Magaia, 2015
Plantlets	MS + 2.0 mg L^{-1} BAP and 0.1 mg L^{-1} NAA	White yam	Nwachukwu *et al.*, 1997

2.5. Establishment of *in vitro* regeneration system

In breeding programmes, implementation of genetic manipulation at molecular and cellular level from somatic tissues requires an efficient

regeneration system. Cassava being vegetatively propagated crop requires efficient plant regeneration from vegetative propagated tissues of well characterized clones (Puonti-Kaerlas *et al.*, 1997; Li *et al.*, 1998).

Embryogenesis, organogenesis and germination are the major types of *in vitro* regeneration systems in cassava, which can be either direct or indirect. Nodes, axillary buds or meristems are used as explants for organogenesis and biological seed for germination. Plant regeneration from immature leaf explants of *in vitro* grown cassava *via* somatic embryogenesis was reported by Le *et al*. (2007). Direct embryogenesis gives rise to hard callus or friable embryogenic callus (FEC) whereas indirect embryogenesis gives rise to FEC *via* secondary embryogenesis (Konan *et al.*, 1994, 1997; Raemarker *et al.*, 1997; Fregene *et al.*, 2000). Secondary somatic embryogenesis was observed by Wongtiem *et al.* (2011) in cassava from fragments of cotyledon-stage somatic embryos which were subcultured on to MS medium supplemented with 2, 4-D.

Although regeneration of cassava through embryogenesis is a standard procedure for some cultivars, it is highly genotype dependent. So there is need of alternative regeneration systems, so that more number of cassava cultivars will be made accessible to regeneration protocols (Iwanaga and Iglesias 1994; Ng *et al.* 1994). Regeneration *via* both organogenesis and somatic embryogenesis in cassava has been reported earlier. Even though, shoot formation through organogenesis was achieved from mesophyll protoplast cultures (Shahin and Shepard, 1980) and stem callus (Tilquin, 1979), the results were not reproducible. On the other hand, regeneration through somatic embryogenesis is well established and has been used successfully by many workers (Stamp and Henshaw 1982, 1987a, 1987b; Szabados *et al.* 1987; Mathews *et al.* 1993; Raemakers *et al.* 1993a; Konan *et al.* 1994).

2.6. *In vitro* mutagenesis

The most important processes contributing towards variability in plants are mutation, hybridization, and recombination, of which mutation is the primary

cause. However, the naturally occurring mutation rate is too low for its practical application in crop improvement (Donini and Sonnino, 1998). Therefore, subtle manipulation of traits of interest in crop plants to enhance genetic variability exploitable for the improvement of agronomic traits such as disease resistance, earliness in bulking, yield and quality (Lee *et al.,* 2003; Guthrie, 2000; El-Sharkawy, 2004; Owoseni *et al.,* 2006) can be carried out by means of induced mutagenesis.

Induced mutations is an effective method to enhance genetic variability and have been used in developing improved cultivars of fruits, cereals and other crops (Lee *et al.,* 2002). Breeders have relied on physical and chemical mutagens to induce mutations and generate genetic variability with relative success, particularly during the 1950s and 1960s (Ahloowalia and Maluszynski, 2001; Ahloowalia *et al.,* 2004). The induction of mutation and selection of mutants provide a efficient, simple, rapid and cheap method by which the genetic make-up can be altered to obtain well-adapted genotypes. Hence, induced mutation techniques in combination with *in vitro* culture methods offer great potential in crop improvement (Lee *et al.,* 2003).

Database on FAO-IAEA mutant varieties listed about 3,100 mutants of 190 plant species including cassava released since 2009 (Asare and Safo-kantanka, 1997: Nwachukwu *et al.,* 1997; Shu *et al.,* 2011). Mutation breeding approach in cassava has been practiced in India by Central Tuber Crops Research Institute (CTCRI) in Trivandrum, for the development of CMD resistant varieties and low level of HCN (Abraham *et al.,* 2002).

Mutation can be induced by using both chemical and physical mutagens. Gamma irradiation of somatic embryos produced through the process of cyclic embryogenesis would be a possible approach for inducing mutations in cassava (Joseph *et al*., 2004). In several induced mutant plants, morphological differences were observed with respect to both quantitative and qualitative traits in storage organs as well as in starch biosynthesis (Ancora and Sonnino 1987; Smith and

Martin 1993; Coleman *et al.,* 1995). In cassava, all the *in vitro* mutated cultures at maturation, desiccation, germination and plant regeneration stages were kept at 25°C with a photoperiod of 16 hours (fluorescent tubes, Sylvania cool white, 90-110 $\mu Mm^{-1}s^{-1}$ PAR) and 60 per cent relative humidity (Mathews *et al.,* 1993).

2.7. Sensitivity of tissues to mutation and dose response of tissues

The success of mutation breeding depends on determination of the sensitivity of the plant material to the mutagen. So the critical factor for the success of mutation induction is the choice of irradiation doses. For instance, at higher doses mutagen may produce high frequencies of mutation but possibly accompanied by lots of undesirable mutations in several segments of the genome (Ndofunsu *et al.,* 2015).

Irradiation dose is the amount of radiation energy absorbed by the material. The unit of measurement of radiation dose is Gray (Gy). One Gy is equal to the absorption of 1 J of energy per kilogram of product irradiated. Mutation frequency usually increases linearly with increasing dose of mutagenic treatment, but survival and regeneration capacity decreases. Additionally, mutation induction aims to optimize genetic variation with minimal plant injuries so as to maintain a balance between achieving mutagenesis and maintaining the integrity of the majority of the genome constitution of mutated material. While determining an optimal dose for treatment, these two aspects should be considered (Ndofunsu *et al.,* 2015).

The chosen dose of mutagen should ensure the highest survival of the irradiated explants with low rate of inhibition of new shoots (Laneri *et al.,* 1990; Magaia, 2015). Based on the study on survival rate of mutagen treated *in vitro* cultures of cassava by Magaia *et al.* (2015), the LD_{50} values for somatic embryos were fixed as 30Gy for gamma radiation and 1.20 per cent for EMS. Similarly, for mutagen treated plantlets LD_{50} value was fixed at 50Gy for irradiation and 0.90 per cent for EMS.

2.8. Irradiation of *in vitro* cultures

Mutation techniques in combination with tissue culture methods provide a powerful technology to improve clonally propagated plants. Induced mutations upgrade well established clones by changing specific traits. Irradiation of micropropagated plants, axillary and adventitious buds, apical meristems, regenerative callus cultures, anthers and microspores, and somatic embryos provide a miniaturized version of trees and seeds in the petri dish instead of the field (Ahloowalia and Maluszynski, 2001).

Induced mutations have been previously attempted in cassava as a means to improve the dry matter content, starch content and cooking quality (Asare and Safo- Kantanka 1997; Nwachukwu *et al.,* 1997). Genetic variations such as chromosomal aberrations or even structural mutations of some genes caused by γ-irradiation may be the reason for overall changes observed in different mutant lines (Lee *et al.,* 2002).

Gamma rays have been reported to affect the morphology, anatomy, biochemistry and physiology of plants, resulting in changes in plant growth and development. The toxic effect of gamma radiation is attributed to the observed low number of leaves and nodes, and length (Suprasanna *et al.,* 2008) of *in vitro* mutated cultures.

The limited number of available reports suggest that callus cultures are much more sensitive to radiation treatment, and require much lower doses (2 to 5 Gy) than stem cuttings or seeds; which require relatively higher doses (15 to 20 Gy). Callus turns necrotic or loses their regenerative capacity under exceeded doses (Ahloowalia *et al.,* 2004). Regenerative callus cultures of date palm above 25 Gy have very poor survival. In potato micropropagated plants, 20 Gy dose gives optimal survival (IAEA, 1998). Hence, the irradiation dose LD_{50} which is not highly inhibitory to plant development, is recommended (Zhou *et al.,* 2006) for inducing mutation.

2.9. *In vitro* mutagenesis using EMS

Mutations are carried out to induce variation in general as well as with respect to specific traits such as pest and disease resistance, salt tolerance, drought tolerance and other traits. Luan *et al.* (2007) obtained salt tolerant lines of sweet potato (*Ipomoea batatas* L.) on callus using EMS. The mutants of chrysanthemum recorded a wide range of variations in petal colour (pink-salmon, light-pink, bronze, white, yellow and salmon colour) (Jain, 2010). In *Arabidopsis*, EMS treatment affected the gene involved in carbohydrate metabolism, which resulted in significant alteration in flowering as well as the leaf starch content (Yu *et al*., 2000). There was reduction in plant height, number of shoots, leaves and length of roots in media incorporated with EMS. Measurable physiological and morphological changes (Muthusamy *et al.*, 2007) were observed in *Arachis hypogaea* (groundnut) when treated with 1-5 mM of ethyl methane sulphonate (EMS) or sodium azide (SA). Thus, the mutagenic effect of EMS is evident on the tissue development in many plants.

EMS treatment on chrysanthemum successfully yielded a frequency of 5.2 per cent mutants (Jain, 2010). There are several reports on reduced survival rate of treated cultures while attempting mutations. According to Magaia *et al.* (2015) the LD_{50} value of EMS for mutagen treated plantlets of cassava was lower than the value for somatic embryos. Hence, for the effective mutagenesis, plantlets should be subjected to lower concentration of EMS than SEs. The treatment of *in vitro* plantlets with 0.9 per cent EMS resulted in mortality of about half of the individual cultures but SE derived plantlets and callus showed an enhanced (59 % and 67 %) survival. However, 1.20 per cent EMS resulted in survival of about half of the individual cultures of SEs. The higher concentration of 1.5 per cent EMS resulted in 100 per cent lethality in both the SE and SE derived plantlets. Hence, the rate of survival for different tissues treated depends up on the dose of EMS used for mutagenesis.

2.10. *In vitro* subculture of irradiated material

In experiments on mutagenesis, whether with chemicals or physical mutagens, it is necessary to advance the treated material through few seed generations or vegetative propagations. Following mutagenesis, in vegetatively propagated plants, several cycles of propagation are needed to 'dissolve' chimeras or to obtain homo-histonts and or to obtain 'solid' mutants (Owoseni *et al.,* 2006). It is also possible to dissociate chimeras through the repeated culturing of somatic embryo tissues, which can be subsequnently followed by shoot tip multiplication of regenerated (Roux *et al.,* 2001).

Mutants require at least two generations of meiosis involving chromosome segregation and recombination (Chopra *et al.,* 2005). The *in vitro* subculture of irradiated material can be achieved rapidly through vegetative stage 2 (V2) to vegetative stage 4 (V4), without loss of any genotype under disease free conditions (Ahloowalia and Maluszynski, 2001). According to Owoseni *et al.* (2006) the resulting population of putative mutants obtained from mutagen treated vegetative explants are multiplied to dissolve chimerism. Finally, the homohistont plants are established and screened for the useful trait. While the mutagenic response is more or less linear with the dose, previous studies affirm that, polyploids are more tolerant than diploids. After EMS treatment, seed set of Malaysian rice reduced significantly with increasing concentrations of EMS. Also, there was a decrease in rice seedling height with increased EMS concentrations (Chopra *et al.,* 2005).

2.11. Plant regeneration

Hormone-free MS medium supplemented with 3 per cent sucrose is used for plant regeneration in cassava. *In vitro* plant regeneration is initiated once shoots reach a size of 1-2 cm. According to Konan *et al*. (1997) further transfer of shoots to the same medium, independent of the type of cytokinin used and regardless of their origin (axillary buds or axillary-bud-derived meristems), all shoots developed into normal plants with roots. The survival rate of *in vitro*

regenerants transferred to the greenhouse was in the range of 90-100 per cent with no apparent differences between regenerants and mother plants (Konan *et al.*, 1997).

2.12. Hardening

The *ex vitro* acclimatisation process can be carried out either in laboratory or conventional green houses or under pad and fan green house condition. The additional advantage in using pad and fan green house is that the temperature conditions and humidity can be kept constant throughout without any fluctuation depending on daily weather conditions (Magaia *et al.* 2015).

In developing countries, the major factor limiting the wider adoption of *in vitro* mutation is the lack of a procedure for hardening and multiplication of the tissue culture plantlets before final transplanting in the production sites. Although reports are available on *in vitro* hardening of cassava in the developed world, the protocols are difficult and expensive to implement in developing countries since the technology incurs high capital, labour and energy (IAEA, 2004).

According to Magaia *et al.* (2015) although the maintenance costs are higher, it is more advantageous to harden *in vitro* plantlets under pad and fan green house. This resulted in 50 per cent establishment of cassava plants, which is much higher compared to laboratory (nil) and conventional green house (three out of 79 plants). It has a fundamental advantage of granting higher survival rates for acclimatization, as high humidity (above 85 per cent) and temperatures as low as about 27 ºC are to be maintained.

2.13. Field evaluation of mutagen treated plantlets of cassava

Field evaluation of *in vitro* mutated plants is to be done as the case with normal genotypes. Hence, the descriptor developed for cassava can be used for evaluation of plants. In cassava, 75 traits have been used for germplasm characterization, of which 54 were morphological and 21 were agronomic traits

(Fukuda and Guevara, 1998). The characterisation of Brazilian cassava germplasm collections were primarily based on the analysis of morphological descriptors (Fukuda and Alves, 1987). Focusing on the documentation and characterization of cassava germplasm, Fukuda *et al.* (2010) published a revised version of the descriptor, to analyze data and to draw comparisons among different countries. The descriptors were divided into four categories *viz.*, 13 minimum, 13 principal, 10 secondary and 15 preliminary agronomic descriptors.

Mutants can be evaluated for survival rate, plant height, thickness of stem, number of nodes, number of leaves, leaf length, number and/or length and thickness of roots under green house conditions (Jorge *et al.*, 2000; Mapayi *et al.*, 2013). Along with qualitative and quantitative differences in starch biosynthesis, in several induced mutant plants of cassava, morphological differences in storage organs have been identified and characterized (Ancora and Sonnino 1987; Coleman *et al.*, 1995; Smith and Martin 1993). Qualitative traits which are not influenced by environment are important in identifying a genotype. Qualitative traits observed on the vegetative parts of cassava plants showed wide variation between the genotypes (Magaia *et al.*, 2014).

According to Rabbi *et al.* (2014), pigmentation of various tissues is the most conspicuous morphological trait distinguishing different varieties of cassava. Two loci control the colour of apical leaves and occur contiguously in linkage group 2. According to them, this trait explains 93 per cent of phenotypic variation in cassava. Hence, colour of apical leaves can be used to identify variants in cassava.

According to the study of Joseph *et al.* (2004), in most of the lines the colour of the petiole was purple-green including the control plants. However, the colour varied in mutant plants from light-purple in S7 and S17 to light-green in S8 and S13. Magaia (2015) reported that petiole colour varied from purple-green to light green in mutated plants. According to the study conducted by Rabbi *et al.*

(2014) anthocyanin pigmentation of the leaf petioles is associated with a single locus which explains 75 per cent of the phenotypic variation.

The result of study conducted by Adjebeng-Danquah *et al.* (2016) showed that the leaf and plant canopy performance play an important role in determining the yield (weight of fresh tuber). A strong, significant positive correlation was observed between leaf retention, number of roots per plant and root yield. Mutation studies in cassava by Joseph *et al*. (2004) showed no significant variation in parameters like plant height, internode length, stem diameter, colour of stem epidermis and periderm between different mutant lines except for S14 and S15, whereas petiole colour and colour of apical leaves varied over a wide range in various mutant lines. According to Khumaidaa *et al.* (2015), in cassava, greener (dark green) leaf colour will increase the weight of tubers per plant and would be well suited for early estimation of yield.

Cassava adapts to water shortage by reducing its leaf canopy (Connor *et al.*, 1981; El-Sharkawy and Cock, 1987) to reduce water use. Hence, leaf shedding is an effective adaptation mechanism as a response to moisture stress. Leaf retention has been reported as one of the desired traits in achieving high yields in crops under limited moisture (El-Sharkawy, 2007; Lenis *et al.* 2006), because of the production of high total fresh biomass and a 33 per cent higher root dry matter (Lenis *et al.*, 2006). Rivero *et al.* (2007) reported that drought accelerates leaf senescence, leading to a decrease in canopy size, loss in photosynthesis and ultimately leading to reduced yields. Cultivars with high leaf longevity are potentially drought tolerant, suiting marginal areas where rainfall is unreliable. Hence, it is desirable to breed and select for high leaf retention when developing genotypes adapted to dry areas (Rivero *et al.*, 2007). So, this trait presents an additional opportunity to increase cassava yields.

The results of the research conducted by Khumaidaa *et al.* (2015) showed the colour variability with respect to petiole, leaf and stem and exterior in mutant plants. The presence of correlation between the leaves colour with the

tuber yield provide an opportunity to get potential mutant genotypes. Hence, variability of leaf colour can be used as a hyperspectral technology to estimate the potential mutant genotypes without waiting for the tuber harvest time, and thus accelerate the plant breeding program.

The colour intensity of the cassava root and the carotene concentration are positively correlated (Iglesias *et al.*, 1997). Hence, the cream or yellow coloured ones may contain carotene and hence, considered nutritionally superior. Paninah *et al.* (2014) reported that the colour of root pulp is controlled by both major and minor genes and hence, both additive and non-additive gene actions operate. Root flesh colour is an indication of the vitamin activity *i.e.,* the yellow genotypes have higher concentrations of β-carotene (carotenoid with 100 per cent pro-vitamin A activity) (Mezette *et al.,* 2009). Yellow (8.7 per cent) and pink (0.3 per cent) root colour genotypes, possibly have lycopene in their roots (Ferreira *et al.,* 2008).

Khumaidaa *et al.* (2015) reported phenotypic variability of mutant lines compared to the parent genotype in the number of lobes, lobe size, mature leaf colour, leaf vein colour, and petiole colour. According to Khumaidaa *et al.* (2015) longer petiole length would increase the lobe length and width and also the lobe size of mature leaf. Petiole length showed positive correlation with number of tubers per plant and number of economic tubers. Hence, an increase in petiole length could increase number of tubers and number of economic tubers. Similarly, positive correlation of harvesting characters, such as stem diameter, number of tubers per plant, and number of economic tubers per plant, indicated that these characters could be used for estimating the cassava yield potential.

According to Adjebeng-Danquah *et al.* (2016), cassava mosaic disease had a significant negative influence on the number of leaves and roots per plant. Earlier studies on cassava mosaic disease by Akinbo (2008) and Egesi *et al.* (2007) showed a significant yield reduction in cassava due to reduction in light interception because of reduction in leaf area. Root number per plant and mean root weight, which are the indication of the size of roots, were positively and

significantly correlated with root yield. This implies that while adopting breeding in cassava, high root number coupled with large root size should be considered for high yield (Aina *et al.*, 2007; Adjebeng-Danquah *et al.*, 2016).

According to Lebot (2009), gamma irradiation of cassava also imparted variability in several tuber characters such as tuber shape, tuber neck type, and tuber taste on several cassava genotypes. Tofiño *et al.* (2011) reported sessile to mixed and pendunculate tuber neck type on gamma irradiation of UJ-5 and Malang-4 cassava genotypes. Cassava cultivars having roots with well developed peduncle are suitable for better storage. They also suggested that genotypes having roots with short peduncle are difficult to separate from the main stem.

Overall shape of tubers is measured from the base to the tip of tuber. Mutants of Ratim genotype of cassava generated cylindrical, cylindrical-conical, and irregular tubers while the parent genotype has cylindrical tuber shape. Ratim genotype was predominantly sweet, while tubers of its mutants generated had sweet and intermediate taste (Tofiño *et al.*, 2011). Similarly, cassava cultivars having compact, cylindrical, or conical roots are suitable for better storage. Hahn *et al.* (1988) suggested that irregular roots of cassava are difficult to harvest and peel by hand. This will result in heavy loss of usable root materials. Hence, plants with cylindrical or conical roots have to be selected.

Harvest index, which is an indication of the accumulation of dry matter into the economic parts of the plant (Alves, 2002; El-Sharkawy, 2004), was positively correlated with root yield. As opposed to selections based solely on fresh root yield, utilization of Harvest Index (HI) as the basis of selection, enable true genetic progress in cassava (Kawano, 1990). HI-based selections were stable across evaluation stages and will truly represent yield potential of genotype under monoculture. So, this can be used as auxiliary criteria for selection of cassava genotypes (Avijala *et al*., 2015).

The magnitude of the association was insignificant between root yield and dry matter content, indicating that the dry matter content can be improved

without any compromise in yield (Aina *et al*., 2007). According to Tofiño *et al.* (2011), the weight of tubers per plant, number of tubers and number of economic tubers of mutants of cassava was not significantly different from original genotype. The high value of cortex thickness reported by them was 0.243 cm, found in V4D1- (1) and the lowest (0.102 cm) in V4D1-2(2), while cortex thickness of original genotype was 0.133 cm.

Gamma irradiation changed the tuber taste of several mutants generated from UJ-5 and Malang-4 genotypes from bitter (parent genotype) to intermediate and sweet (Tofiño *et al.*, 2011). Nwachukwu *et al.* (1997) reported reduction in the cyanogen content of storage root of selected mutant lines on irradiation of cassava stem cuttings. There was a reduction in yield and variation in the colour of the storage root phelloderm while no significant increase in starch content was observed in any of the mutant lines. In addition, mutant lines also showed altered levels of photosynthetic pigments (Joseph *et al.*, 2004). In contrast Joseph *et al.* (1999) observed no significant alteration in cyanogen content in storage roots of mutant lines of γ-irradiated stem cuttings.

Additionally, the number of mutations that occur in a mutant population depends on the interaction of multiple factors and the likelihood that the mutation produces an altered phenotype (Wu *et al*., 2005; Nag and Smerdon, 2009).

2.14. Sensory evaluation of cassava tubers

Cooking quality in cassava in terms of mealiness of cooked tubers, elasticity and freedom from lumpiness of pounded paste was investigated by Safo-Katanka *et al*. (1997). The studies conducted by Raji *et al*. (2007) inferred that improved cultivars were having low mealiness of boiled tubers compared to landraces. They substantiated the preference of landraces of cassava for sensory attributes over improved cultivars with the presence of genes to adaptation to local condition.

Low or high HCN is an indication of the sweetness of fresh tuber contents on chewing which can be used to categorize cassava into sweet or bitter (Frank *et al.,* 2011). Magaia (2015) suggested that before cooking, fresh tuber colour and chewing sweetness are the most important quality traits preferred by cassava consumers. Similarly, taste, texture and fibre content are preferred quality traits after cooking cassava tubers. Similar results were obtained by Gonzalez and Johnson (2009). In addition, Safo- Katanka *et al.* (1997) observed that these traits were highly correlated with cumulative ranking.

Materials and methods

3. MATERIALS AND METHODS

The present study on '*In vitro* mutagenesis and evaluation of somatic embryo derived plantlets in cassava (*Manihot esculenta* Crantz.)' was undertaken to induce variability for yield and related traits in cassava. The study comprised of two major experiments:

1. Evaluation of mutagen treated somatic embryo derived plantlets
2. *In vitro* induction of mutation

The materials used and the methodologies followed in the present investigation are detailed below:

3.1. Experimental location

The present investigation was carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University, Vellanikkara P.O., Thrissur, 680 656, 40 MSL at 10°31' N Latitude and 76°13' E longitude, experiencing humid tropical climate, during 2014-2016.

The laboratory and field facilities under Department of Plant Breeding and Genetics, College of Horticulture were used for study. Gamma irradiation was done in the Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore.

3.2. Experimental Material

1. Hardened plants derived from mutated callus cultures maintained in the pad and fan system and pre-existing mutated *in vitro* plantlets available in the tissue culture laboratory of the Department of Plant Breeding and Genetics
2. Cassava genotypes Sree Jaya and CC1 (Table 2) were used for the initiation of *in vitro* cultures.

Table 2. Details of genotypes used

Sree Jaya	**CC1**
Duration: Short (180-210 days) Yield: 26-30 t ha^{-1} Dry matter: 32-34 per cent Susceptibility to CMV: Moderate	Duration: Short (180-210 months) Yield: 31-34 t ha^{-1} Dry matter: 34-37 per cent Susceptibility to CMV: Tolerant

Detailed descriptor of Sree Jaya and CC1 is given in the Appendix 1.

3.3. Methods

3.3.1. Experiment 1: Evaluation of mutagen treated somatic embryo derived plantlets

A total of 10 *in vitro* mutagen treated plants maintained in the pad and fan green house and 58 *in vitro* plantlets ready for hardening available at different stages of establishment in the Department of Plant Breeding and Genetics were used for the study. An illustration of hardening undertaken in the present experiment is given in Figure 1 and Plate 1.

3.3.1.1. Hardening of treated *in vitro* mutated plantlets

3.3.1.1.1. Primary hardening under pad and fan green house

In vitro regenerated plantlets of cassava having height of 3 cm and 3 roots were shifted to pad and fan green house as described below. Observations were recorded on height of the plant, number of roots, number of leaves and girth of plant at the time of planting out for hardening.

1. Plastic pots of 10 cm radius and 12 cm height having pores for water drainage were filled up to three fourth of their volume with a 1: 1 mixture of commercial product Soilrite™ (consisting of perlite, vermiculite and Irish moss) and sand.

2. *In vitro* plantlets were gently removed from the test tubes or jam bottles and the root portion was rinsed with fungicide (Megasten 0.02 % WP) in distilled water to remove agar and prevent fungal diseases.
3. One plantlet was planted per pot and water was sprinkled up to field capacity
4. The transplanted plantlets were transferred to pad and fan green house and kept in a moist tray. The pots were covered with transparent plastic bags to prevent loss of humidity.
5. Plantlets were supported with wire mesh on four sides. The temperature and humidity inside the chamber were maintained at 24.0-27.0 °C and RH of 80 - 85 per cent, respectively.
6. Water was sprinkled regularly to ensure humid environment for the plantlets and excess water accumulated in the trays were removed periodically.
7. Three or four weeks later, or when the plantlets reached 25 cm height, a water soluble fertilizer concentrate Greencare ^{TM} ($N\text{-}P_20_5\text{-}K_2O$ 30:10:10, secondary and micro nutrients like Boron, Calcium, Copper, Iron, Magnesium, Manganese, Molybednum, Sulphur, Zinc) was applied as foliar spray at a concentration of 0.01 per cent. Plants were then irrigated once in two days.
8. Initially, nutrient preparation was applied only once in a week. Subsequently the frequency was increased to thrice a week up to third week and then it was applied on daily basis.

Observations were recorded on plant height , number of leaves and girth of plants at two months after planting under pad and fan green house. Survival of the plant was periodically recorded.

3.3.1.1.2. Secondary hardening in rain shelter

When each plant attained a growth of three months under primary hardening, the pots with the plantlets were transferred to rain shelter and placed

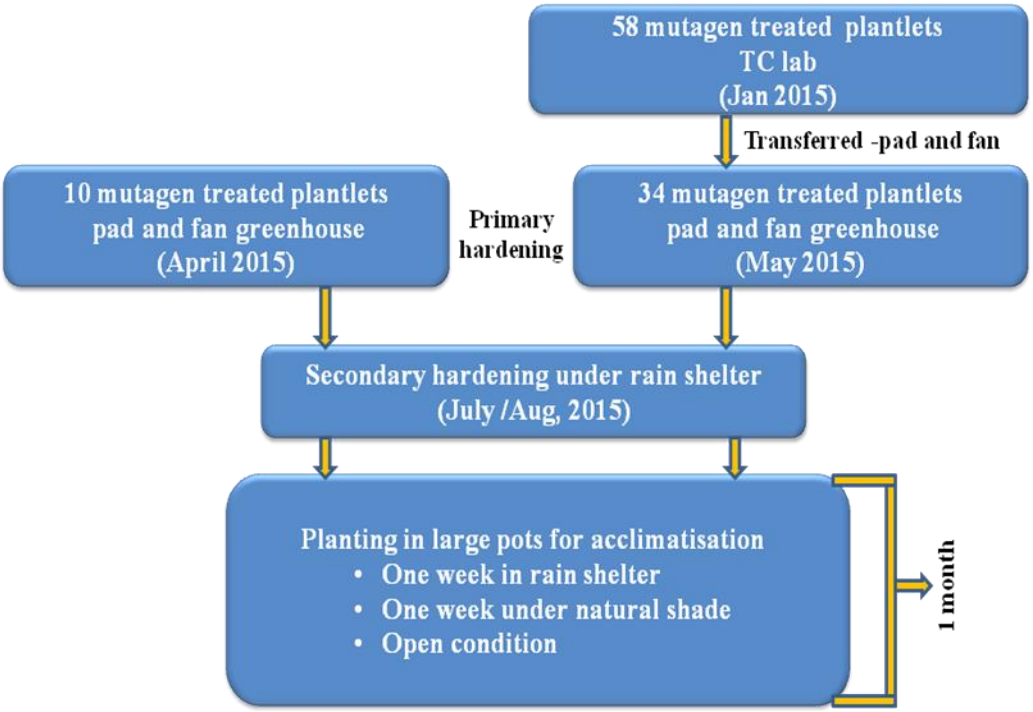


Figure 1. Illustration of hardening of *in vitro* mutagen treated plantlets in cassava

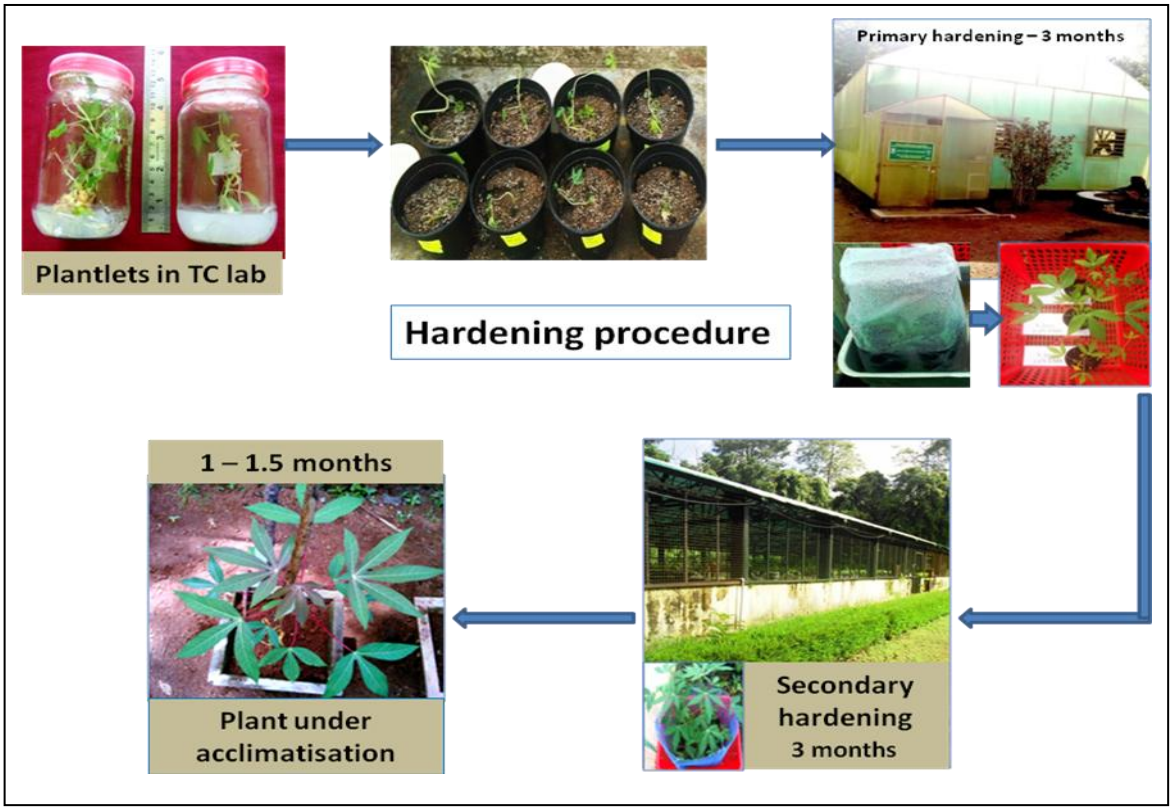


Plate 1. Stages of hardening of *in vitro* mutagen treated plantlets in cassava

inside moist tray supported by a cage made of chicken mesh. The plantlets were maintained at a temperature of 26 - 32 °C with a relative humidity of 60 per cent. Nutrient was applied similar as before, but duration of irrigation was changed to twice in a week. The survival of plants under secondary hardening was recorded.

3.3.1.2. Planting out of hardened plantlets in pots for acclimatisation

The hardened plants, after 3 months of growth in rain shelter were transplanted into large pots for acclimatisation. The planting media used was a mixture of red loamy soil, dried cow dung and vermi compost. Irrigation was ensured twice a week for two weeks and later once in a week. The process of acclimatisation was done in three phases. Initially, the pots were placed in net house for one week and later transferred to the field under shade for another week and then finally to open condition. After two weeks, one tenth of the recommended dose of fertilizer for cassava (KAU, 2011) was applied, followed by another dose by fourth week.

3.3.1.3. Evaluation of *in vitro* mutated plants in field

3.3.1.3.1. Planting of *in vitro* mutated plants in field

After 1 to 1.5 months of growth in the pots, the *in vitro* mutated plants were planted in the field and were used for characterisation and performance evaluation against the original genotype (Table 3). Since each mutant plant had the potential to become a clone, the observations on various parameters were taken on individual basis. A total of 50 plants including the mutants and its control checks were planted at a spacing of 0.75 m × 0.75 m as detailed below:

Table 3. *In vitro* mutated plants of cassava used for field evaluation

Plant number	Genotype	Treatment
1	Sree Jaya	0.3 % EMS
2	Sree Jaya	0.3 % EMS
3	Sree Jaya	0.3 % EMS
4	Sree Jaya	0.3 % EMS
5	Sree Jaya	0.3 % EMS
6	Sree Jaya	0.3 % EMS
7	Sree Jaya	0.3 % EMS
8	Sree Jaya	0.3 % EMS
9	Sree Jaya	0.3 % EMS
10	Sree Jaya	0.3 % EMS
11	Sree Jaya	0.3 % EMS
12	Sree Jaya	0.3 % EMS
13	Sree Jaya	0.6 % EMS
14	Sree Jaya	0.6 % EMS
15	Sree Jaya	0.6 % EMS
16	CC1	0.6 % EMS
17	CC1	0.6 % EMS
18	CC1	0.6 % EMS
19	CC1	0.6 % EMS
20	Sree Jaya	1.2 % EMS
21	Sree Jaya	1.2 % EMS
22	Sree Jaya	1.2 % EMS
23	Sree Jaya	1.2 % EMS
24	Sree Jaya	1.2 % EMS
25	Sree Jaya	1.2 % EMS
26	Sree Jaya	1.2 % EMS
27	Sree Jaya	1.2 % EMS
28	Sree Jaya	1.2 % EMS
29	Sree Jaya	1.2 % EMS
30	Sree Jaya	1.2 % EMS
31	Sree Jaya	1.2 % EMS
32	Sree Jaya	1.2 % EMS
33	Sree Jaya	1.2 % EMS
34	CC1	0.9 % EMS
35	CC1	0.9 % EMS

36	CC1	0.9 % EMS
37	CC1	0.9 % EMS
38	CC1	0.9 % EMS
39	CC1	0.9 % EMS
40	CC1	0.9 % EMS
41	CC1	0.9 % EMS
42	CC1	0.9 % EMS
43	CC1	0.9 % EMS
44	CC1	0.9 % EMS
45	Sree Jaya	Control
46	Sree Jaya	Control
47	Sree Jaya	Control
48	CC1	Control
49	CC1	Control
50	CC1	Control

All the plants evaluated were treated with EMS. The field evaluation trial was conducted between September, 2015 and June, 2016. The recommended agronomic practices (KAU, 2011) were followed during the growth period.

3.3.1.3.2. Observations recorded

Data on quantitative and qualitative traits of individual plants were taken by modifying the standard guidelines of descriptor for morphological and biometrical traits, suggested by International Institute of Tropical Agriculture (IITA) (Fukuda *et al.,* 2010) for cassava. The scales of various descriptors are enlisted in Table 4 and Appendix II.

3.3.1.3.2.1. Observations recorded at three months after planting

3.3.1.3.2.1.1. Colour of apical leaves

Damages caused by cassava green mite can obscure the colour of apical leaves (Fukuda *et al.,* 2010). So, it is recommended to score for this trait earlier. Observations were recorded based on most frequent occurrence.

3.3.1.3.2.1.2. Pubescence on apical leaves

Most frequent occurrence on the basis of scale for scoring was recorded.

3.3.1.3.2.2. Observations recorded six month after planting

3.3.1.3.2.1.1. Leaf retention

Leaf retention is a trait that presents an additional opportunity to increase cassava yield. The leaf retention was measured six months after planting.

Visual scoring for leaf retention for clone was done using a scale of 1-5 as enumerated below:

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

A plant with average leaf retention is the one with leaves covering about half of the plant (Fukuda *et al.*, 2010).

3.3.1.3.2.1.2. Shape of central leaflet

The shape of central leaflet was taken from leaves at mid-height position. Most frequent occurrence was recorded as per the scale for the trait.

3.3.1.3.2.1.3. Petiole colour

For scoring on colour of petiole the leaves were chosen from mid-height position and most frequent occurrence was recorded based on the scale of the trait. For this trait intermediate descriptor states are allowed (Fukuda *et al.*, 2010).

3.3.1.3.2.1.4. Leaf colour

Observation was taken from leaves from the middle of the plant. The most frequent occurrence as per the scale for the trait was recorded. In case of leaf colour intermediate descriptor states are not allowed (Fukuda *et al.,* 2010).

3.3.1.3.2.1.5. Number of leaf lobes

A leaf from the middle of the plant was observed to record the observation for this trait. Only one score was recorded as per the scale for the trait given in the descriptor. Assessment was made based on five leaves and the predominant number of lobes was recorded.

3.3.1.3.2.1.6. Length of leaf lobe

Measurement was taken from the point of intersection of all lobes to the end of the middle lobe. Two leaves from the middle of the plant were measured. The length was measured in centimetres and recorded to one decimal place.

3.3.1.3.2.1.7. Width of leaf lobe

Measurement was taken from two leaves form the middle of the plant. The widest part of the middle lobe was measured. The width was also measured in centimetres and recorded to one decimal place.

3.3.1.3.2.1.8. Ratio of lobe length to lobe width of central leaf lobe

Based on the observations taken for length and width of leaf lobe, the ratio was computed and recorded to two decimal place.

3.3.1.3.2.1.9. Lobe margins

The observation on lobe margin was taken from the middle third portion of the plant. Most frequent observation was recorded as per the scale and no intermediates were allowed (Fukuda *et al.,* 2010).

3.3.1.3.2.1.10. Petiole length

Petiole lengths of leaves were recorded from the middle third portion of the plant. The length was measured for two leaves per plant and was expressed in centimetres.

3.3.1.3.2.1.11. Colour of leaf vein

The upper side of central leaf lobe of the leaf selected from middle part of the plant was used for taking observation. Vein near the base of the leaf lobe was observed and the most frequent occurrence was recorded based on the scale provided in the descriptor. Intermediates are not allowed in the descriptor scale for this character.

3.3.1.3.2.1.12. Orientation of petiole

The middle part of the plant was observed and recorded based on a general picture across the row. Intermediates are not allowed for this descriptor scale also (Fukuda *et al.,* 2010).

3.3.1.3.2.3. Observations recorded at nine month after planting

3.3.1.3.2.3.1. Prominence of foliar scars

The middle third of the plant was observed and the most frequent occurrence was recorded as per the scale of the descriptor.

3.3.1.3.2.3.2. Colour of stem cortex

A small shallow cut was made at the middle third part of the plant and the epidermis was peeled back. Observation was recorded as per the scale suggested by Fukuda *et al.* (2010).

3.3.1.3.2.3.3. Colour of stem epidermis

The epidermis of the middle third part of the plant was peeled back and observed the underside of the epidermis (skin) was observed. Observations were recorded as per the scale provided in the descriptor.

3.3.1.3.2.3.4. Colour of stem cortex

Observation for this trait was taken from the middle part of the plant as per the scale of descriptor.

3.3.1.3.2.3.5. Distance between the leaf scars

Measurement for this trait was taken from the middle third part of the plant from the middle stem, where the scars are not flat. A measurement of the length of the stem was made and the value was divided with number of scars present in the measured part.

3.3.1.3.2.3.6. Growth habit of the stem

Based on the growth habit the cassava genotypes were recorded either straight or zig-zag.

3.3.1.3.2.3.7. Length of the stipules

Observations are taken from the top third portion of the plant and the most frequent occurrence was recorded. Intermediates are not allowed for this trait also (Fukuda *et al.*, 2010).

3.3.1.3.2.3.8. Stipule margin

Stipule margin was observed from the top third portion of the plant. Most frequent observation was recorded and no intermediate scales are allowed.

3.3.1.3.2.3.9. Plant height

Vertical height from the ground to top of the canopy was measured and expressed in centimetres.

3.3.1.3.2.3.10. Height of first branching

The vertical height of the plant from the ground to first primary branch was measured and the observation was expressed in centimetres.

3.3.1.3.2.3.11. Shape of the plant

The most frequent occurrence as per the scale of the descriptor was recorded.

3.3.1.3.2.3.12. Number of storage roots per plant

The number of storage roots for each plant was recorded.

3.3.1.3.2.3.13. Number of commercial roots per plant

The number of commercial roots with length greater than 20 cm from each plant was recorded.

3.3.1.3.2.3.14. Extend of root peduncle

The observation for this trait was taken only for main roots. The most frequent occurrence as per the scale given in the descriptor.

3.3.1.3.2.3.15. Root constrictions

Root constrictions were taken from a mature root. The most frequent observation based on the scale for the trait was recorded.

3.3.1.3.2.3.16. Root shape

The most frequent occurrence for root shape was recorded as per the scale given in the descriptor was recorded.

3.3.1.3.2.3.17. External colour of storage root

The most frequent occurrence based on the descriptor scale was recorded.

3.3.1.3.2.3.18. Colour of root pulp (parenchyma)

Colour of root pulp was recorded based on the most frequent occurrence as per the scale.

3.3.1.3.2.3.19. Colour of root cortex

The most frequent occurrence of colour of root cortex was recorded as per the scale. The intermediate scales are allowed (Fukuda *et al.*, 2010).

3.3.1.3.2.3.20. Texture of root epidermis

The texture of root epidermis was analysed by touch and feel and recorded the most common root type.

3.3.1.3.2.3.21. Cortex thickness

The thickness from the roots at the proximal (closest to stem), mid and distal (furthest from the stem) end was measured using callipers is estimated and scored according to the scale of the descriptor.

3.3.1.3.2.3.22. Harvest index (HI)

Harvest index was estimated to study the genetic progress in the treated *in vitro* mutated cassava plants. The roots and the aboveground biomass (stems, branches, and leaves) were weighed separately.

HI was computed as:

$$\frac{\text{Weight of roots}}{\text{Weight of roots} + \text{Weight of above ground biomass}}$$

3.3.1.3.2.3.23. Disease scoring

As described by Hahn *et al.* (1989) and Hahn *et al.*, (1980) all the plants were scored for the incidence of *Cercospora* Leaf Spot (CLS) and Cassava Mosaic Disease (CMD). Scoring was done by taking observations of two leaves from bottom, middle and top part of the plant and separate scoring was done for each leaves. Scoring was done using 0-5 scale given by Hahn *et al*, (1989) according to the symptoms produced.

3.3.1.3.2.3.24. Assessment for dry matter (DM) and starch content

Estimation of starch content and DM in cassava is based on the principle of a linear relationship between specific gravity with DM and/or starch content (Fukuda *et al.*, 2010).

Percentage DM = 158.3x – 142

Percentage starch content = 112.1x – 106.4; where x = specific gravity.

Specific gravity was measured according to the following methodology:

1. Root samples to be weighed were ensured free of soil and other debris.
2. The samples were weighed both in air (Wa) and water (Ww) using a suitable balance. A sturdy wire basket, as it allows the soil debris to fall through and also allows easy measurement in water was used as container to hold the tubers.
3. Specific gravity was computed using the equation:

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

4. Dry matter (DM) and starch content were then computed using the formulae mentioned earlier.

Table 4. Descriptor for qualitative and quantitative trait evaluation in cassava

Sl No.	Trait	Schedule	Unit/score	Reference
1	Colour of apical leaves	3 MAP	3,5,7,9	IITA-2010 (Fukuda *et al.*, 2010)
2	Pubescence on apical leaves	3 MAP	0,1	
3	Leaf retention	6 MAP	1-5	
4	Shape of central leaflet	6 MAP	0-10	
5	Colour of petiole	6 MAP	1-9	
6	Colour of leaf	6 MAP	3,5,7,9	
7	Number of leaf lobes	6 MAP	3,5,7,9,11	
8	Length of leaf lobe	6 MAP	cm	
9	Width of leaf lobe	6 MAP	cm	
10	Length to width ratio of leaf lobe	6 MAP	-	
11	Lobe margins	6 MAP	3,7	
12	Petiole length	6 MAP	cm	
13	Colour of leaf vein	6 MAP	3,5,7,9	
14	Orientation of petiole	6 MAP	1,3,5,7	
15	Prominence of foliar scars	9 MAP	3,5	
16	Colour of stem cortex	9 MAP	1-3	
17	Colour of stem epidermis	9 MAP	1-4	
18	Colour of stem exterior	9 MAP	3-9	
19	Distance between leaf scars	9 MAP	3,5,7	
20	Growth habit of stem	9 MAP	1,2	
21	Length of stipule	9 MAP	3,5	
22	Stipule margin	9 MAP	1,2	
23	Height of plant	Harvest	cm	
24	Height of first branching	Harvest	cm	
25	Shape of plant	Harvest	1-4	
26	Number of storage roots/plant	Harvest	Number	
27	Number of commercial roots/plant	Harvest	Number	
28	Extent of root peduncle	Harvest	0,3,5	
29	Root constriction	Harvest	1-3	
30	Root shape	Harvest	1-4	

31	External colour of root	Harvest	1-4	
32	Colour of the root pulp	Harvest	1-5	
33	Colour of root cortex	Harvest	1-4	
34	Cortex ease of peeling	Harvest	1,2	
35	Txture of root epidermis	Harvest	3,5,7	
36	Harvest index (HI)	Harvest	-	
37	Tuber weight per plant	Harvest	kg	
38	Tuber girth	Harvest	cm	
39	Length of neck	Harvest	0,3,5	Mahangu *et al.*, 1983
40	Stem girth	Harvest	cm	
41	Dry matter content per plant	Harvest	kg	
42	Incidence of CMD	-	1-5	Hahn *et al.*, 1989
43	Incidence of CLS	-	0-5	Hahn *et al.*, 1980

3.3.1.4. Sensory evaluation of cassava

Sensory evaluation of tubers from each plant was done immediately after the harvest. The references and scores for different attributes followed for evaluation are enumerated in Table 5 and the score card is provided in Appendix II.

Table 5. Sensory evaluation of cassava genotypes

Sl no.	Sensory attributes	Score	Reference
1	Easiness in peeling	1-5	Gonzalez and Johnson, 2009 Fukuda *et al.*, 2010
2	Colour before cooking	1-5	
3	Colour after cooking	1-5	-
4	Sweetness on chewing	1-5	Frank *et al.*, 2011;Fukuda *et al.*, 2010
5	Texture after cooking	1-5	Gonzalez and Johnson, 2009
6	Taste after cooking	1-5	
7	Fibrelessness	1-5	
8	Cooking ability	1-5	

The sensory evaluation of different attributes in freshly harvested cassava tubers (both cooked and uncooked) was done by a group of randomly selected individuals among teaching and non teaching staffs, research assistants and students of different age groups. Scoring was done based on the above score card. Freshly peeled cassava tubers weighing 70 g was cut in to small pieces of uniform

size and cooked by boiling in 175 ml water in ordinary cooking pans on gas stove. The tuber was cooked till it was easy to pick with a fork. Then time required to attain softness was recorded with the help of a stop watch. Scoring was done as given in Table 6.

Table 6. Score card for cooking ability of cassava

Score	Cooking time (minutes)	Trait associated
1	20	Very bad
2	16-20	Bad
3	11-15	Medium
4	5-10	Good
5	Up to 5	Very good

3.3.2. Experiment 2: *In vitro* induction of mutation

An illustration of *in vitro* mutagenesis under taken in the present experiment is given in figure 2.

3.3.2.1. Explant used

Young leaves of Sree Jaya and CC1 were used in the study.

3.3.2.2. Culture conditions for *in vitro* regeneration in cassava

Explants of cassava were cultured on the MS nutrient medium (Murashige and Skoog, 1962) as basal medium, supplemented with 30 g sucrose. The pH of the medium was adjusted to 5.7 before autoclaving at 121°C for 15 min at 15 psi.

3.3.2.3. Preparation of stock solutions

Five stock solutions were prepared for MS media preparation *viz.*, Stock I: Macronutrients, Stock II: Calcium stock, Stock III: Iron-EDTA stock, Stock IV: Micronutrients and Stock V: Vitamin Stock (Table 7). Stocks for different plant growth regulators were prepared separately at the concentration of 25 mg per 100ml.

Table 7. Stock solutions, ingredients and concentration of MS media

Stock	Chemical	mg L^{-1}	Stock concentration	Stock for 1000ml (g L^{-1})	Stock for 500ml (g L^{-1})
I	NH_4NO_3	1600	50x	82.5	41.25
	KNO_3	1900		95	47.5
	KH_2PO_4	170		8.5	4.25
	$MgSO_4$. $7H_2O$	370		18.5	9.25
II*	$CaCl_2$. $2H_2O$	440	50x	22	11
III**	Na_2EDTA	37.3	100x	3.7	1.85
	$FeSO_4$	27.8		2.8	1.4
(Dissolved separately) and then mix					
IV	$MnSO_4.4H_2O$	22.3	100x	2.23	1.115
	$ZnSO_4$. $7H_2O$	8.6		0.86	0.43
	H_3BO_3	6.2		0.62	0.31
	KI	0.83		0.083	0.042
	Na_2MoO_4	0.25		0.025	0.013
	$CoCl_2$. $6H_2O$	0.025		0.003	0.001
	$CuSO_4$	0.025		0.003	0.001
	Vitamins				
V	Glycine	2	100x	0.2	0.1
	Nicotinic Acid	0.5		0.05	0.025
	Pyridoxine HCl	0.5		0.05	0.025
	Thiamine HCl	0.1		0.01	0.005
	Myoinositol	100	**Measure and mix to media at the time of preparation**		
	Sucrose	30 g L^{-1}			
	Agar	8 g L^{-1}	**Adjust pH to 5.7 using 1N HCl or 1 N NaOH**		

* Prepare Stocks separately or otherwise it may precipitate

** Store Stock III in Amber coloured bottles or cover bottle with Aluminium foil

3.3.2.4. Preparation of culture media

Basal medium of full strength MS was prepared using Stock I and Stock II of fifty times concentration (50x) and Stock III, Stock IV, and Stock V of hundred times concentration (100x), prepared in 500 ml volumetric flasks. Thus, the MS basal media was constituted using 20 ml Stock I (macronutrients), 20 ml Stock II (calcium stock), 10 ml Stock III: (Iron-EDTA stock), 10 ml Stock IV (micronutrients) and 10 ml Stock V (vitamin stock). Optionally the vitamins can

be added separately from a stock solution of 25 mg $L^{-1}$of each vitamin. The mixture was supplemented with required concentration of the growth regulators, from a stock of 25 mg per 100 ml. Then 100 mg myoinositol and 30 g L^{-1} sucrose (3 per cent) were dissolved into the mixture, and the volume was made up to 1 L using distilled water. The pH was adjusted to 5.7 using 1 N HCl or 1 N NaOH. Finally, 8 g agar was added and melted until the media become clear. The media was poured into air dried clean test tubes and sealed with non-absorbent cotton. The media was autoclaved at 121°C at 100 kPa (15 psi) pressure for 15 minutes (Berhanu and Feyissa, 2013).

3.3.2.5. Preparation of explants

Based on earliness, tuber yield, biotic stress tolerance and cooking quality, Sree Jaya and CC1 were identified as the best genoytpes by Magaia, (2015). The shoot cuttings of these two cassava genotypes were planted in red loam soil and the plants were maintained in the rain shelter at Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University. These plants were used as explants source for *in vitro* mutagenesis.

3.3.2.5.1. Sterilisation and *in vitro* propagation in cassava

Newly sprouted shoot tips with unfolded leaf lobe of Sree Jaya and CC1 were collected at four weeks interval and sterilised using standardised protocol (Magaia, 2015) as follows:

1. The newly sprouted shoots of four to five centimetres were collected, properly labelled and washed once with tap water to remove mud and dirt.
2. Leaf bits with veins of about 1cm × 1cm from unfolded to partially unfolded leaves, were collected and kept in distilled water.
3. Primary sterilisation of the explants were done in 5 per cent detergent solution (Teepol) prepared in distilled water by gentle shaking for four to five minutes, followed by treatment with 0.02 per cent carbendazim (Megasten 50 % WP).

4. Explants were washed and rinsed with tap water until the last trace of detergent and fungicides were removed and then taken to laminar air flow (LAF) cabinet.
5. Under the LAF, the explants were wiped or immersed in 70 per cent ethanol for one or two minutes and rinsed thrice with sterile distilled water.
6. Further, disinfection was done with mercuric chloride 0.05 per cent for one or two minutes, followed by three times washing with sterile distilled water.
7. The inoculation was carried out in LAF under sterile environment by placing the leaf explants in solid media supplemented with required growth regulators. The leaf lobes were kept such that the abaxial surface touches the media.
8. The labelled test tubes were then transferred to the culture room and kept under controlled conditions of 27 ± 1°C at 40 per cent relative humidity with 12 hours of illumination at 3000 lux.
9. Contaminated cultures were periodically noted and removed from the culture room.

3.3.2.5.2. Establishment of callus cultures in cassava

Media supplemented with the concentrations of different growth regulators *viz.*, MS + 8 mg L^{-1} 2, 4-D, MS + 8 mg L^{-1} 2, 4-D + 0.5 mg L^{-1} NAA + 1 mg L^{-1} BA and MS + 8 mg L^{-1} Picloram which were found best in earlier experiment conducted in Department of Plant Breeding and Genetics were used for regeneration of callus in both Sree Jaya and CC1 genotypes of cassava. The observations were recorded twice a week as scores of necrosis, hard callus formation and friable callus formation based on the area covered on the surface of the medium, ranging from zero to four. The best media was identified for callus initiation and regeneration for further experiments. The calli produced in all the media were used for further experiments. The scoring patterns followed are enumerated below:

Scoring for necrosis (based on percentage of callus turned necrotic):

0- Absence of necrosis
1- Necrosis of 1-25 per cent
2- Necrosis of 26-50 per cent
3- Necrosis of 51-75 per cent
4- Necrosis of 76-100 per cent

Scoring for hard callus formation (based on percentage of callus turned hard):

0- Absence of necrosis
1- Hard callus of 1-25 per cent
2- Hard callus of 26-50 per cent
3- Hard callus of 51-75 per cent
4- Hard callus of 76-100 per cent

Scoring for friable embryogenic callus formation (FEC) (based on percentage spread):

0- Absence of growth
1- Spread of 1-25 per cent
2- Spread of 26-50 per cent
3- Spread of 51-75 per cent
4- Spread of 76-100 per cent

3.3.2.6. *In vitro* induction of mutation in cassava

Calli derived from explants of two genotypes *viz.,* Sree Jaya and CC1 were exposed to mutagens (physical mutagen gamma ray (γ ray) and chemical mutagen Ethyl Methane Sulphonate (EMS)) at the doses which were already standardised. The callus of each genotype was exposed to four doses of γ rays 30Gy, 40Gy, 50Gy and 60Gy. EMS was incorporated into the liquid media of MS at a dosage ranging from 0.1 to 0.9 per cent at an interval of 0.1 per cent. The illustration of protocol followed is given in Figure 2.

3.3.2.6.1. Effect of gamma irradiation on the callus cultures of cassava

In order to accommodate in the gamma chamber, the callus cultures were inoculated into test tubes of size 25 mm × 100 mm of 45 ml capacity with 15 ml MS. The test tubes of both the genotypes were then wrapped separately in bundles of seven and tied with rubber bands for each treatment exposure. Irradiation was done in gamma chamber with Co^{60} as the source (Plate 2). Soon

after irradiation, the treated callus cultures were transferred into fresh media. After a week the survived callus were subcultured into fresh medium. Contaminated cultures were noted. Then observations on survival of both genotypes on each treatment exposure were made as enumerated below:

1. Percentage of necrosis
2. Percentage of callusing
3. Percentage of hard callus

The scoring was done as in previous experiment.

3.3.2.6.2. Effect of EMS on callus cultures of cassava

The callus cultures of Sree Jaya and CC1 were treated with EMS by incorporating EMS in liquid media of MS (Plate 3). The callus was treated for 24 hours. To avoid death of cells due to lack of aeration, bridge method was adopted for treatment (M shaped blotting paper strip placed in test tube containing liquid media and callus for treatment was placed in its groove above media). Calli after 24 hours were then transferred into fresh MS medium without EMS. Observations were taken twice a week and survival rate was estimated for each treatment of both genotypes as in previous experiments.

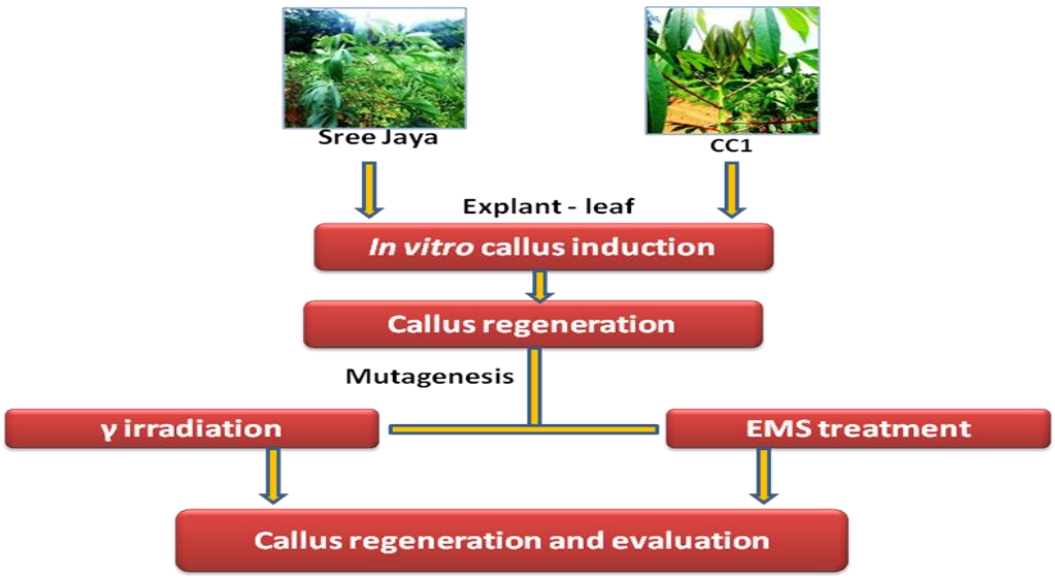


Figure 2. Illustration of steps followed for *in vitro* mutagenesis in cassava

Plate 2. Gamma irradiation of cassava callus cultures

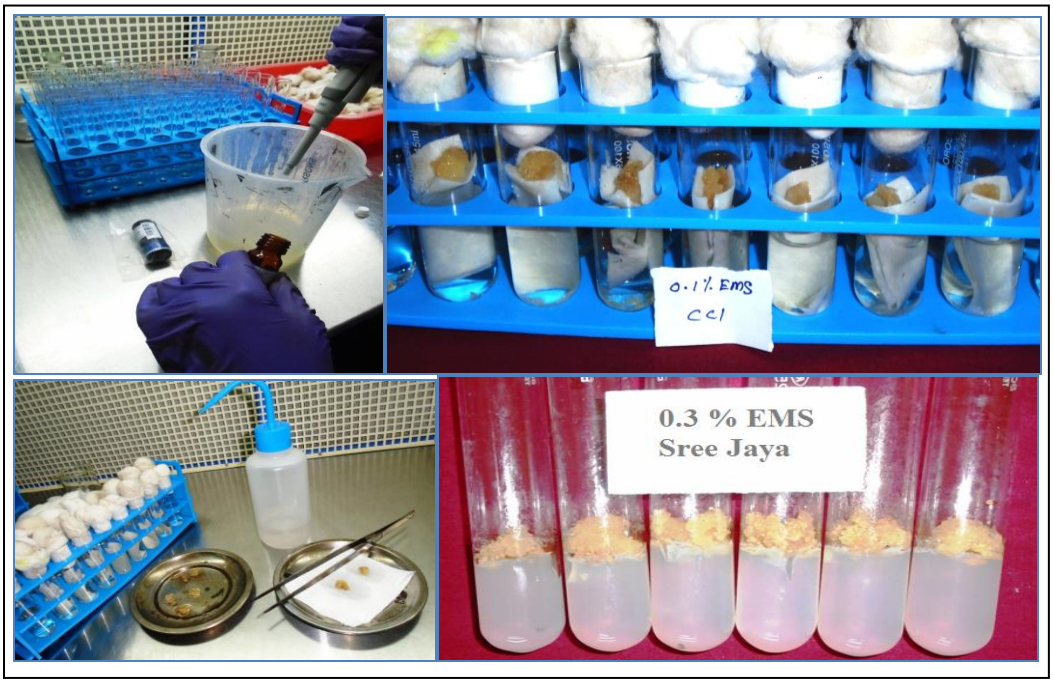


Plate 3. EMS treatment and subculturing of cassava callus cultures

3.3.2.6.3. Regeneration of mutated callus for somatic embryogenesis

The treated callus survived after mutagenesis was repeatedly subcultured in MS + 8 mg L^{-1} Picloram for induction of SEs.

Results

4. RESULTS

The present study was conducted to induce i*n vitro* mutation and to evaluate mutagen treated *in vitro* plantlets in cassava (*Manihot esculenta* Crantz.). The mutagen treated *in vitro* plantlets were evaluated under pad and fan green house during hardening and in field on individual plant basis. Concurrently, callus derived from leaf explants of cassava genotypes, Sree Jaya and CC1 were used for *in vitro* induction of mutation in a completely randomised block design (CRD).

4.1. Experiment 1: Evaluation of mutagen treated somatic embryo derived plantlets

Ten mutagen treated plantlets under different stages of primary hardening and 58 plantlets yet to be hardened available in the Department of Plant Breeding and Genetics were hardened and evaluated under filed conditions. The results of the experiment are presented below:

4.1.1. Hardening of mutagen treated *in vitro* plantlets

Observations on morphological characters taken for mutagen treated *in vitro* plantlets of cassava at the time of planting out for hardening and two months after planting in pad and fan green house are presented in Table 8 and Appendix III.

4.1.1.1. Primary hardening under pad and fan green house

4.1.1.1.1. Evaluation of mutagen treated *in vitro* plantlets of cassava at the time of planting out

4.1.1.1.1.1. Evaluation of *in vitro* mutagen treated Sree Jaya plantlets

The result of evaluation of *in vitro* mutagen treated plantlets of Sree Jaya at the time of transferring to pad and fan green house are presented in Table 8. The height of these plants varied from 3.2 to 18.3 cm with a mean value of 11.02 cm and length of the roots varied from 1.7 to 8.3 cm at the time of transferring

with a mean value of 6.06 cm. The girth of the stem at the time of planting ranged from 0.1 to 0.22 cm with a mean value of 0.15 cm. The plantlets produced a maximum of 5 leaves and minimum of 2 with a mean value of 3.07.

4.1.1.1.1.2. Evaluation of *in vitro* mutagen treated CC1 plantlets

Variations in morphological characters of *in vitro* mutagen treated plantlets of CC1 at time of transferring to pad and fan greenhouse are presented in Table 8. Height of the plantlets varied from 3.2 to 11.8 cm with a mean value of 8.12 cm and stem girth varied from 0.10 to 0.20 cm with a mean value of 0.15 cm. The length of the root ranged from 2.8 to 8.9 cm with a mean value of 5.22 cm. Plantlets produced up to 6 leaves and the minimum number of leaves observed at the time of planting was 2 with a mean value 3.38.

4.1.1.1.2. Evaluation of *in vitro* mutagen treated plantlets of cassava two months after planting out

4.1.1.1.2.1. Evaluation of *in vitro* mutagen treated Sree Jaya plantlets

The result of evaluation of *in vitro* mutagen treated plantlets of Sree Jaya at two months after transferring to pad and fan green house are presented in Table 8. The height of these plants varied from 12.20 cm to 55.50 cm with a mean value of 30.64 cm and stem girth ranged from 0.60 cm to 1.20 cm with a mean value of 0.86 cm. The plantlets produced a maximum of 11 leaves and minimum of 2.

4.1.1.1.2.2. Evaluation of *in vitro* mutagen treated CC1 plantlets

The evaluation of *in vitro* mutagen treated plantlets of CC1 at two months after transferring to pad and fan greenhouse (Table 8) showed that height of the plantlets varied from 16.3 cm to 55.5 cm and stem girth varied from 0.1cm to 0.5 cm. Some plantlets produced up to 4 shoots and 9 leaves with a mean value of 5.33.

Table 8. Variations in morphological characters at the time of planting out for hardening and after two months

Observations	Time of observation (month)	Sree Jaya			CC1		
		Min	Max	Mean	Min	Max	Mean
Length of roots (cm)	0	1.7	8.3	6.06	2.8	8.9	5.22
Plant height (cm)	0	3.2	18.3	11.02	3.2	11.8	8.12
	2	12.2	55.2	30.64	16.3	55.5	42.95
Girth of stem (cm)	0	0.1	0.22	0.15	0.1	0.2	0.15
	2	0.6	1.2	0.86	0.1	0.5	0.75
Number of leaves	0	2	5	3.07	2	6	3.38
	2	2	11	5.71	4	9	5.33

4.1.1.1.3. Survival of mutagen treated *in vitro* plantlets

The first batch of 10 plants which were already in primary hardening was successfully moved to rain shelter for secondary hardening. In the other batch of 58 plantlets kept for hardening, 34 plantlets survived and got established under pad and fan green house. Among the 36 mutagen treated plantlets of Sree Jaya kept for primary hardening, 22 got established. The number of established plantlets forwarded to secondary hardening in CC1 was only 12 out of 22 CC1 plants (Appendix III).

4.1.1.2. Secondary hardening under rain shelter

4.1.1.2.1. Survival of mutagen treated *in vitro* plantlets

All the *in vitro* mutagen treated plants of the genotypes Sree Jaya and CC1 were transferred to rain shelter for secondary hardening and observation on survival of the transferred plants during the course of secondary hardening was recorded. All the 44 plants (10 plantlets already available in pad and fan green house + 34 plantlets hardened from TC laboratory) survived under secondary hardening.

4.1.2. Assessment of field level variability of *in vitro* mutagen treated cassava genotypes

Forty four *in vitro* mutagen treated plants were evaluated on individual plant basis. Mutagen treated plants included 29 plants of Sree Jaya and 15 plants of CC1. Along with them, three plants each of Sree Jaya and CC1 were used as control. The variability was assessed in both qualitative and quantitative characters based on standard descriptor (Fukuda *et al.*, 2010) and the observations are presented in Appendix IV and the results of evaluation are presented below:

4.1.2.1. Field evaluation of qualitative traits of mutagen treated *in vitro* plants of cassava

The evaluation of *in vitro* mutagen treated plants of cassava was done at three, six and nine months after planting and the results are presented below:

4.1.2.1.1. Evaluation at three months after planting

Observations on colour and pubescence of apical leaves were taken.

4.1.2.1.1.1 Colour of apical leaf

Colour of apical leaf in control plants of Sree Jaya was purplish green (score 7) and control plants of CC1 had light green (score 3) apical leaf. Variations were observed in *in vitro* mutagen treated plants on comparison with control genotypes. Out of the mutagen treated plants, 9 Sree Jaya plants scored 3 for apical leaf colour. In addition to the colours scored in the descriptor of Fukuda *et al.* (2010), more variants were observed. They were one plant each of mutagen treated Sree Jaya with colours greenish purple, dark green with purple margin and light green with purple margin. One mutagen treated CC1 plant had purplish green (score7) and another had dark green (score 5) apical leaves. Nine plants of CC1 treated with mutagens had light green (score 3) and one plant had purple (score 9) for apical leaf. The

intermediary apical leaf colour was observed in three mutagen treated CC1 plants which had greenish purple apical leaves.

4.1.2.1.1.2. Pubescence of apical leaves

Irrespective of treated or control, all plants showed a score of 0 (absence) of pubescence on apical leaves.

4.1.2.1.2. Evaluation at six months after planting

Observations on leaf retention, shape of central leaflet, colour of petiole, colour of leaf, number of leaf lobes, lobe margins, petiole length, colour of leaf vein and orientation of petiole were taken. In both control and mutagen treated plants the colour of mature leaves had score of 5 (dark green) in Sree Jaya and score 3 (light green) in CC1. Smooth lobe margin (score 3) and reddish-green in more than half of the leaf lobe (score 7) for colour of leaf vein were seen in all *in vitro* mutagen treated plants similar to control plants.

4.1.2.1.2.1. Leaf retention

Visual scoring for leaf retention for clone was done using a scale of 1-5 as enumerated below:

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

A plant with average leaf retention is the one with leaves covering about half of the plant. This is taken as the bench mark and scoring was done based on the descriptor given above. The results of leaf retention in the *in vitro* mutagen treated plants of cassava are presented in Table 9.

Table 9. Leaf retention in *in vitro* mutagen treated cassava plants

Treatment	**No. of plants**					
	Sree Jaya		**CC1**			
Score	**4**	**5**	**2**	**3**	**4**	**5**
Control	All	-	All	-	-	-
Mutagen treated	9	20	-	2	4	9

Control plants of Sree Jaya showed better than average leaf retention while CC1 had less than average leaf retention. Among mutagen treated plants nine plant of Sree Jaya and four plants of CC1 showed better than average leaf retention. Only two mutagen treated CC1 plants had average leaf retention. Most of the mutagen treated plants of both Sree Jaya and CC1 had outstanding leaf retention (9 plants of CC1 and 20 plants of Sree Jaya).

4.1.2.1.2.2. Shape of central leaflet

Control plants of CC1 showed a score of 5 (lanceolate) and CC1 scored 1 (ovoid) with respect to shape of central leaflet. On comparison with control plants, two mutagen treated CC1 plants showed variation *i.e.,* lanceolate shaped central leaflet.

4.1.2.1.2.3. Colour of petiole

Control plants of Sree Jaya had red petiole (score7) while CC1 had purple petiole (score 9). Variation in petiole colour was observed for all mutagen treated *in vitro* plantlets of CC1 of which one plant had scored 3, one plant had very deep purple (score 9) and the rest had red (score 7) petiole.

4.1.2.1.2.4. Colour of mature of leaf

Control plants of Sree Jaya had a dark green (score 5) leaf, CC1 and was with light green (score 3) leaf. There was no difference with respect to leaf colour in mutagen treated *in vitro* plantlets when compared with control types.

4.1.2.1.2.5. Number of leaf lobes

For all plants evaluated, the number of leaf lobes ranged from five to seven.

4.1.2.1.2.6. Lobe margin

The lobe margin for all the cassava plants evaluated was smooth (score 3).

4.1.2.1.2.7. Colour of leaf vein

Irrespective of the treatments there was no variation with respect to colour of vein in all cassava plants evaluated. All plants had a score of 7 (reddish-green in more than half of the lobe) for colour of leaf vein.

4.1.2.1.2.8. Orientation of petiole

For control plants of Sree Jaya and CC1 the petiole orientation was inclined downwards (score 3). Four mutagen treated plants of Sree Jaya had horizontal petiole (score 5).

4.1.2.1.3. Evaluation at nine months after planting

The observations on prominence of foliar scars, colour of stem cortex, colour of stem epidermis, growth habit of stem, colour of end branches, length of stipule, stipule margin, shape of plant, extent of root peduncle, root constriction, texture of root epidermis, colour of stem exterior, root shape, external colour of root, colour of root cortex, colour of the root pulp, extend of root peduncle, incidence of CMD and incidence of CLS were taken.

For all plants evaluated at nine months after planting, there was prominent leaf scar (score 5), straight stem (score 1), and long stipule (score 5) with split stipule margin (score 2).

4.1.2.1.3.1. Colour of stem cortex

Control plants of Sree Jaya had light green (score 2) stem cortex and CC1 had dark green (score 3). Mutagen treated *in vitro* plantlets did not show any variation for this trait.

4.1.2.1.3.2. Colour of stem epidermis

Control plants of Sree Jaya and CC1 scored 6 (light brown) for colour of epidermis. Mutagen treated *in vitro* plantlets of both Sree Jaya and CC1 showed no variation with respect to colour of epidermis when compared to its control.

4.1.2.1.3.3. Colour of stem exterior

Control plants of Sree Jaya scored 8 (grey) and CC1 scored 7 (silver) for colour of stem exterior. One mutagen treated CC1 plant scored 5 (golden) for colour of stem exterior. Similarly one plant of CC1 treated by mutagen scored 4 (greeny-yellowish) for stem colour. All other plants showed no variation when compared with control types.

4.1.2.1.3.4. Distance between leaf scars

Irrespective of mutagen treated *in vitro* plantlets or control plants, all cassava plants evaluated had leaf scars at a medium distance (8-15 cm).

4.1.2.1.3.5. Growth habit of the stem

All plants of cassava evaluated had straight (score 1) stem.

4.1.2.1.3.6. Length of stipule and stipule margin

Irrespective of mutagen treated *in vitro* plantlets or control plants all cassava plants evaluated had forked (score 2) stipule margin.

4.1.2.1.3.7. Height at branching

Branching was not observed in all plants evaluated.

4.1.2.1.3.8. Cassava Mosaic Disease (CMD) and *Cercospora* Leaf Spot (CLS)

Irrespective of control or mutagen treated plants, CMD symptoms scored moderate mosaic (score 3) to severe distortion of entire leaf (score 5) and for CLS the symptom appeared as angular leaf spots (score 2). The exception was one plant of Sree Jaya treated with 0.3 per cent EMS, which scored 1 (no symptom) for CLS and score 2 (mild chlorotic pattern of leaf) for CMD.

4.1.2.1.3.9. Texture of root epidermis

The observation on texture of root epidermis showed that, all plants evaluated had smooth (score 5) textured roots.

4.1.2.1.3.10. Extend of root peduncle

Control plants of Sree Jaya had pedunculate roots (score 3) and for CC1 it was sessile (score 0). Among mutagen treated *in vitro* plantlets, three plants of Sree Jaya treated with mutagen scored 0 for extend of root peduncle. One mutagen treated plant of Sree Jaya and a plant of CC1 had mixed root peduncle (score 5). Two mutagen treated CC1 plants produced tubers with peduncle.

4.1.2.1.3.11. Root constriction

Root constriction varied from none to a few (score 1-3) for mutagen treated as well as control plants of both Sree Jaya and CC1.

4.1.2.1.3.12. Root shape

Control plants of Sree Jaya and CC1 had conical roots (score 1). Out of the mutagen treated plants, four plants of mutagen treated Sree Jaya plants produced cylindrical roots (score 3) and all other mutagen treated Sree Jaya plants produced conical roots. One mutagen treated plant of CC1 had irregular

roots while another had cylindrical roots. All other CC1 plants produced conical roots.

4.1.2.1.3.13. External colour of root

All control plants had dark brown (score 4) external colour for roots. One plant each of mutagen treated Sree Jaya had yellow (score 2) and light brown (Score 3) external colour for roots. All other mutagen treated plants, both of Sree Jaya and CC1 had dark brown roots (score 4) exterior.

4.1.2.1.3.14. Colour of root pulp

All irrespective of control or treated plants of Sree Jaya and CC1 produced white root pulp except one mutagen treated plant of CC1 which showed variation in colour of root pulp with respect to its control type. The variant had a root with cream pulp (score 2).

4.1.2.1.3.15. Colour of root cortex

Control plants and most of the mutagen treated *in vitro* plantlets of cassava genotypes had pink (score 3) coloured root cortex. Even though, the score for colour was same there was observable variation in gradation of colour of root cortex. The gradation in cortex colour observed in mutagen treated Sree Jaya plants, four with light pink root cortex, one plant with creamish-pink and two plants with deep pink coloured root cortex. All other mutagen treated Sree Jaya plants produced tubers with pink coloured cortex.

4.1.2.2. Field evaluation of quantitative traits

The observations on field evaluation of quantitative traits in mutagen treated *in vitro* plantlets of cassava genotypes are presented in Table 10, Table 11 and Appendix V.

4.1.2.2.1. Quantitative traits at six months after planting

Observations on length of leaf lobe, width of leaf lobe, ratio of leaf lobe and petiole length were taken. Variations in quantitative characters at 6 MAP are presented in Table 10.

4.1.2.2.1.1. Length of leaf lobe

The control plants of Sree Jaya had leaf lobe of length 16.8 cm and CC1 had 18.2 cm. Mutagen treated Sree Jaya had leaf lobe length at the range of 15.2 to 23.4 cm with a mean value 19.2 cm. Mutagen treated CC1 plants had a leaf lobe length ranging from 15.6 to 23.2 cm with a mean value 17.3 cm.

4.1.2.2.1.2. Width of leaf lobe

The control plants of Sree Jaya had leaf lobe of width 4.5 cm and CC1 had 5.2 cm. Mutagen treated Sree Jaya plants had leaf lobe width at the range of 3.4 to 5.6 cm with a mean value of 4.6 cm. Mutagen treated plants of CC1 had width of leaf lobe at the range of 3.2 to 5.5 cm with a mean value 4.3 cm.

4.1.2.2.1.3. Length to width ratio of leaf lobe

Based on length and width of leaf lobes, ratio was estimated. The length to width ratio of leaf lobe for Sree Jaya and CC1 was 3.9 and 3.2, respectively. Mutagen treated Sree Jaya plants had a leaf lobe ratio in the range 3.5 to 5.3 with a mean value of 4.6 while mutagen treated CC1 plants had lobe ratio 3.6 to 4.9 with a mean value of 4.3.

4.1.2.2.1.4. Petiole length

Control plants of Sree Jaya had a mean petiole length of 23.3 cm, while CC1 had 24.0 cm. Mutagen treated CC1 plants had length of petiole ranging from 21.6 to 30.6 cm with a mean value of 25.2 cm while that of Sree Jaya ranged from 20.6 to 36.6 cm with a mean value of 29.2 cm.

Table 10. Variations in quantitative characters 6 MAP

Observations	Sree Jaya				CC1			
	Mutagen treated			Control	Mutagen treated			Control
	Min	Max	Mean	Mean	Min	Max	Mean	Mean
Length of leaf lobe (cm)	15.2	23.4	19.2	16.8	15.6	23.2	17.3	18.2
Width of leaf lobe (cm)	3.4	5.6	4.6	4.5	3.2	5.5	4.3	5.2
Ratio of leaf lobe	3.5	5.3	4.6	3.9	3.6	4.9	4.3	3.2
Petiole length (cm)	20.6	36.6	29.2	23.3	21.6	30.6	25.2	24.0

4.1.2.2.2. Quantitative traits at nine months after planting

Observations on height of first branching, distance between leaf scars, height of plant, stem girth, number of storage roots per plant, number of commercial roots per plant, tuber weight per plant and tuber girth were taken. Based on the observations taken harvest index (HI), starch and dry matter content were estimated. The data are presented in Table 11.

4.1.2.2.2.1. Distance between leaf scars

The mean distance between the leaf scars of the control plants of Sree Jaya was 3.6 cm and for CC1 it was 3.9 cm. For mutagen treated Sree Jaya plants it ranged from 1.3 to7.6 cm with a mean value 4.3 cm. For mutagen treated CC1 plants the distance ranged from 1.6 to 6.1 with a mean value of 3.8 cm.

4.1.2.2.2.2. Stem girth

The mean stem girth of control plants of Sree Jaya and CC1 and Hraswa were 1.6 cm and 1.1 cm, respectively. Stem girth of mutagen treated Sree Jaya plants ranged from 1.0 to 2.8 cm with a mean value of 1.8 cm. Mutagen treated plants of CC1 had stem girth at a range of 1.1 to 2.5 cm with a mean value of 1.5 cm.

4.1.2.2.2.3. Height of the plant

Mean height in control plants of Sree Jaya was 135.0 cm and CC1 was 196.0 cm, respectively. Height of mutagen treated CC1 plants ranged from 141 to 313 cm with a mean value of 196 cm. Mutagen treated plants of Sree Jaya had height at range of 52.5 to 556 cm with a mean value of 177.9 cm.

4.1.2.2.2.4. Tuber characters

Few plants did not produce tubers under field condition. For those plants which produced tubers, quantitative characters for tubers were observed.

4.1.2.2.2.5. Number of storage roots and commercial roots per plant

Number of storage roots in the control plants of Sree Jaya and CC1 were 2.00 and 3.00, respectively. Mutagen treated Sree Jaya plants produced storage roots ranging between 0.00 and 6.00 with a mean value of 2.27. However, the mutagen treated CC1 plants produced on an average 2.27 no. of storage roots ranging between 0.00 and 4.00.

Storage roots longer than 20 cm are considered to be commercial roots. The number of commercial roots ranged between 0.00 and 3.00 in the mutagen treated Sree Jaya plants with a mean value of 0.73 and that of the

mutagen treated CC1 plants ranged between 0.00 and 1.00 with a mean of 0.27. The control plants did not produce any commercial root.

4.1.2.2.2.6. Thickness of tuber

The control plants of Sree Jaya and CC1 produced tubers of 8.90 cm and 3.90 cm mean thickness, respectively. The mutagen treated plants of Sree Jaya produced tubers with thickness ranging between 3.24 cm and 6.40 cm and a mean thickness of 14.00 cm. Tuber thickness in the mutagen treated CC1 plants ranged between 2.30 cm and 7.10 cm with a mean thickness of 4.50 cm.

4.1.2.2.2.7. Tuber length

The mutagen treated Sree Jaya and CC1 plants produced tubers with a mean length of 20.49 cm and 18.06 cm, respectively ranging between 10.20 cm and 30.10 cm in Sree Jaya and 12.30 cm and 34.20 cm in CC1. The control plants of Sree Jaya produced tubers with a mean length of 19.10 cm and that of CC1 with 17.30 cm mean tuber length.

4.1.2.2.2.8. Average cortex thickness

Average thickness of cortex in the control plants of Sree Jaya and CC1 were 1.50 mm and 1.60 mm, respectively. The mutagen treated plants of CC1 exhibited cortex with thickness ranging between 0.80 mm and 2.00 mm and a mean value of 1.32 mm. The mean cortex thickness of the mutagen treated Sree Jaya plants were 0.23 mm ranging between 1.00 mm and 2.20 mm.

4.1.2.2.2.9. Fresh tuber weight

Fresh tuber weight of the mutagen treated Sree Jaya plants ranged between 0.09 kg and 1.19 kg with a mean weight of 0.29 kg. The mutagen treated CC1 plants produced tubers with mean weight of 0.40 kg ranging between 0.12 kg

and 1.05 kg. The control plants of Sree Jaya and CC1 had 1.40 kg and 0.32 kg for mean tuber weight.

4.1.2.2.2.10. Above ground plant weight

The control plants of Sree Jaya and CC1 had mean above ground plant weight of 1.50 kg and 0.40 kg, respectively. The above ground plant weight of mutagen treated Sree Jaya plants ranged between 0.33 kg and 1.72 kg with a mean value of 0.70 kg. The mutagen treated CC1 plants had above ground plant weight ranging between 0.26 kg and 1.77 kg with a mean value of 0.58 kg.

4.1.2.2.2.11. Harvest index (HI)

Harvest Index was estimated based on weight of fresh tuber and above ground plant weight. The mutagen treated Sree Jaya plants exhibited harvest index in the range between 0.21 and 1.16 with a mean value of 0.35, whereas its control plant had a harvest index of 1.00. The mutagen treated CC1 plants were with a mean harvest index of 0.57 ranging between 0.24 and 1.35 and its control plants had harvest index of 0.30.

4.1.2.2.2.12. Dry matter (DM)

Dry matter was estimated based on the standard formula given by Fukuda *et al*. (2010). Dry matter content in the control plants of Sree Jaya and CC1 were 23.20 per cent and 30.60 per cent, respectively. The mutagen treated Sree Jaya however, had mean dry matter content of 20.31 per cent ranging between 21.05 per cent and 49.54 per cent respectively. Dry matter content in the mutagen treated plants of CC1 ranged between 16.30 per cent and 32.13 per cent with a mean value of 18.95 per cent.

4.1.2.2.2.13. Starch

Starch was also estimated based on standard formula given by Fukuda *et al.* (2010). Starch content in the mutagen treated plants of Sree Jaya ranged between 9.06 per cent and 29.24 per cent with a mean value of 10.49 per cent and the control plant had 10.60 per cent starch in it. The control plants of CC1 had 15.80 per cent starch in the tubers while the mutagen treated CC1 plants had a starch content of 8.64 per cent ranging between 5.7 per cent and 16.91 per cent.

Table 11. Variations in quantitative characters 9 MAP.

Observations	Sree Jaya				CC1			
	Mutagen treated			Control	Mutagen treated			Control
	Min	Max	Mean	Mean	Min	Max	Mean	Mean
Leaf scar distance (cm)	1.30	7.60	4.30	3.60	1.60	6.10	3.80	3.90
Stem girth (cm)	1.00	2.80	1.80	1.60	1.10	2.90	1.50	1.10
Plant height (cm)	52.50	556.00	177.90	185.00	141.00	313.0	176.00	196.00
No. of storage roots	0.00	6.00	2.27	2.00	0.00	4.00	2.27	3.00
No. of commercial roots	0.00	3.00	0.73	0.00	0.00	1.00	0.27	0.00
Tuber thickness (cm)	3.24	6.40	14.00	8.90	2.30	7.10	4.50	3.90
Tuber length (cm)	10.20	30.10	20.49	19.10	12.30	34.20	18.61	17.30
Cortex thickness (mm)	1.00	2.20	0.23	1.50	0.80	2.00	1.32	1.60
Fresh tuber weight (kg)	0.09	1.19	0.29	1.40	0.12	1.05	0.40	0.32
Above ground weight (kg)	0.33	1.72	0.70	1.50	0.26	1.77	0.58	0.40
HI	0.21	1.16	0.35	1.00	0.24	1.35	0.57	0.3
Dry matter (%)	21.05	49.54	20.31	23.2	16.3	32.13	18.95	30.6
Starch (%)	9.06	29.24	10.49	10.6	5.7	16.91	8.64	15.8

4.1.3. Sensory evaluation of mutagen treated *in vitro* plants of cassava

All the plants evaluated in the field did not produce tubers. The plants which produced tubers were evaluated for eight sensory attributes by twelve panellists and the results are presented in Table 12, Table 13 a, b, and Table 14 a, b.

Kendall's coefficient of concordance was used to study the significance of perception of taste between the panellists and to rank the plants based on the mean rank of different sensory attributes evaluated.

The result of Kendall's W^a presented in Table 12 confirmed that there was no significant difference with respect to attributes like colour before cooking, peeling easiness, chewing sweetness, colour after cooking, taste after cooking and bitterness, among the panellists.

Table 12. Kendall's W^a of sensory evaluation of cassava

Sl no.	Character	Kendall's W^a	Significance
1	Colour before cooking	0.515	NS
2	Peeling easiness	0.512	NS
3	Chewing sweetness	0.142	NS
4	Colour after cooking	0.300	NS
5	Texture after cooking	0.364	NS
6	Taste after cooking	0.362	NS
7	Fiberlessness	0.213	NS
8	Bitterness	0.338	NS

*Significance at 5 % level

The result in Table 13 (a, b) showed that among 31 plants evaluated, the highest rank for peeling easiness was recorded for plant 42 (24.83) followed by control plant of Sree Jaya (22). Control plant of Sree Jaya recorded highest rank of 25.21and 23.25 for colour before cooking and chewing sweetness, respectively. Similarly, plant 32 scored highest for both taste after cooking (25.17) and bitterness (20.88). Regarding the fiberlessness, plant 3 (23.08) was most acceptable by panellists followed by plant 32 (22.13). Plant 31 (25.5) had the highest rank for texture after cooking followed by plant 32 (24.63). With regard to colour after cooking, plant 5 (22.54) recorded highest rank followed by plant 28 (21).

Table 13 a. Mean rank for sensory evaluation in mutagen treated *in vitro* plants of cassava

Plant no.	Colour before cooking	Peeling easiness	Chewing sweetness	Colour after cooking	Texture after cooking	Taste after cooking	Fiberlessness	Bitterness
3	14.63	16.67	16.96	19.63	17.17	20.63	23.08	16.71
4	19.38	14.79	18.21	15.58	17.75	16.42	17.00	16.13
5	21.00	16.38	16.83	22.54	17.33	14.29	15.00	16.13
6	17.96	19.88	20.54	13.58	18.21	19.67	17.58	19.42
7	12.63	16.54	12.21	14.58	16.00	15.54	16.13	17.42
10	14.63	19.21	12.83	16.33	17.50	15.58	19.42	19.67
11	8.50	10.50	22.46	16.63	14.92	14.17	14.83	14.75
12	10.92	20.88	12.54	15.92	23.88	23.29	19.88	17.13
13	15.13	16.63	19.42	16.46	15.92	18.33	21.5	18.92
14	14.96	15.75	17.08	17.25	14.42	18.67	16.92	19.79
15	21.83	17.83	16.58	17.75	16.88	22.33	17.83	17.96
21	20.04	16.33	14.75	13.17	12.96	13.13	13.38	14.21
23	19.13	16.88	16.92	16.38	16.25	18.38	16.58	18.88

Table 13 b. Mean rank for sensory evaluation in mutagen treated *in vitro* plants of cassava

Plant no.	Colour before cooking	Peeling easiness	Chewing sweetness	Colour after cooking	Texture after cooking	Taste after cooking	Fiberlessness	Bitterness
24	21.13	16.79	17.71	15.42	17.42	16.54	16.46	16.67
28	20.42	11.13	15.71	21.00	16.25	12.83	14.50	18.46
29	14.46	15.67	16.33	19.04	16.04	11.79	14.17	13.63
30	16.71	16.63	12.96	14.29	15.63	10.75	12.96	12.50
31	14.46	18.92	21.46	20.63	25.50	24.58	20.96	19.92
32	18.08	21.33	20.63	20.83	24.63	25.17	22.13	20.88
33	14.50	16.29	21.58	18.75	11.00	16.08	17.38	18.63
34	18.29	11.83	12.38	20.04	10.17	11.92	14.58	14.96
35	17.46	10.67	14.54	19.08	14.71	17.04	16.58	15.75
36	17.42	19.21	12.63	20.42	10.96	10.67	19.83	16.25
37	17.71	10.58	17.58	15.83	14.13	13.13	20.42	15.33
41	20.21	21.71	19.79	13.50	17.63	16.83	12.42	16.21
42	21.79	24.83	21.42	20.50	23.50	22.13	19.63	20.71
43	12.17	21.42	13.46	13.38	17.75	15.58	16.79	17.58
44	12.88	14.29	14.38	15.71	14.71	16.29	19.13	17.33
CC1*	12.42	19.54	20.54	15.83	19.33	18.17	14.50	19.71
Sree Jaya*	25.21	22.00	23.25	12.46	19.17	20.42	13.25	18.50

Table 14 a. Ranking based on cumulative score for sensory evaluation in *in vitro* mutagen treated plants of cassava

Plant no.	Colour after cooking	Peeling easiness	Chewing sweetness	Colour after cooking	Texture after cooking	Taste after cooking	Fiberlessness	Bitterness	Cumulative score	Rank
32	3.25	4.17	3.42	3.92	3.92	4.00	4.00	4.08	**30.76**	1
31	3.00	3.92	3.58	3.92	4.08	4.00	3.92	4.00	**30.42**	2
42	3.50	4.42	3.42	3.83	3.75	3.67	3.83	4.00	**30.42**	3
15	3.58	3.83	3.17	3.75	3.33	3.75	3.75	3.92	**29.08**	4
6	3.33	3.92	3.42	3.33	3.42	3.50	3.67	3.92	**28.51**	5
Sree Jaya*	3.92	3.92	3.33	3.53	3.36	3.22	3.39	3.72	**28.39**	6
3	3.00	3.75	3.17	3.83	3.25	3.50	4.00	3.75	**28.25**	7
CC1*	2.75	4.00	3.42	3.50	3.42	3.33	3.42	4.00	**27.84**	8
5	3.50	3.75	3.08	4.08	3.33	3.00	3.42	3.67	**27.83**	9
12	2.67	4.08	2.75	3.50	3.75	3.75	3.50	3.83	**27.83**	10
4	3.33	3.75	3.25	3.50	3.33	3.17	3.58	3.75	**27.66**	11
41	3.42	4.17	3.33	3.33	3.25	3.17	3.17	3.75	**27.59**	12
13	3.08	3.67	3.42	3.58	3.17	3.33	3.33	4.00	**27.58**	13
33	3.00	3.67	3.50	3.75	2.83	3.17	3.67	3.92	**27.51**	14
24	3.50	3.75	3.17	3.42	3.25	3.08	3.50	3.75	**27.42**	15

Table 14 b. Ranking based on cumulative score for sensory evaluation in *in vitro* mutagen treated plants of cassava

Plant no.	Colour after cooking	Peeling easiness	Chewing sweetness	Colour after cooking	Texture after cooking	Taste after cooking	Fiberlessness	Bitterness	Cumulative score	Rank
23	3.33	3.58	3.08	3.50	3.17	3.33	3.50	3.92	**27.41**	**16**
14	3.00	3.58	3.25	3.58	3.08	3.33	3.58	4.00	**27.40**	**17**
10	3.00	3.92	2.75	3.52	3.33	3.08	3.75	4.00	**27.35**	**18**
28	3.50	3.25	3.00	3.92	3.17	2.83	3.42	3.92	**27.01**	**19**
36	3.25	3.91	2.75	3.92	2.75	2.67	3.83	3.75	**26.83**	**20**
21	3.25	3.25	3.17	3.50	3.08	2.83	3.92	3.67	**26.67**	**21**
43	2.75	4.17	2.75	3.33	3.33	3.08	3.37	3.83	**26.61**	**22**
35	3.17	3.17	2.92	3.75	3.17	3.17	3.58	3.67	**26.60**	**23**
44	2.83	3.42	2.92	3.50	3.00	3.17	3.75	3.83	**26.42**	**24**
29	3.00	3.67	3.08	3.75	3.17	2.67	3.33	3.50	**26.17**	**25**
7	2.83	3.67	2.67	3.42	3.17	3.00	3.50	3.83	**26.09**	**26**
21	3.42	3.67	2.92	3.25	3.00	2.92	3.25	3.58	**26.01**	**27**
11	2.50	3.17	3.67	3.58	3.08	2.92	3.42	3.58	**25.92**	**28**
30	3.33	3.75	2.75	3.42	3.17	2.67	3.25	3.33	**25.67**	**29**
34	3.25	3.25	2.67	3.83	2.67	2.75	3.25	3.58	**25.25**	**30**

The least rank for colour before cooking (8.50) and peeling easiness (10.5) was observed in plant 11. Control plant of Sree Jaya had the lowest rank for colour after cooking (12.46) and plant 30 had lowest score for bitterness (12.50). The lowest rank for chewing sweetness was obtained in plant 36 (12.63) and for fiberlessness it was plant 41 (12.42). The least rank for texture after cooking was obtained for plant 34 (10.17) followed by plant 36 (10.96). Panellists scored least for plant 36 (10.67) with respect to taste after cooking.

The result of cumulative scoring of the different attributes of cassava tubers of each plant evaluated are presented in Table 14 (a, b) .The highest cumulative score was recorded in plant 32 (30.76) followed by plant 31 and 42 with a rank of 30.42. Plant 34 had the least cumulative score (22.25) followed by plant 30 (25.67).

4.2. Experiment 2: *In vitro* induction of mutation

In vitro mutation being a potential tool to induce variability in cassava, mutagenesis of callus cultures of already identified superior genotypes, Sree Jaya and CC1 was attempted to produce more variants. The results of effect of media and mutagen on production of necrosis, hard callus and friable embryogenic callus are presented below:

4.2.1. Effect of media on callusing in cassava

The first step towards *in vitro* mutagenesis was the production of callus cultures of Sree Jaya and CC1 genotypes. For the establishment of callus cultures, three media which were already identified superior were used. The ANOVA and result for evaluation of effect of different media are presented in Table 15 and Table 16. The treatments were T1 (MS + 8 mg L^{-1} 2, 4-D), T2 (MS + 8 mg L^{-1} Picloram) and T3 (MS + 8mg L^{-1} 2,4-D + 0.5mg L^{-1} NAA + 1mg L^{-1} BA).

All the treatments (T1, T2, and T3) were on par with respect to necrosis and hard callus production of explants. However, the treatments showed significant difference with respect to friable embryogenic callus production.

Table 15. ANOVA for effect of media on callusing from leaf explants in cassava

ANOVA						
Trait	**Sources of variation**	**df**	**Sum of Squares**	**Mean Square**	**F**	**Significance**
Callus necrosis	Treatment	2	6.22	3.11	0.69	0.50
	Error	105	473.44	4.51		
	Total	107	479.67			
Hard Callus	Treatment	2	4.22	2.11	0.69	0.50
	Error	105	320.78	3.05		
	Total	107	325.00			
Friable embryogenic callus	Treatment	2	24.2	12.11	3.20*	0.04
	Error	105	397.78	3.79		
	Total	107	422.0			

* Significance at 5% level

The effect of different growth regulators on production of friable embryogenic callus from leaf explants are presented in Table 16. The mean score for friable embryogenic callus production was highest in T3 (3.00) followed by T2 and T1 which were on par.

Table 16. Effect of media on friable embryogenic callus production

Trait	**Treatments**		
	T1	**T2**	**T3**
Necrosis	2.17[a]	1.22[a]	1.94[b]
Hard callus	2.06[a]	1.67[a]	2.06[b]
Friable embryogenic callus	1.61[a]	1.61[a]	3.00[a]

T1 - MS + 8 mg L^{-1} 2,4-D T2 - MS + 8 mg L^{-1} Picloram T3 - MS + 8mg L^{-1} 2,4-D + 0.5mg L^{-1} NAA + 1mg L^{-1} BA

4.2.2. *In vitro* induction of mutation

In vitro mutation of the established callus cultures of Sree Jaya and CC1 were irradiated with fixed doses of gamma rays and treated with EMS to induce mutation. The results of effect of mutagens on treated callus are presented below:

4.2.2.1. Effect of gamma irradiation on the callus cultures of cassava

ANOVA and the results of evaluation of callus cultures of Sree Jaya and CC1 irradiated with γ rays is shown in Table 17 and Table 18, respectively.

Table 17. ANOVA for effect of gamma irradiation on cassava callus cultures

ANOVA						
Trait	**Sources of variation**	**Sum of Squares**	**df**	**Mean Square**	**F**	**Significance**
Necrosis	Treatment	52.02	4	13.01	6.02*	0.24
	Error	355.77	165	2.16		
	Total	367.79	169			
Hard callus	Treatment	64.79	4	16.20	6.79*	0.00
	Error	393.50	165	2.38		
	Total	458.89	169			
Friable embryogenic callus	Treatment	6.45	4	1.61	0.43	0.79
	Error	616.26	165	3.73		
	Total	622.71	169			

* Significance at 5 % level

The effect of γ irradiation on callus cultures of Sree Jaya and CC1 (Table 17) showed that the treatments were significantly different for formation of necrosis and hard callus. The result showed no significant difference with respect to friable embryogenic callus formation.

The effect of γ irradiation on callusing is presented in Table 18. Out of the total cultures irradiated, highest mean score for necrosis was observed on treatment with 60 Gy (T4). All doses for irradiation 30Gy, (T1), 40 Gy (T2), 50Gy, (T3) and 60 Gy, (T4) were on par with respect to hard callus formation.

Table 18. Effect of gamma irradiation on cassava callus cultures

Trait	**Treatments**				
	T1	**T2**	**T3**	**T4**	**T5**
Callus necrosis	1.588^{ab}	1.618^{ab}	1.618^{ab}	1.912^{a}	1.088^{b}
Hard callus	2.088^{a}	1.882^{a}	2.324^{a}	2.588^{a}	0.794^{b}
Friable embryogenic callus	3.206^{a}	2.394^{a}	3.147^{a}	3.324^{a}	3.564^{a}

T1 - 30 Gy T2 - 40 Gy T3 - 50 Gy T4 - 60 Gy T5 - Control

4.2.2.2. Effect of EMS treatment on *in vitro* cassava callus cultures

ANOVA of evaluation of callus cultures of Sree Jaya and CC1 treated with EMS is shown in Table 19. There was significant difference with respect to necrosis, hard callus production and production of friable embryogenic callus when treated with EMS at 0.1 to 0.9 per cent at 0.1 per cent interval.

The effect of treatment of callus cultures with EMS (Table 20) showed that treatments T5, T6, T7, T8 and T9 resulted in highest mean score for necrosis ranged from 0.39 to 0.55 while, treatments T1, T2, T3 and T4 were on par with mean score of necrosis ranging from 0.22 to 0.39. Treatment T10 had lowest mean score of 0.08 for hard callus formation and all other treatments were on par in terms of hard callus formation. Treatments T1, T2, T3, T4 and T10 were on par with respect to friable embryogenic callus formation with mean score ranging from 1.16 to 2.07. The treatments T5, T6, T7, T8 and T9 were on par with mean score ranging from 1.89 to 2.37.

Table 19. ANOVA for effect of EMS treatment on cassava callus cultures

ANOVA						
Trait	**Sources of variation**	**Sum of Squares**	**df**	**Mean Square**	**F**	**Significance**
Necrosis	Treatment	29.06	9	3.23	1.05*	0.402
	Error	460.19	150	3.07		
	Total	489.24	159			
Hard callus	Treatment	2.42	9	0.27	3.32*	0.001
	Error	12.16	150	0.08		
	Total	14.59	159			
Friable embryogenic callus	Treatment	13.31	9	1.48	5.21*	0.000
	Error	42.59	150	0.28		
	Total	55.91	159			

* Significance at 5% level

Table 20. Effect of EMS treatment on cassava callus cultures

Trait	**Treatments**									
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
Callus necrosis	0.22^{cd}	0.30^{cd}	0.31^{cd}	0.29^{cd}	0.39^{bcd}	0.43^{abcd}	0.44^{abcd}	0.50^{abc}	0.55^{ab}	0.03^{e}
Hard callus	1.82^{b}	1.31^{b}	1.56^{b}	1.56^{b}	2.06^{ab}	2.44^{a}	2.69^{a}	1.69^{a}	2.06^{a}	0.08^{b}
Friable embryogenic callus	2.02^{a}	1.89^{a}	2.07^{a}	2.09^{a}	1.69^{abc}	1.16^{abcd}	1.45^{bcd}	1.38^{cd}	1.26^{d}	2.37^{a}

T1 - 0.1 % EMS T2 - 0.2 % EMS T3 - 0.3 % EMS T4 - 0.4 % EMS T5 - 0.5 % EMS
T6 - 0.6 % EMS T7 - 0.7% EMS T8 - 0.8 % EMS T9 - 0.9 % EMS T10 - Control

Discussion

5. DISCUSSION

Cassava (*Manihot esculenta* Crantz.) is an important source of carbohydrates both in tropics and sub-tropics. The main constraints for crop improvement are low fertility, low hybrid seed set, poor germination rate, in-breeding depression and the polygenic and recessive nature of many desirable traits. Conventional breeding was attempted to improve the agronomic traits of cassava. It has also been used successfully to produce few resistant varieties to CMD. However, inter-specific hybridization is slow and requires scientific expertise. Hence, one of the strategies to increase the genetic variability of cassava is through induction of mutation. Considering these factors *in vitro* induction of mutation was attempted using chemical mutagen EMS and physical mutagen γ rays. Mutagenesis with an optimum dose was used to maintain a balance between achieving mutagenesis without compromising the integrity of majority of its genetic constitution. The present study aimed to undertake *in vitro* mutagenesis in CC1 and Sree Jaya genotypes of cassava and to evaluate the mutagen treated plantlets in cassava under hardening and in field condition. Hence, characterization of the qualitative and quantitative characters of mutagen treated cassava plants was done for assessing the variation.. The results obtained are discussed in detail below:

5.1. Experiment 1: Evaluation of mutagen treated somatic embryo derived plantlets

5.1.1. Hardening of mutagen treated *in vitro* plantlets

5.1.1.1. Evaluation of *in vitro* mutagen treated cassava plantlets at the time of planting out and two months after planting

To monitor somaclonal variation in cassava a simple and preliminary option is the visual examination of tissue culture plants under *in vitro* conditions. Subsequent evaluation can be done under green house and field for confirmation or identification of additional mutated traits (Reed *et al.*, 2004). Magaia (2015)

observed variations with respect to plantlet height, number of shoots, number of leaves and roots as well as length of the roots on treatment with different doses of EMS. The effect of EMS treatment on the tissue development was evident, resulting in measurable physiological and morphological changes in the plantlets regenerated.

Magaia (2015) suggested that higher doses of any of the mutagen can suppress the root development in cassava. EMS treated somatic embryo derived plantlets of Sree Jaya resulted in reduction in the number of shoots along with reduction in the number of leaves. In genotype CC1, gamma irradiation resulted in reduction in the plantlet height along with reduction in the number of shoots, number of leaves and in the number of roots. Hence, treatment of callus with both the mutagens resulted in reduction in vegetative characters of the plantlets produced.

Minimal growth parameters of at least 2.0 - 4.0 cm height and 3-5 roots are required for planting out of *in vitro* regenerated plantlets of cassava as suggested by Jorge *et al.* (2000). Mutants can be evaluated for survival rate, plant height, thickness of stem, length of leaves and roots, number of nodes and leaves, number and/or thickness of roots as per the report of Jorge *et al.* (2000) and Mayapi *et al.* (2013).

In the present study, most of the plants of variety Sree Jaya had 1 to 3 leaves at the time of planting out while CC1 had 3 to 5 leaves (Figure 3). Plantlets with more number of leaves were higher in Sree Jaya compared to CC1 at two months after planting (Figure 4). More number of taller plants at the time of planting out was observed in Sree Jaya. However, plants with long roots were exhibited by CC1 (Figure 5). More number of individuals with taller plants at the time of planting out was observed in Sree Jaya whereas the number of taller plants was less in CC1 at the time of planting (Figure 6). After two months of planting more number of taller plants were observed in CC1 plants treated with mutagen (Figure 7). Hence, it is not necessary that plants having more leaves and height at

the time of planting will have more growth rate under hardening. The longer roots of CC1 plants treated with mutagen might have resulted in more absorption of nutrients leading to a faster growth rate. Slightly higher number of plants of Sree jaya had stem girth in the range of 0.1 to 0.6 cm compared to the plants of CC1 (Figure 8). More number of mutagen treated plants of Sree Jaya exhibited increased stem girth at two months after planting while most of the CC1 plants did not show an increase in stem girth (Figure 9).

These results obtained thus indicate that variability was observed with respect to morphological traits under primary hardening. Hence, there is a need of evaluation of these variants observed for better understanding the variation at field level.

5.1.1.2. Survival of *in vitro* mutagen treated plantlets of cassava under hardening

The fundamental advantage of hardening in pad and fan green house is that it grants higher survival rates for acclimatization. The advantage is due to constant maintenance of temperature conditions and humidity throughout the period without any fluctuation (Magaia *et al.* 2015). Hardening as per the guidelines of IITA and CIAT guarantees only 35-50 per cent survival of cassava in Mozambique and Zimbabwe (Jorge *et al.*, 2000). According to the study conducted by Magaia (2015), there was 50 per cent survival of *in vitro* mutant plantlets of cassava under pad and fan green house condition.

In the present study, the *ex vitro* acclimatisation of *in vitro* mutant plantlets of cassava was carried out under pad and fan green house condition (RH > 85 %; temperature 27°C). The protocol of Dumet *et al.* (2007) was used for hardening the *in vitro* cassava plants. The success of this protocol was confirmed in cassava by Magaia (2015). The study showed that the rate of survival after hardening of 58 *in vitro* mutagen treated plantlets transferred from tissue culture laboratory was 58.62 per cent under pad and fan green house whereas all the 10 plantlets which was already in primary hardening, survived primary hardening.

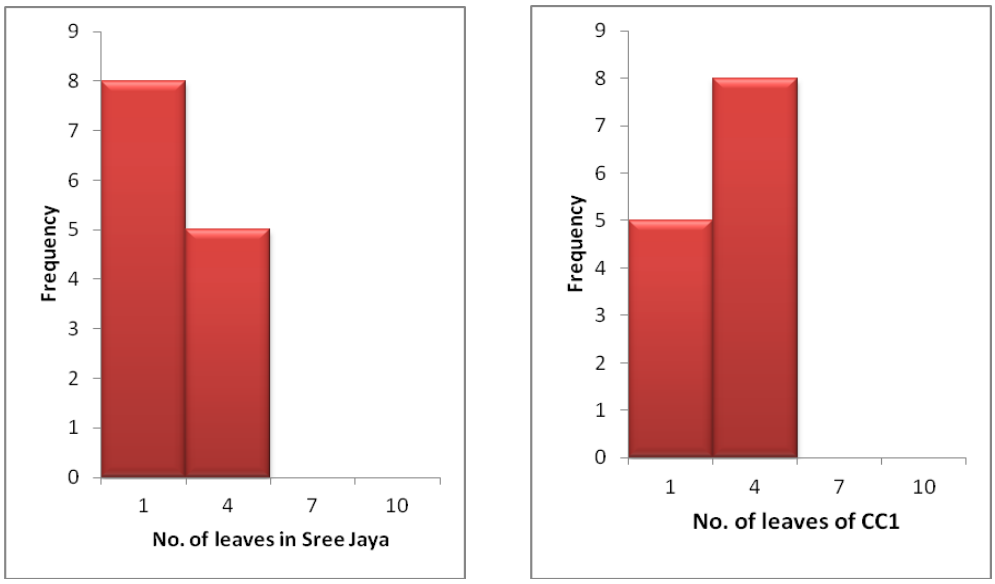


Figure 3. Frequency distribution of number of leaves in Sree Jaya and CC1 at the time of planting out

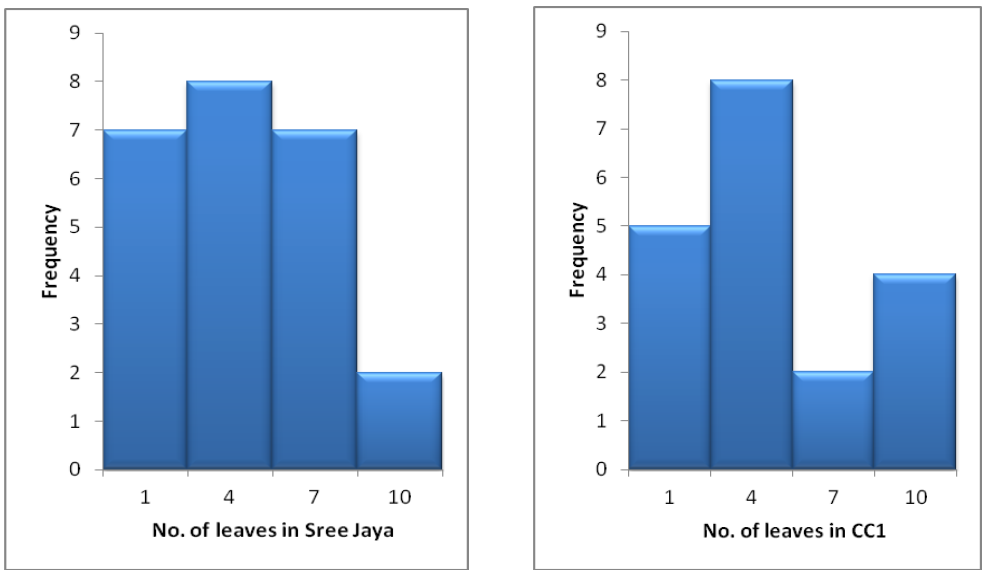


Figure 4. Frequency distribution of number of leaves in Sree Jaya and CC1 two months after planting out

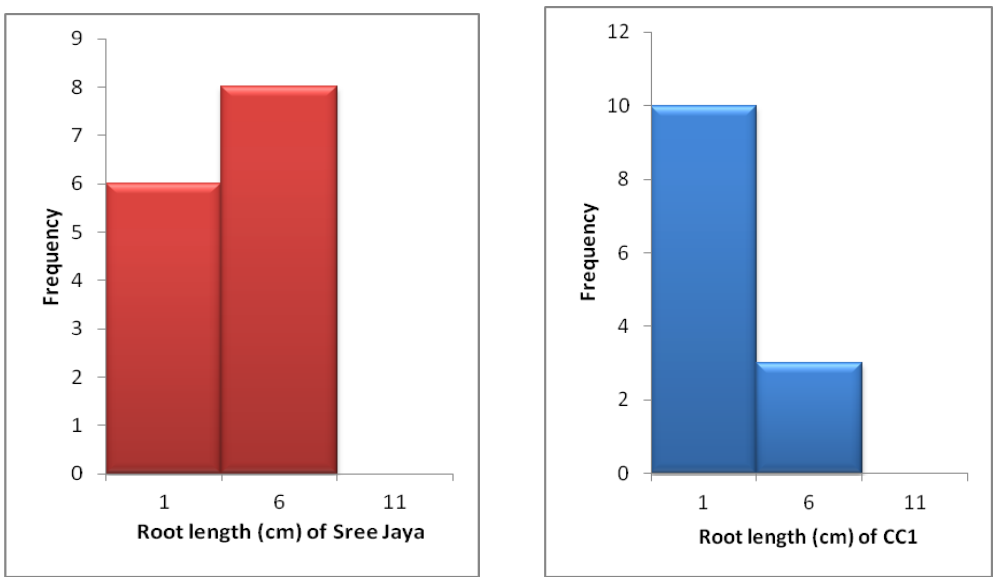


Figure 5. Frequency distribution of length of roots in Sree Jaya and CC1 at the time of planting out

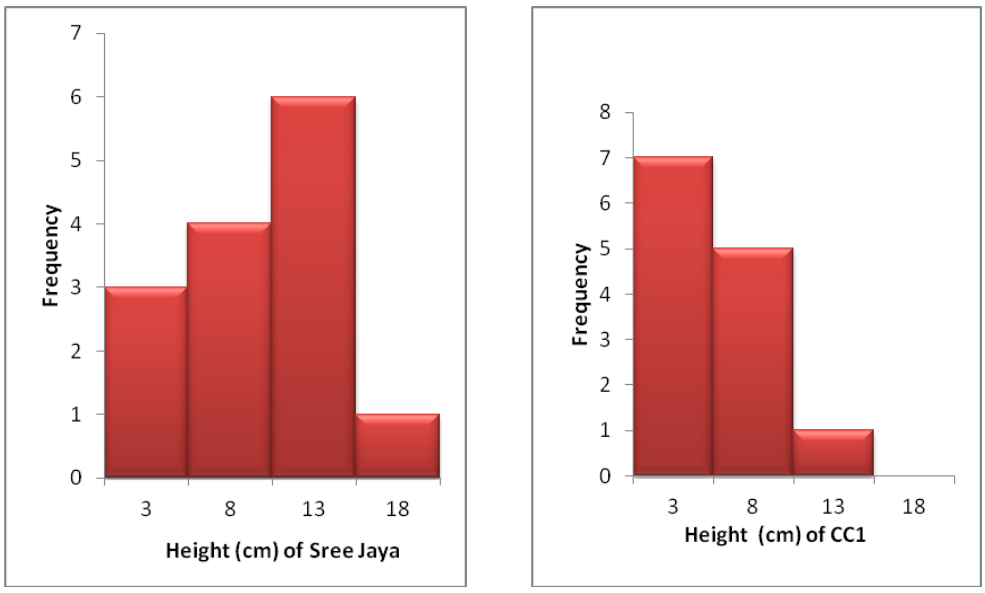


Figure 6. Frequency distribution of plantlet height in Sree Jaya and CC1 at the time of planting out

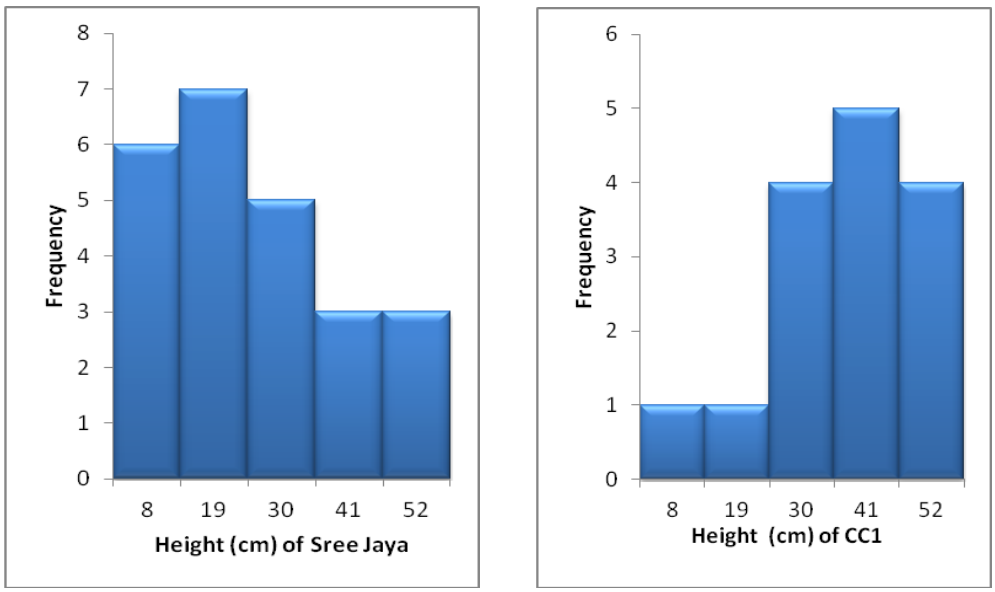


Figure 7. Frequency distribution of plantlet height in Sree Jaya and CC1 two months after planting out

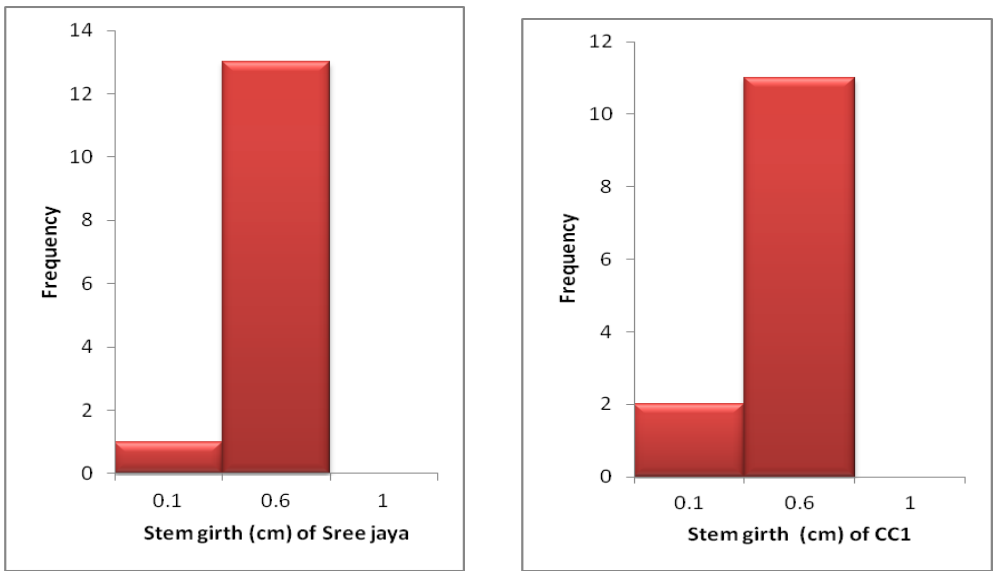


Figure 8. Frequency distribution of stem girth in Sree Jaya and CC1 at the time of planting out

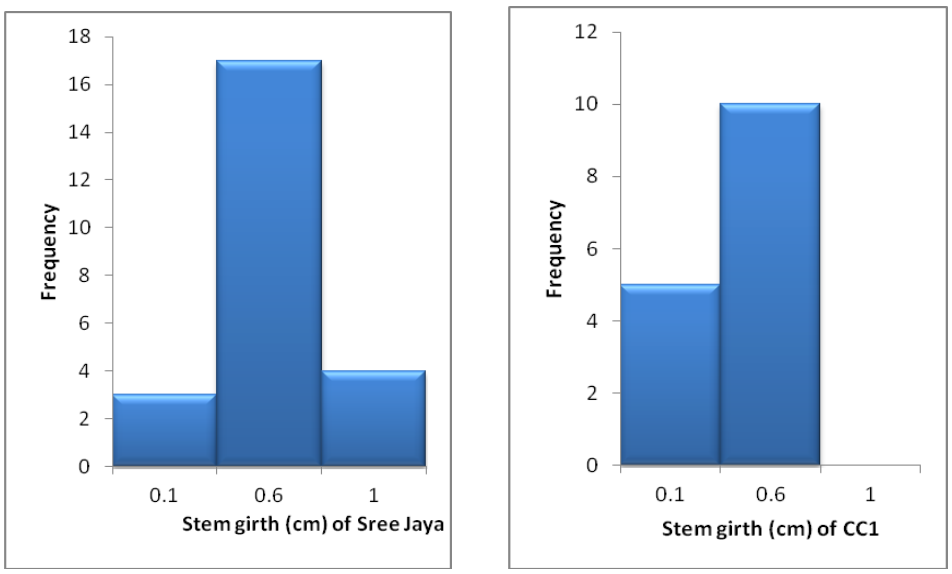


Figure 9. Frequency distribution of stem girth in Sree Jaya and CC1 two months after planting out

Secondary hardening was done under rain shelter condition. The plantlets were maintained at a temperature of 27- 32 °C and at a relative humidity of 60 per cent. It was observed that all the plants survived under secondary hardening.

5.1.2. Assessment of field level variability of *in vitro* mutagen treated cassava genotypes

5.1.2.1. Field evaluation of *in vitro* mutagen treated plants of cassava for qualitative traits

After hardening, once the mutants are established in field conditions, the cassava descriptors suggested by IITA might be used for both characterization and performance evaluation of genotypes against the original ones. Since each mutant plant is capable of becoming a new clone, the data should be recorded on individual basis (Magaia, 2015).

5.1.2.1.1. Field evaluation of qualitative traits at three months after planting

Colour and pubescence of apical leaf was recorded at three month old plants according to the descriptor developed by Fukuda *et al.* (2010). Variations in apical leaf colour were recorded in *in vitro* mutagen treated plants of both the genotypes. Control plants of Sree Jaya had purplish green apical leaf while, light green apical leaves were observed in mutagen treated plants of Sree Jaya at different levels of EMS. In addition to the colours scored for apical leaves in the descriptor of Fukuda *et al.* (2010), more variants were observed like greenish purple, dark green with purple margin and light green with purple margin (Plate 4). Similarly *in vitro* mutagen treated plants of CC1 also showed variation in colour of apical leaves compared to the control plants which had light green apical leaves (Plate 5). All CC1 plants treated with 0.6 per cent EMS produced purplish green apical leaves. One each of 0.9 per cent EMS treated CC1 plants produced dark green and purple leaves. The gradation of purple colour varied from deep purple, greenish purple to purplish green.

In the study conducted Joseph *et al.* (2004), colour of apical leaf varied from purple-green in control plants to light green. Magaia (2015) also reported that the emerging leaf colour varied from purple-green to light green in his studies. Rabbi *et al.* (2014) illustrated that two loci control this trait and occur contiguously in linkage group 2. According to them, this trait explains 93 per cent of phenotypic variation in cassava. This indicates the presence variability created by mutation in the present study. Rabbi *et al.* (2014) also suggested that pigmentation of various tissues is the most conspicuous morphological trait distinguishing different varieties of cassava.

Irrespective of the treatment, all the plants evaluated showed no variation with respect to pubescence in the apical leaves.

5.1.2.1.2. Field evaluation of qualitative traits at six months after planting

As per the descriptor developed by Fukuda *et al.* (2010) observations were recorded on eight qualitative traits of field grown *in vitro* mutagen treated plants. The characters namely, leaf lob margin, colour of mature leaves and colour of leaf vein did not show variation between the *in vitro* mutagen treated plants and its control.

Leaf retention is a trait that presents an additional opportunity to increase cassava yields. A plant with average leaf retention is the one with leaves covering about half of the plant (Fukuda *et al.,* 2010) which is scored as 3. Accordingly, score 1 represents very poor retention, score 2 is less than average retention, score 4 better than average retention and score 5 outstanding leaf retention. Cassava adapts to water shortage by reducing its leaf canopy (Connor and cock, 1981; El-Sharkawy and Cock, 1987) to reduce water use. Hence, leaf shedding is an effective adaptation mechanism as a response to moisture stress.

The green leaf retention in plants showed that control plants of Sree Jaya had better than average leaf retention while CC1 had less than average leaf retention. Most of the mutagen treated plants of both Sree Jaya and CC1 had outstanding leaf retention (9 plants of CC1 and 20 plants of Sree Jaya). Even though, the crop was under moisture stress, the plants showed variation in leaf retention and the plants with outstanding green leaf retention indicate presence of more chlorophyll leading to more photosynthetic effeciency and may have yield advantage over other types under moisture stress.

Plants of Sree Jaya had lanceolate leaf lobe and CC1 had ovoid leaf lobe. On comparison with these plants, two mutagen treated CC1 plants showed variation in shape of central leaflet and it was lanceolate (Plate 6).

According to Joseph *et al.* (2004), in most of the lines the colour of the petiole was purple-green including the control plants. However, the colour varied from light-green in S8 and S13 to light-purple in S7 and S17. Magaia (2015)

reported that petiole colour varied from purple-green to light green. In the present study, control plants of Sree Jaya had red petiole and CC1 had purple petiole. Mutagen treated plants of Sree Jaya had no variation in petiole colour. Except two plants of i*n vitro* mutagen treated CC1 plants all others had red coloured petiole. One mutagen treated CC1 plant produced very deep purple colour petiole and another plant produced reddish green petiole (Plate 7). According to the study conducted by Rabbi *et al.* (2014) anthocyanin pigmentation of the leaf petiole is associated with a single locus which explains 75 per cent of the phenotypic variation. Hence, this trait also can be used to distinguish potential variants in cassava.

The evaluated plants were given scores of 5 and 7 based on the number of most frequent occurrence of number of lobes. It was observed that in the same plant there were leaves with 5 and 7 numbers of lobes. Hence, it was not considered as a variable trait.

There were two types of leaf orientation observed between the plants evaluated. Majority of the plants were having leaf orientation inclined downwards. However, four plants of Sree Jaya treated with different doses of EMS had horizontal leaves (Plate 8). Williams and Ghazali (1969) have found variation in orientation of upper leaves in cassava clones. There upper leaves move towards vertical orientation at night and a change to moderate angles of display during daylight hours. Hence, the four plants observed to have horizontal leaf orientation in the present study cannot be used as a typical character to distinguish between genotypes of cassava as this character is influenced by environment.

All these variations observed in the *in vitro* mutagen treated cassava plants indicate that variability is created by mutagen on different qualitative traits of the selected cassava varieties.

5.1.2.1.3. Field evaluation of qualitative traits at nine months after planting

Observations taken on the qualitative characters on cassava as per the descriptor at nine month after planting showed that for the traits like prominence of leaf scar, distance between leaf scar, growth habit, colour of stem cortex, length of stipule, and stipule margin exhibited no variation between the plants.

The exterior colour of stem of Sree Jaya was grey and that of CC1 was silver. One mutagen treated plant of CC1 had golden coloured stem exterior. Similarly one plant of CC1 treated with EMS had greeny-yellowish stem colour (Plate 9). All other plants showed no variation when compared with control plants. Rabbi *et al.* (2014) suggested that stem exterior colour likely to be controlled by a single locus with dominant effect. This indicates that whatever variations are observed in these traits can be considered as true variations in the population.

Irrespective of control or mutagen treated plants, all plants showed moderate to severe CMD symptoms because of dry climate and high infestation of white fly. CLS was also observed as angular leaf spots. The exception was one plant of Sree Jaya (Plant 8) treated with 0.3 per cent EMS, which produced no symptom for CLS and only mild chlorotic pattern of leaf towards the end of crop season for CMD (Plate 10). Hence, this plant needs to be evaluated further for confirming the disease response reaction.

5.1.2.1.3.1. Evaluation of qualitative traits of roots

Evaluation of qualitative traits of the roots showed that texture of root epidermis was the same in all the tested plants. Plants of Sree Jaya had pedunculate root while, CC1 sessile root neck. Among *in vitro* mutant plants, one plant of Sree Jaya treated and one plant of CC1 treated with EMS had sessile root peduncle. Also, one plant each of Sree Jaya and CC1 treated with different doses of EMS had mixed root (Plate 11, Plate12). According to Lebot (2009), cassava cultivars having roots with well developed peduncle are suitable for better storage.

They also suggested that genotypes having roots with short peduncle are difficult to separate from the main stem. Hence, the plants having pedunculate roots have to be identified and selected.

Root constriction varied from few to none for various treatments. Out of the plants which showed many constrictions most were of Sree Jaya. The root constriction can be caused by nematodes and/or cassava brown streak disease (Fukuda *et al.,* 2010).

Sree Jaya and CC1 plants had conical roots. Some variants were observed with respect to this trait. Most of the mutagen treated plants of both Sree Jaya and CC1 genotypes had conical roots as that of its control. Irregular roots were also observed in one mutagen treated plant of CC1 (Plate13, 14). This variation can be attributed to genetic factors as well environmental factors such as incidence of CMV, soil moisture stress *etc.* According to Lebot (2009), cassava cultivars having compact, cylindrical, or conical roots are suitable for better storage. Hahn *et al.* (1988) suggested that irregular roots of cassava are difficult to harvest and peel by hand. This will result in heavy loss of usable root materials. Hence, plants with cylindrical or conical roots have to be selected.

In the case of root external colour, control plants had dark brown colour. One plant of Sree Jaya treated with 1.2 per cent EMS resulted in yellow root. Also, one plant of Sree Jaya treated with 0.3 per cent EMS resulted in light brown root exterior colour (Plate 15). Magaia (2015) observed three colour variations in external colour of root in cassava varieties as light brown, dark brown and white. In his studies majority of plants were having dark brown root.

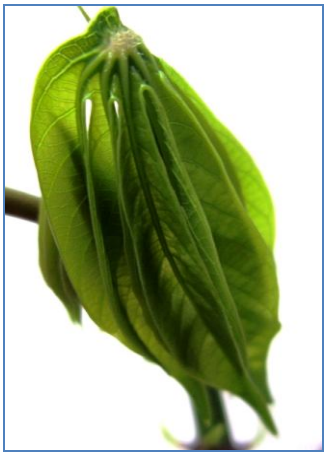
In the present study, one 0.9 per cent EMS treated plant of CC1 showed variation in colour of root pulp with respect to control. The variant had a root with cream pulp in contrast to white coloured root pulp in control plants (Plate 16). Magaia (2015) observed three classes of colour for root pulp namely, cream, white and yellow with majority falling under white colour. The colour intensity of the cassava root and the carotene concentration are positively

correlated (Iglesias *et al*., 1997). Hence, the cream coloured ones may contain carotene and may be nutritionally superior. Paninah *et al.* (2014) reported that the colour of root pulp is controlled by both major and minor genes and hence, both additive and non-additive gene actions operate. Similarly, Iglesias *et al.* (1996) reported that a few genes control the production of carotenoids, mostly β-carotene in cassava storage roots. The evaluated plants show variability irrespective of gene action.

Control plants and most of the *in vitro* mutant plants of cassava genotypes had pink coloured root cortex (Plate 17). An exception was a plant of Sree Jaya treated with 0.3 and 1.2 per cent EMS, which resulted in deep pink to purple root cortex. Even though, the score for colour was same there was observable variation in gradation of colour of root cortex. This included creemish pink, light pink, pink and deep pink. Magaia (2015) observed four classes for root cortex colour namely, pink, yellow, cream and purple with half of them being pink. In communities that prefer mealy varieties for boil-and-eat consumption, tubers with pinkish inner skin are given a premium value (Rabbi *et al.*, 2014).

According to Rabbi *et al.* (2014) pigmentation of various tissues is the most conspicuous morphological trait distinguishing different varieties of cassava. Evaluation of qualitative traits in *in vitro* mutagen treated plants at various stages of growth revealed presence of variation with respect to pigmentation of various parts of plant as well as other qualitative traits. The pigmentation with respect to colour of apical leaves, petiole colour and external stem colour can be used to distinguish variants in cassava. All the results obtained indicate that wide variability was created by *in vitro* mutagenesis in the cassava genotypes.

Sree Jaya control
Purplish green



Plant no. 14
Light green

Plant no. 2
Light green

Plant no. 34
Light green

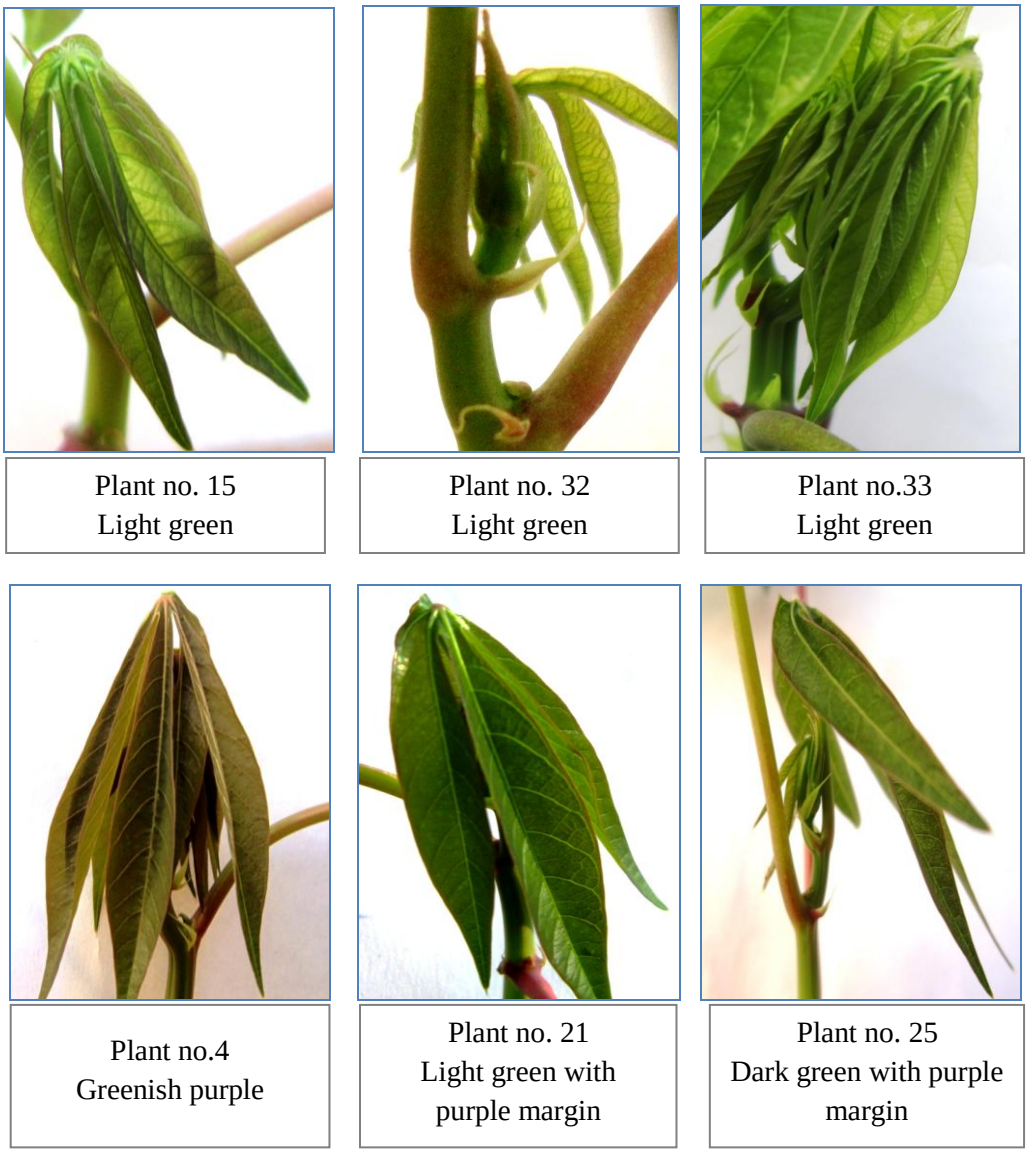
Plant no. 12
Light green

Plant no. 3
Light green

Plant no. 1
Light green

Contd...

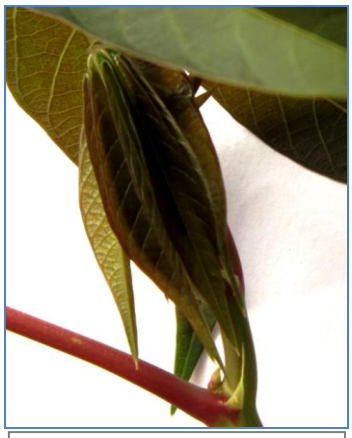
Plate 4. Variation of apical leaf colour in Sree Jaya



Plant no. 15 Light green	Plant no. 32 Light green	Plant no.33 Light green
Plant no.4 Greenish purple	Plant no. 21 Light green with purple margin	Plant no. 25 Dark green with purple margin

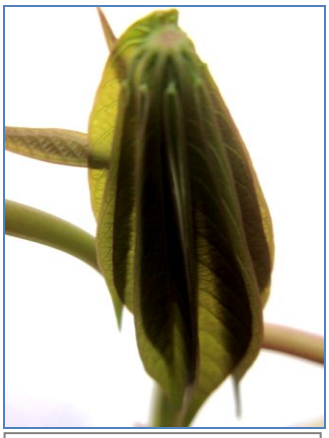
Plate 4. Variation of apical leaf colour in Sree Jaya

CC1control
Light green



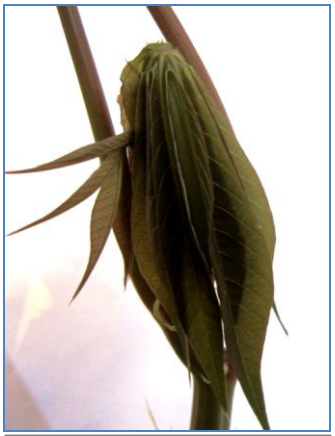
Plant no. 38
Greenish purple

Plant no. 40
Greenish purple



Plant no. 39
Greenish purple

Plant no. 36
Light green



Plant no.
Deep purple

Plant no. 34
Dark green

Plate 5. Variation of apical leaf colour in CC1

CC1 control
Ovoid

Plant no. 18
Lanceolate

Plant no. 19
Lanceolate

Plate 6. Variation in shape of central leaflet of CC1

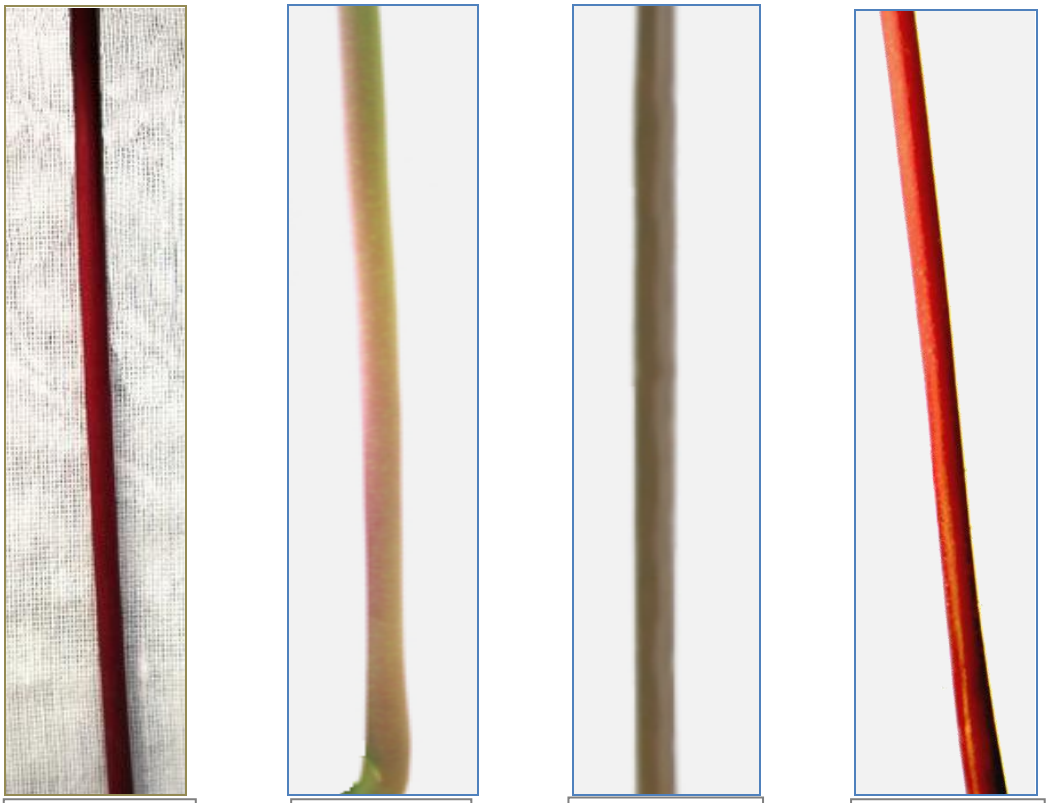


Plate 7. Variation in petiole colour of CC1

Sree Jaya control
Inclined downwards

Plant no.1
Horizontal

Plant no.13
Horizontal

Plant no.15
Horizontal

Plant no.25
Horizontal

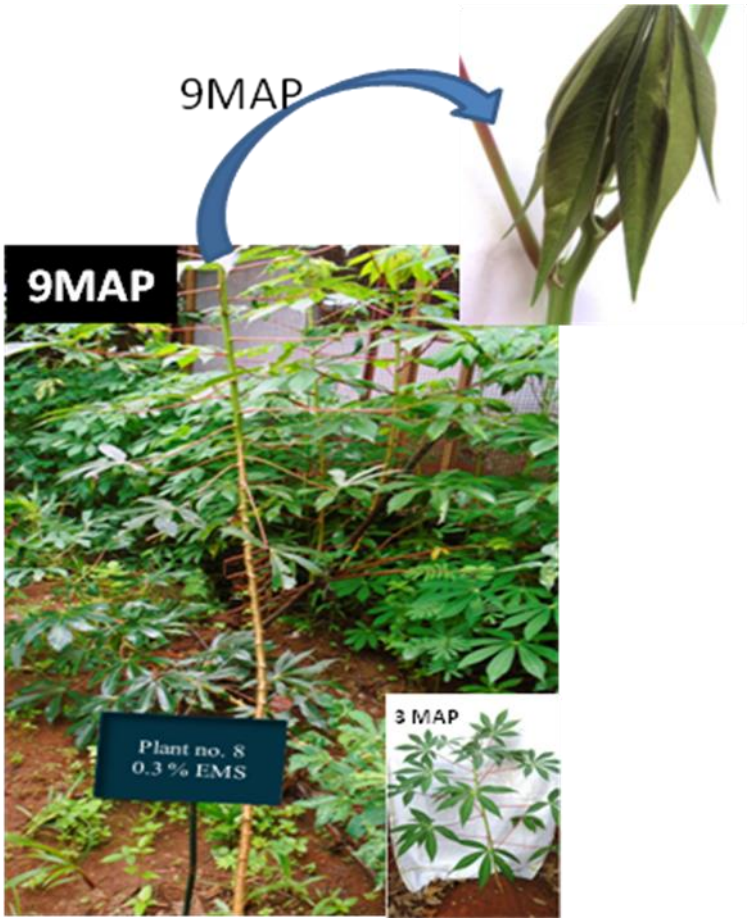
Plate 8. Variation in leaf orientation of Sree Jaya

CC1control
Silver

Plant no.42
Golden

Plant no.19
Greeny yellowish

Plate 9. Variation in external stem colour of CC1

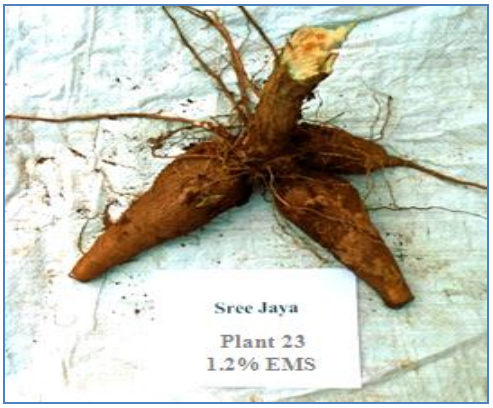


Character	Descriptor
Colour of apical leaf	Purplish green
Leaf retention	Outstanding
Shape of central leaflet	Lanceolate
Colour of petiole	Red
Colour of leaf	Dark green
Colour of leaf vein	Reddish green in more than half of the lobe
Colour of stem exterior	Grey
CMV	Mild chlorotic pattern on leaf
CLS	No symptom
Ratio of leaf lobe	4.25
Petiole length	20.6 cm
Stem girth	1.51 cm
Plant height	105 cm

Plate 10. Tolerance to CMD and CLS

Sree Jaya control
Pedenculate

Plant no.4
Sessile

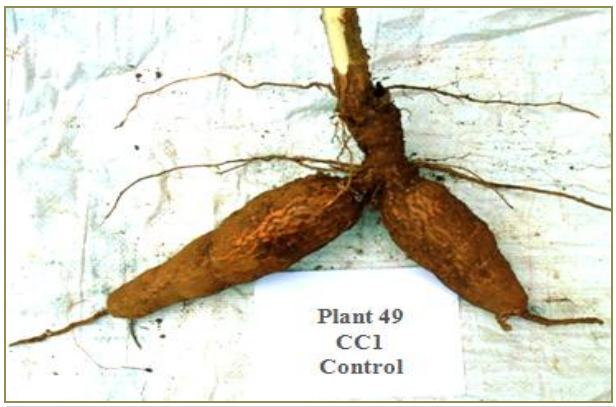


Plant no.23
Sessile

Plant no.7
Sessile

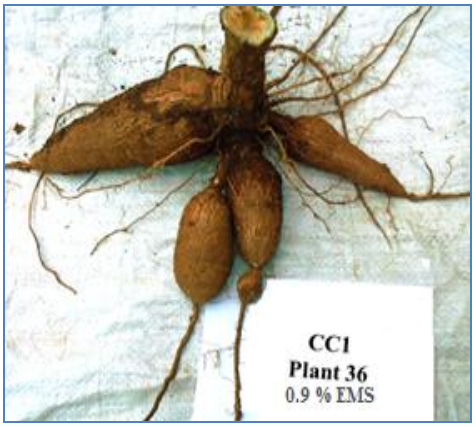
Plant no.14
Mixed

Plate 11. Variation in extend of root peduncle of Sree Jaya



CC1control
Sessile

Plant no.37
Pedenculate



Plant no.36
Mixed

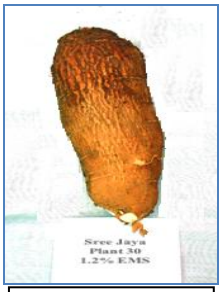
Plate 12. Variation in extend of root peduncle of CC1

Plant no.
Conical tuber

Plant no.15
Cylindrical

Plant no.21
Cylindrical

Plant no.12
Cylindrical

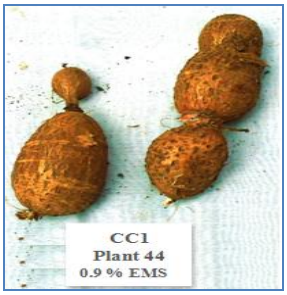


Plant no.30
Cylindrical

Plate 13. Variation in tuber shape in Sree Jaya

Control
Conical tuber

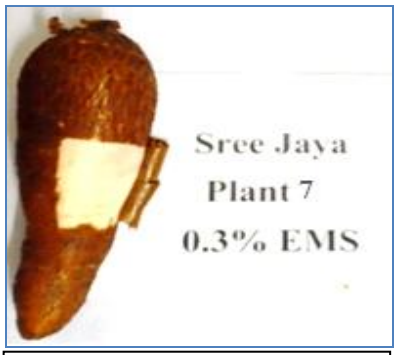
Plant no.36
Cylindrical tuber



Plant no.44
Irregular tuber

Plate 14. Variation in tuber shape in CC1

Sree Jaya control
Dark brown

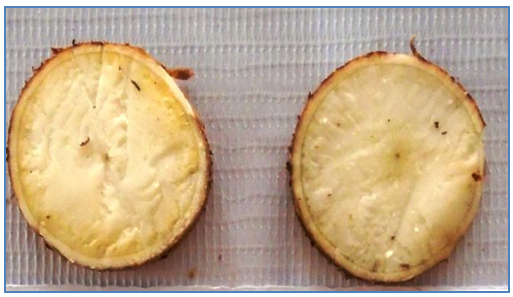


Plant no.7
Light brown

Plant no.21
Yellow

Plate 15. Variation in colour of external root epidermis of Sree Jaya

Plant no.
Cream root pulp



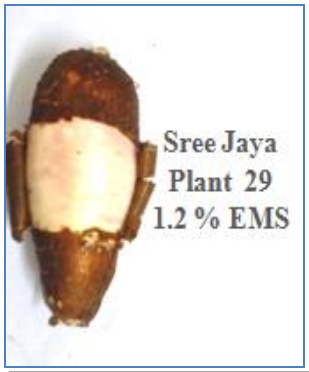
Control
White root pulp

Plate 16. Variation in root pulp colour in CC1

Sree Jaya control
Pink

Plant no.25
Light pink

Plant no.3
Light pink



Plant no.29
Light pink

Plant no.10
Deep pink

Plant no.21
Deep pink

Plant no.4
Creamish pink

Plate 17. Variation in root cortex colour of Sree Jaya

5.1.2.2. Field evaluation of quantitative traits of *in vitro* mutagen treated plants of cassava

5.1.2.2.1. Field evaluation of quantitative traits at six months after planting

Leaf lobe size of cassava leaves are estimated as the ratio of length to width of leaf lobe. As per the studies of Khumaidaa *et al.* (2015) in Gamma ray irradiated population of cassava genotypes, reported mature leaf lobe size (ratio of length: width lobe) on mutants from Jame-jame, Ratim, and Malang-4 was relatively equal, while mutants from UJ-5 had larger lobe length, and mutants from Adira-4 had narrower lobes width. The present study also showed variation with respect to the leaf lobe size in the *in vitro* mutagen treated plants. The control plants were having low ratio of length of leaf lobe to width of leaf lobe (3.22 - 3.86) compared to many of the *in vitro* mutagen treated plants. Nine *in vitro* mutagen treated plants of Sree Jaya belonging to different EMS treatment had a ratio of more than 4.50. Plant number 31 was having the highest value of 5.31. According to Ghosh *et al.* (1988) petiole length in cassava ranged between 5 and 30 cm. The present study showed few *in vitro* mutagen treated plants with longer petiole. Also it was observed that the range of petiole length was narrow (20.6 - 36.6cm) (Appendix V).

On comparison of mean values of length, width and length to width ratio of leaf lobe and petiole length, mutagen treated Sree Jaya plants were having greater values compared to its control whereas, mutagen treated CC1 plants showed varied response on comparison with its control for these characters.

5.1.2.2.2. Field evaluation of quantitative traits at nine months after planting

Distance between leaf scars in *in vitro* mutagen treated plants of cassava also showed variation between plants. The range was between 1.30 cm to 7.60 cm in different mutagen treated plants of Sree Jaya and CC1. In the control plants it was between 3.15 cm to 3.9 cm. Agre *et al.* (2015) and Nadjiam *et al*. (2016) grouped plants based on distance between leaf scars as short (≤8 cm), medium (8 -

15 cm) and long (> 15cm). Hence, all the presently evaluated plants had short distance between leaf scars. Stem girth of control plants ranged between 1.57 cm and 1.34 cm. In the case of *in vitro* mutagen treated plants the range was broader (1.00 -2.86 cm), indicating the variability induced through *in vitro* mutagenesis.

Height of the plants was ranged between 185 cm and 215 cm in control plants while the *in vitro* mutagen treated plants had a wider range of plant height ranging from 52.50 cm to 556.00 cm. Range for number of storage roots in control plants was 2 to 3 while it ranged from 0 to 6 numbers in *in vitro* mutagen treated plants. There were no commercial roots present in the control plants while, it ranged between 0 and 3 in *in vitro* mutagen treated plants. Thickness of tuber also showed wider variation between *in vitro* mutagen treated plants (2.31 – 7.10 cm) compared to control plants (3.90 - 4.87 cm). In case of tuber length the range in control plants was 15.1 to 19.63 cm while the *in vitro* mutagen treated plants had a range of 10.20 cm to 34.20 cm. Average cortex thickness in control plants showed a range of 1.5 mm to 1.6 mm and in *in vitro* mutagen treated plants it was between 0.8 mm to 2.2 mm. Fresh tuber weight exhibited a range of 0.32 kg to 1.44 kg while the *in vitro* mutagen treated plants had a range of 0.09 to 1.19 kg (Appendix V).

Range for above ground plant weight in control plants was 0.40 to 1.47 kg and in *in vitro* mutagen treated plants it was between 0.26 kg and 1.77 kg. Similarly harvest index, dry matter content and percentage of starch also showed wider variability in *in vitro* mutagen treated plants compared to control (Appendix V). In the studies by Magaia (2015), on evaluation of 15 cassava genotypes the range for plant height observed was between 224.40 cm and 254.15 cm, and the stem girth 21.01 mm 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. Not all the *in vitro* mutagen treated plants produced tubers. This may be because of different growth stages of plants as the plants atre field planted on same day or may be because of the deleterious effect of mutagen. Mature plants can adapt more to water stress by means of regulatory

mechanisms such as reduction of leaf canopy, utilization of deep soil water and thus maintains photosynthetic activity (Nesreen *et al.,* 2013). Hence, Water stress may not have an effect as cassava is a crop that rather reduce leaf canopy than limiting tuber production. The present study indicated wider variability of *in vitro* mutagen treated plants in comparison with control plants as well as with the genotypes studied by Magaia (2015) for quantitative traits.

The dry matter content of tubers was between 23.16 per cent and 30.55 per cent in control plants while the *in vitro* mutagen treated plants had a wider range of dry matter ranging from 16.3 to 49.54 per cent. Range for starch content of roots in control plants was 10.56 to 20.27 per cent while it ranged from 5.7 to 29.24 in *in vitro* mutagen treated plants. Both starch yield and quality are dependent on planting date and time of harvest (Sriroth *et al.,* 2001). The HI of control plants ranged from 1.00 to 0.30. The mutagen treated plants had HI at the range 0.21 to 1.35 (Appendix V).

The mean values of distance between leaf scars, plant height, stem girth, length and thickness of tuber, cortex thickness, fresh tuber weight, above ground weight, HI, dry matter and starch content and number of tubers and commercial roots per plant in mutagen treated Sree Jaya plants were having greater values compared to its control whereas, mutagen treated CC1 plants showed varied response on comparison with its control for these characters.

The treatment of cassava with EMS resulted in more variability at morpho-physiological level (Magaia, 2015). The quantum of variability expressed in the *in vitro* mutagen treated plants of cassava with respect to qualitative and quantitative traits shows the efficiency of *in vitro* mutagenesis in creating variability in cassava.

5.1.3. Sensory evaluation of *in vitro* mutagen treated plants of cassava

All the plants evaluated for tubers were scored for different attributes like colour before cooking, peeling easiness, chewing sweetness, colour and taste after

cooking and bitterness by 12 panellists of different age groups. The scoring was done on a five point Hedonic scale which ranged from dislike extremely (1) to like extremely (5).

Magaia (2015) suggested that before cooking, fresh tuber colour and chewing sweetness are the most important quality traits preferred by cassava consumers. Similarly, taste, texture and fibre content are the preferred quality traits after cooking cassava tubers. Similar results were obtained by Gonzalez and Johnson (2009). In addition, these traits were highly correlated with cumulative ranking (Safo- Katanka *et al.,* 1997). The analysis showed that there was no significant difference between the taste perceptions of the panellists. Hence, the outcome of the analysis is expected to be highly reliable.

The mean rank of the best plants under analysis showed in Figure 10 and best plants for different attributes in sensory evaluation is presented in Table 21.

Table 21. Top ten plants obtained in sensory evaluation

Character	**Plant no.**									
Rank	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Colour before cooking	47	15	42	24	5	28	45	41	21	4
Peeling easiness	42	47	41	43	32	12	6	ree Jaya	CC1*	36
Chewing sweetness	47	11	33	31	42	32	6	48	41	13
Colour after cooking	5	28	32	31	42	36	45	34	3	35
Texture after cooking	31	32	12	42	48	Sree Jaya*	47	6	4	43
Taste after cooking	32	31	12	15	42	3	47	6	14	15
Fiberlessness	3	32	13	31	37	12	36	42	10	44
Bitterness	31	42	31	14	CC1*	10	6	13	23	33

* Control plants

The cumulative scoring of the different attributes of cassava tubers of each plant showed that plant 32 (30.76) scored highest on over all basis followed by plant 31 and 42 with a rank of 30.42 (Table 14 a, b). This plant was most

preferred by panellists because of their better taste, less fiber content and good texture after cooking and was least bitter compared to all other plants evaluated for its tuber. Plant 34 had the least cumulative score (22.25) followed by plant 8 (25.67).

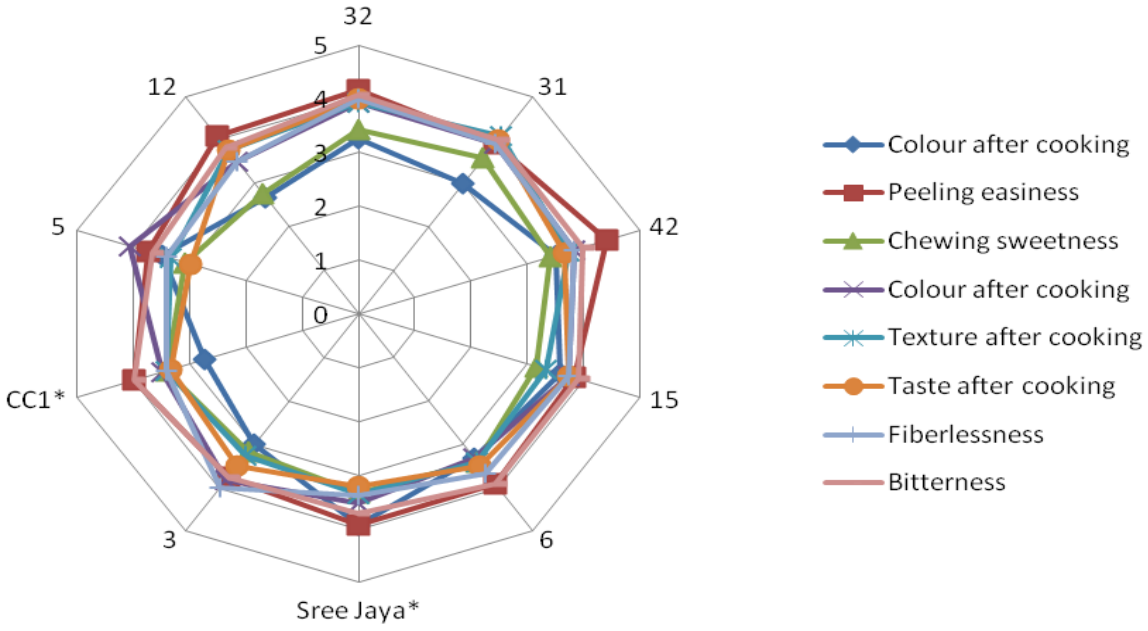


Figure 10. Radar showing mean rank of sensory attributes of top ten cassava plants

5.2. Experiment 2: *In vitro* induction of mutation

5.2.1. Effect of media on callusing in cassava

Study conducted to investigate the effect of different growth regulators on callus formation and regeneration from leaf explants of cassava showed that use of MS + 8 mg L^{-1} 2, 4-D, MS+ 8 mg L^{-1} Picloram or MS + 8 mg L^{-1}2,4-D + 0.5 mg L^{-1}NAA + 1 mg L^{-1}BA had no significant effect on necrosis and hard callus production. Whereas, MS + 8 mg L^{-1} 2,4-D + 0.5 mg L^{-1} NAA + 1 mg L^{-1} BA resulted in highest rate of production of friable embryogenic callus (Plate 18).

Yasmin *et al.* (2003) found that 95 per cent callusing, 80 per cent callus regeneration and 16 plantlets per callus were obtained in a medium supplemented with 2.5 mg L^{-1} NAA + 2 mg L^{-1} BAP.

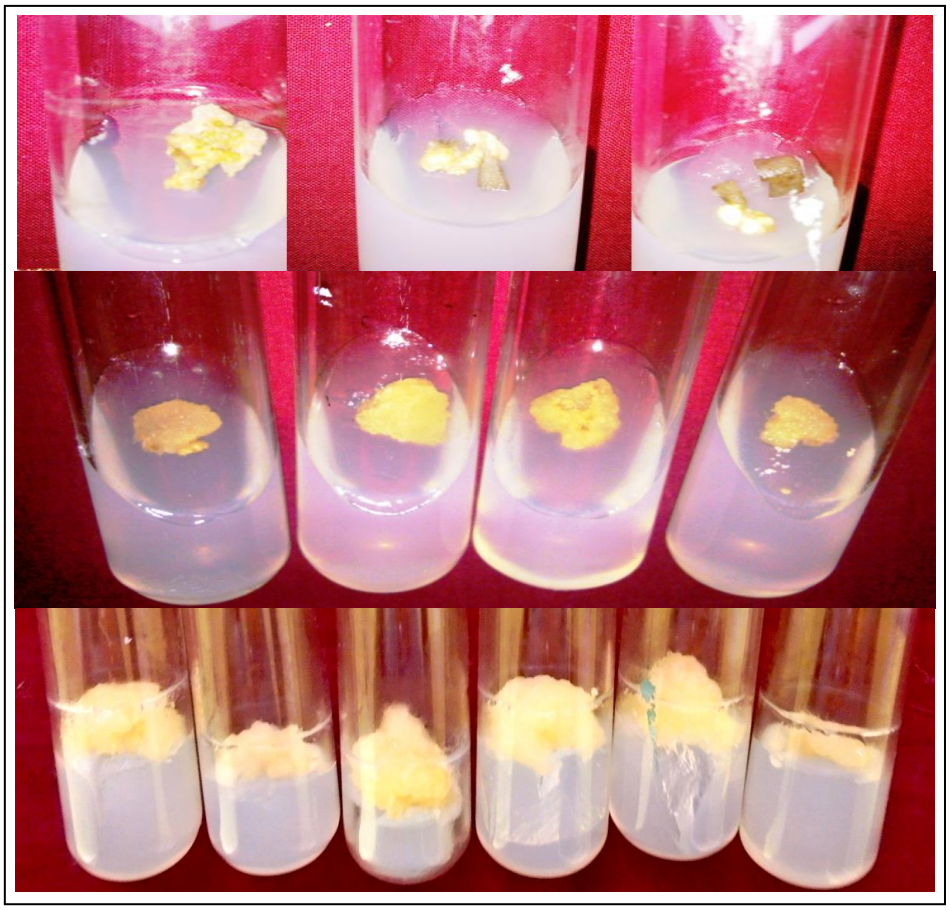


Plate 18. Stages of callusing in cassava (MS + 8 mg L^{-1} 2,4-D + 0.5 mg L^{-1} NAA + 1 mg L^{-1} BA)

In the study conducted by Vidal *et al.* (2014) the highest frequency of callus induction and the greatest number of somatic embryos per callus was obtained in media MS supplemented with 8 mg L^{-1} of Picloram.

Naema *et al*. (2013) suggested that, compared with other supplements, MS medium supplemented with 15 mg L^{-1} 2,4- D was the best media combination for cassava callus production.

Taylor *et al.* (1996) and Bull *et al.* (2009) suggested that, friable embryogenic callus (FEC) clusters can be induced from secondary somatic embryos by continuous incubation on a growth and development medium supplemented with 50µM Picloram. However, in the present study, with all the treatments, there was no somatic embryo production.

5.2.2. *In vitro* induction of mutation in cassava

5.2.2.1. Effect of gamma irradiation on the callus cultures of cassava

Induced mutagenesis can be carried for the subtle manipulation of traits of interest in crop plants and to enhance genetic variability exploitable for the improvement of agronomic traits such as disease resistance, earliness in bulking, yield and quality (Lee *et al.,* 2003; Guthrie, 2000; El-Sharkawy, 2004; Oweseni *et al.,* 2006). Regarding the use of physical mutation, Wi *et al*. (2007) emphasised that low dose irradiation will induce growth stimulation by making a change in the hormonal signalling pathways in plant cells or by increasing the anti-oxidative capacity of the cells.

The study was conducted to confirm the optimum dosage of gamma rays for irradiation as suggested by Magaia (2015). Survival of callus cultures and their ability to induce friable embryogenic callus was considered as the parameter to study the effect of treatment. Magaia (2015) suggested the LD_{50} of the cassava callus as 40 Gy. In the present study, the highest mean score for necrosis was observed on treatment with 60 Gy (T4). All doses for irradiation

30Gy, (T1), 40 Gy (T2), 50Gy, (T3) and 60 Gy, (T4) were on par with respect to hard callus formation. Similarly, irradiation with already standardised doses 30 Gy, 40 Gy, 50 Gy and 60 Gy of gamma rays resulted in same rate of production of friable callus. Subculturing the friable callus in the regeneration media MS + 8 mg L^{-1} Picloram however, failed to produce somatic embryos in all cultures.

Induction of mutation aims to optimize genetic variation with minimal plant injuries so as to maintain a balance between achieving mutagenesis and maintaining the integrity of the majority of the genome constitution of mutagen treated material (Ndofunsu *et al.,* 2015). Hence, the chosen dose should result in the highest survival of the irradiated explants (Laneri *et al.,* 1990).

5.2.2.2. Effect of EMS treatment on *in vitro* cassava callus cultures

Inducing mutations upgrade well established clones by changing specific traits. The success of mutation breeding depends on determination of the sensitivity of the plant material to the mutagen. According to Magaia *et al.* (2015) the LD_{50} value of EMS was 1.2 per cent for friable embryogenic callus. In the present study, treatment with 0.6 to 0.9 per cent EMS resulted in high necrosis. Treatment with lower doses EMS, 0.1 to 0.4 percent resulted in lower mean value for necrosis. Response of cultures to friable embryogenic callus was more on treatment with 0.1 to 0.4 per cent EMS. On subculturing the survived callus in regeneration media, the callus failed to respond and somatic embryogenesis was not observed.

The evaluation of *in vitro* mutagen treated plantlets in field showed wide variation with respect to most morphological characters as well as biometrical traits. All the plants evaluated can hence be advanced to next generation of evaluation (M1V1) with replication to identify the mutants. On *in vitro* mutation of fresh callus cultures of Sree Jaya and CC1, friable embryogenic callus was produced in the regeneration media, MS + 8 mg L^{-1} Picloram. Since no embryogenesis was observed, the survived callus can be cultured in alternate regeneration media for somatic embryo production and plantlet regeneration.

Summary

SUMMARY

A study was conducted in Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University during 2014-2016 on *in vitro* mutagenesis and evaluation of somatic embryo derived plantlets in cassava (*Manihot esculenta* Crantz.). The salient findings are summarised below:

1. Plants with minimum of 3 cm height and 3 to 5 roots were found to be better for transfer to pad and fan green house for hardening.
2. Variability existed with respect to quantitative traits like height, number of shoots and number of leaves for *in vitro* mutated plantlets of Sree Jaya and CC1 at the time of planting and two months after transferring to pad and fan green house.
3. It is not necessary that plants having more leaves and height at the time of planting will have more growth rate.
4. The rate of survival after hardening of *in vitro* mutated plantlets of cassava was 58.62 per cent under pad and fan green house at temperature of 24.0-27.0 °C and relative humidity of 80-85 per cent.
5. Under rain shelter condition cent per cent survival was obtained on secondary hardening at a temperature of 27-32 °C and at a relative humidity of 60 per cent.
6. Variations could be induced by mutagen treatment in terms of qualitative and quantitative characters.
7. Control plants of Sree Jaya had purplish green apical leaves and mutagen treated plants of Sree Jaya at different levels of EMS produced light green apical leaves. All CC1 plants treated with 0.6 per cent EMS produced purplish green apical leaves. One each of 0.9 per cent EMS treated CC1 plants produced dark green and purple leaves.
8. Majority of mutagen treated plants of both Sree Jaya and CC1 had outstanding leaf retention.
9. Two of the CC1 plants treated with EMS produced lanceolate central leaflet in contrast to ovoid in control.

10. Control plants of Sree Jaya had red petiole and CC1 had purple petiole. Majority of mutagen treated Sree Jaya as well as CC1 had red coloured petiole. One EMS treated plant of CC1 produced very deep purple colour petiole and another CC1 plant produced greenish red coloured petiole.
11. Majority of the plants had leaf orientation inclined downwards. Four plants of Sree Jaya treated with EMS produced horizontal leaves.
12. The exterior stem colour of control plants of Sree Jaya was grey and CC1 was silver. One CC1 plant treated with EMS resulted in golden coloured stem exterior. Similarly another plant of CC1 treated with EMS had greeny-yellowish stem.
13. Plant 8 (Sree Jaya treated with 0.3 per cent EMS) produced no symptom for CLS and only mild chlorotic pattern of leaf towards the end of crop season for CMV.
14. Control plants of Sree Jaya had pedunculate root. Among *in vitro* mutated plants, three plants of Sree Jaya treated with EMS had sessile root. Two mutagen treated CC1 plants had pednculated tubers. Also, one plant each of Sree Jaya and CC1 treated with EMS had mixed root.
15. Control plants of Sree Jaya produced conical root. Out of the mutagen treated plants, four plants of Sree Jaya treated with EMS had cylindrical roots. Irregular roots as well as cylindrical roots were observed in one plant each of mutagen treated CC1.
16. External colour of root for control plants was dark brown. One plant of Sree Jaya treated with EMS resulted in yellow root. Also, another plant of Sree Jaya treated with EMS resulted in light brown colour for root exterior.
17. One CC1 plant treated with EMS showed cream colour for root pulp in contrast to white coloured root pulp in control plants.
18. Control plants and most of the *in vitro* mutated plants of cassava genotypes had pink coloured root cortex. One plant each of Sree Jaya was treated with different doses of EMS, resulted in deep pink root cortex. The

colour gradation of root cortex varied from creemish pink, light pink, pink to deep pink.

19. The pigmentation with respect to colour of apical leaves, petiole colour and external stem colour can be used to distinguish mutants in M1V1 evaluation of *in vitro* mutagen treated cassava.
20. Wider variability was observed in *in vitro* mutated plants in comparison with control plants for quantitative traits. The control plants were having low length to width ratio of leaf lobe (3.22-3.86) compared to many of the in *vitro* mutated plants. Nine *in vitro* mutated plants of Sree Jaya belonging to different EMS treatments had a ratio more than 4.50. Plant number 31 (Sree Jaya 1.2% EMS) was having the highest value of 5.31.
21. A few *in vitro* mutated plants were with longer petiole and all the evaluated plants had short distance between leaf scars.
22. Stem girth of control plants ranged between 1.57 cm and 1.34 cm while stem girth of i*n vitro* mutated plants had a broader range (1.00 -2.86 cm).
23. Height of the plants ranged between 185 cm to 215 cm in control plants while the *in vitro* mutated plants had a wider range of plant height ranging from 52.50 cm to 556.00 cm.
24. The number of storage roots in control plants ranged from 2 to 3 numbers while from 0 to 6 in *in vitro* mutated plants. There were no commercial roots present in the control plants while it ranged from 0 to 3 in *in vitro* mutated plants.
25. Thickness of tuber also showed wider variation between *in vitro* mutated plants (2.31-6.4 cm) and control plants (2.30-7.10 cm).
26. The range of tuber length of control plants was 15.1 to 19.63 cm while the *in vitro* mutated plants had a range of 10.20 cm to 34.20 cm.
27. Average cortex thickness in control plants showed a range of 1.5 mm to 1.6 mm and in *in vitro* mutated plants it was between 0.8 mm to 2.2 mm.
28. Fresh tuber weight exhibited a range of 0.32 kg to 1.44 kg for control plants while the *in vitro* mutated plants had a range of 0.09 to 1.19 kg.

29. Range for above ground weight in control plants was 0.40 to 1.47 kg and in *in vitro* mutated plants it was between 0.26 kg to 1.77 kg.
30. The dry matter of tubers ranged between 23.16 per cent and 30.55 per cent in control plants while the *in vitro* mutated plants shared a wider range (16.3-49.54 %).
31. Range for starch content of roots in control plants was 10.56 to 20.27 per cent. However, it ranged from 5.7 to 29.24 in *in vitro* mutated plants.
32. Range for harvest index of control plants was from 0.3 to 1.0 while that of mutagen treated plants was from 0.21 to 1.35.
33. The mean value for quantitative characters in mutagen treated Sree Jaya was higher than its control.
34. The plant 32, followed by plant 31 and plant 42 were selected as the most preferred ones based on sensory attributes.
35. The best media for friable embryogenic callus induction was MS3 basal media supplemented with a combination of 8 mg L^{-1} 2,4-D + 0.5 mg L^{-1} NAA + 1 mg L^{-1} BA.
36. Highest necrosis was observed on irradiation with 60 Gy.
37. Friable calli was produced on irradiation with 30 Gy, 40 Gy, 50 Gy and 60 Gy
38. Necrosis was high on treatment with 0.6, 0.7, 0.8 and 0.9 per cent EMS. Friable calli was produced on treatment with 0.1, 0.2, 0.3 and 0.4 per cent EMS.

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Appendices

Appendix I

Descriptor of Sree Jaya and CC1

Trait	Genotype	
	Sree Jaya	**CC1**
Stipule colour	Light green	Light green
Petiole colour	Red	Purple
Emerging Leaf colour	Purplish green	Purplish green
Shape of central leaf lobe	Lanceolate	Ovoid
Stem Exterior colour	Light brown	Grey
Flowering	Absent	Present
Root external colour	Dark Brown	Dark Brown
Root pulp colour	White	White
Root cortex colour	Pink	Pink
Plant height (cm)	282.92	231.25
Branch height (cm)	107.5	151.88
Stem girth (mm)	26.56	25.71
Inter nodal length (cm)	2.82	1.68
Number of leaf scar in 30cm	11.00	17.88
Number of branches	2.25	3.25
Tuber no. plant^{-1}	4.42	5.38
Tuber length (cm)	43.36	47.31
Tuber fresh weight (kg plant^{-1})	3.49	3.31
Tuber girth (mm)	43.71	52.58
Tuber yield (t ha^{-1})	34.92	33.13
CMD Score (1-5)	2.21	1.67
CLS Score (0-5)	0.78	0.65
Dry matter of tubers (kg plant^{-1})	1.25	1.12
Plant upper biomass (kg)	3.39	3.14
Harvest Index (HI)	0.52	0.51

Appendix II

Score card for sensory evaluation of cassava tubers

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

Assessor:

Please score accordingly

	Item\Genotype	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
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	Bitterness										
10	Other:										

Date Signature

Appendix III

Observations on *in vitro* mutagen treated plantlets at the time of planting out for hardening

Plant no	Genotype	Explant source	Media	Treatment	Plantlets no.	Height (cm)	Length of roots (cm)	Stem girth (cm)	No. of leaves	Plantlet survived
1	Sree Jaya	SE	MS + 8 mg L^{-1} Picloram	1.2 % EMS	5	12.1	5.4	0.12	3	2
2	Sree Jaya	SE	MS + 8 mg L^{-1} Picloram	40 Gy	1	10.8	5.1	0.22	2	0
3	Sree Jaya	SE	MS + 8 mg L^{-1} Picloram	0.9 % EMS	1	5.9	1.7	0.13	3	0
4	Sree Jaya	SE	MS + 8 mg L^{-1} Picloram	0.9 % EMS	2	5.3	2.9	0.14	3	0
5	Sree Jaya	SE	MS + 8 mg L^{-1} Picloram	0.3 % EMS	2	8.7	8.2	0.10	2	0
6	Sree Jaya	SE	MS + 8 mg L^{-1} Picloram	0.9 % EMS	1	3.2	5.1	0.21	4	0
7	Sree Jaya	SE	MS + 8 mg L^{-1} Picloram	1.2 % EMS	4	9.3	5.7	0.20	4	1
8	Sree Jaya	SE	MS + 8 mg L^{-1} Picloram	0.3 % EMS	6	15.2	8.1	0.11	2	4
9	Sree Jaya	SE	MS + 8 mg L^{-1} Picloram	1.2 % EMS	4	10.2	6.7	0.18	3	4
10	Sree Jaya	SE	MS + 8 mg L^{-1} Picloram	0.3 % EMS	3	16.1	8.3	0.13	4	3
11	Sree Jaya	SE	MS + 8 mg L^{-1} Picloram	0.6 % EMS	3	18.3	6.9	0.17	5	3
12	Sree Jaya	SE	MS + 8 mg L^{-1} Picloram	0.3 % EMS	4	16.0	7.5	0.16	3	1
13	Sree Jaya	SE	MS + 8 mg L^{-1} Picloram	1.2 % EMS	4	10.4	6.7	0.12	3	2
14	Sree Jaya	SE	MS + 8 mg L^{-1} Picloram	1.2 % EMS	4	12.8	6.5	0.15	2	2

Observations on *in vitro* mutagen treated plantlets at the time of planting out for hardening

Plant no	Genotype	Explant source	Media	Treatment	Plantlets no.	Height (cm)	Length of roots (cm)	Stem girth (cm)	No. of leaves	Plantlet survived
1	CC1	SE	MS + 8 mg L^{-1} Picloram	0.3 % EMS	4	8.3	8.9	0.14	3	0
2	CC1	SE	MS + 8 mg L^{-1} Picloram	0.9 % EMS	2	7.2	5.6	0.10	6	2
3	CC1	SE	MS + 8 mg L^{-1} Picloram	30 Gy	1	11.3	5.3	0.14	3	0
4	CC1	SE	MS + 8 mg L^{-1} Picloram	0.6 % EMS	5	9.6	5.1	0.12	3	2
5	CC1	SE	MS + 8 mg L^{-1} Picloram	0.6 % EMS	1	11.8	4.2	0.10	2	1
6	CC1	SE	MS + 8 mg L^{-1} Picloram	0.9 % EMS	4	9.9	6.5	0.18	4	0
7	CC1	SE	MS + 8 mg L^{-1} Picloram	0.9 % EMS	2	7.4	2.8	0.20	4	2
8	CC1	SE	MS + 8 mg L^{-1} Picloram	0.9 % EMS	4	4.5	6.1	0.19	2	1
9	CC1	SE	MS + 8 mg L^{-1} Picloram	0.9 % EMS	5	7.8	4.6	0.15	3	0
10	CC1	SE	MS + 8 mg L^{-1} Picloram	0.9 % EMS	4	3.2	5.1	0.14	5	3
11	CC1	SE	MS + 8 mg L^{-1} Picloram	1.2 % EMS	1	16.9	5.4	0.17	3	0
12	CC1	SE	MS + 8 mg L^{-1} Picloram	0.6 % EMS	2	3.2	5.1	0.12	4	0
13	CC1	SE	MS + 8 mg L^{-1} Picloram	0.9 % EMS	5	4.5	3.2	0.14	2	1

Observations of *in vitro* mutated plantlets two months after planting out

Genotype	Treatment	Height (cm)	Leaf no.	Girth of stem (cm)
Sree Jaya	0.3 % EMS	12.3	3	0.9
Sree Jaya	0.3 % EMS	15.5	5	0.8
Sree Jaya	0.3 % EMS	16.4	3	0.6
Sree Jaya	0.3 % EMS	45.1	3	0.9
Sree Jaya	0.3 % EMS	53.1	2	1.0
Sree Jaya	0.3 % EMS	54.4	5	0.9
Sree Jaya	0.3 % EMS	15.7	5	1.1
Sree Jaya	0.3 % EMS	24.7	10	0.7
Sree Jaya	0.3 % EMS	56.5	11	0.8
Sree Jaya	0.6 % EMS	44.4	5	0.6
Sree Jaya	0.6 % EMS	21.2	6	1.2
Sree Jaya	0.6 % EMS	31.3	4	1.2
Sree Jaya	1.2 % EMS	23.5	9	0.9
Sree Jaya	1.2 % EMS	35.1	8	0.9
Sree Jaya	1.2 % EMS	12.2	6	0.6
Sree Jaya	1.2 % EMS	26.1	7	0.7
Sree Jaya	1.2 % EMS	32.6	3	0.9
Sree Jaya	1.2 % EMS	19.7	2	0.8
Sree Jaya	1.2 % EMS	38.6	5	1.0
Sree Jaya	1.2 % EMS	49.8	8	0.8
Sree Jaya	1.2 % EMS	27.9	3	0.7
Sree Jaya	1.2 % EMS	35.5	9	0.8
Sree Jaya	1.2 % EMS	25.5	8	0.7
Sree Jaya	1.2 % EMS	18.4	7	1.1
CC1	0.6 % EMS	34.2	3	0.9
CC1	0.6 % EMS	39.5	5	0.7
CC1	0.6 % EMS	38.7	4	0.9
CC1	0.6 % EMS	54.4	5	0.7
CC1	0.9 % EMS	49.9	8	0.6
CC1	0.9 % EMS	47.8	9	0.5
CC1	0.9 % EMS	42.1	5	0.6
CC1	0.9 % EMS	16.3	2	0.9
CC1	0.9 % EMS	55.5	2	0.7
CC1	0.9 % EMS	54.4	3	0.8
CC1	0.9 % EMS	34.6	3	0.6
CC1	0.9 % EMS	52.6	8	1.0
CC1	0.9 % EMS	47.0	9	0.9
CC1	0.9 % EMS	49.0	7	0.8
CC1	0.9 % EMS	28.3	7	0.6

Appendix IV

Observations on qualitative traits of *in vitro* mutagen treated plantlets of cassava at 2 and 6 MAP

Plant no.	Genotype	Colour of apical leaf	Pubescence of apical leaf	Leaf Retention	Shape of central leaflet	Colour of Petiole	Colour of leaf	No. of leaf lobes	Lobe margins	Colour of leaf vein	Orientation of petiole
		Two month after planting		Six months after planting							
1	SJ	3	0	5	5	7	5	5	3	7	3
2	SJ	3	0	5	5	7	5	7	3	7	5
3	SJ	3	0	5	5	7	5	7	3	7	5
4	SJ	7	0	4	5	7	5	7	3	7	5
5	SJ	7	0	5	5	7	5	7	3	7	5
6	SJ	7	0	5	5	7	5	7	3	7	5
7	SJ	7	0	5	5	7	5	7	3	7	5
8	SJ	7	0	5	5	7	5	7	3	7	5
9	SJ	7	0	5	5	7	5	5	3	7	5
10	SJ	7	0	4	5	7	5	7	3	7	5
11	SJ	7	0	4	5	7	5	7	3	7	5
12	SJ	3	0	5	5	7	5	7	3	7	5
13	SJ	7	0	5	5	7	5	7	3	7	3
14	SJ	3	0	5	5	7	5	7	3	7	5
15	SJ	3	0	5	5	7	5	7	3	7	3
16	CC1	7	0	3	1	7	5	7	3	7	5
17	CC1	7	0	3	1	7	5	7	3	7	5
18	CC1	7	0	5	5	7	5	7	3	7	5
19	CC1	7	0	5	5	7	5	7	3	7	5
20	SJ	7	0	4	5	7	5	7	3	7	5
21	SJ	7	0	4	5	7	5	7	3	7	5
22	SJ	7	0	4	5	7	5	7	3	7	5
23	SJ	7	0	4	5	7	5	7	3	7	5

Observations on qualitative traits of *in vitro* mutagen treated plantlets of cassava at 2 and 6 MAP

Plant no.	Genotype	Colour of apical leaf	Pubescence of apical leaf	Leaf Retention	Shape of central leaflet	Colour of Petiole	Colour of leaf	No. of leaf lobes	Lobe margins	Colour of leaf vein	Orientation of petiole
24	SJ	7	0	5	5	7	5	7	3	7	5
25	SJ	7	0	5	5	7	5	7	3	7	3
26	SJ	7	0	5	5	7	5	7	3	7	5
27	SJ	7	0	5	5	7	5	7	3	7	5
28	SJ	7	0	4	5	7	5	7	3	7	5
29	SJ	7	0	5	5	7	5	7	3	7	5
30	SJ	7	0	4	5	7	5	7	3	7	5
31	SJ	3	0	5	5	7	5	7	3	7	5
32	SJ	3	0	5	5	7	5	7	3	7	5
33	SJ	3	0	5	5	7	5	7	3	7	5
34	CC1	5	0	5	5	7	3	7	3	7	5
35	CC1	3	0	5	5	7	3	7	3	7	5
36	CC1	7	0	4	5	7	3	7	3	7	5
37	CC1	3	0	5	5	7	3	5	3	7	5
38	CC1	7	0	4	5	7	3	7	3	7	5
39	CC1	7	0	4	5	7	3	7	3	7	5
40	CC1	7	0	4	5	7	3	7	3	7	5
41	CC1	3	0	5	1	3	3	7	3	7	5
42	CC1	3	0	5	1	7	3	7	3	7	5
43	CC1	3	0	5	1	7	3	7	3	7	5
44	CC1	9	0	5	1	7	3	7	3	7	5
	SJ*	7	0	4	5	7	5	7	3	7	5
	CC1*	3	0	2	1	9	3	7	3	7	5

* Mode score of control plants

Observations on qualitative traits of *in vitro* mutagen treated plantlets of cassava at 9 MAP

Plant no.	Genotype	Prominence of foliar scar	Colour of stem cortex	Colour of epidermis	Colour of stem exterior	Growth habit of stem	Length of stipule	Stipule margin	CMV	CLS
1	SJ	5	2	2	8	1	5	2	4	2
2	SJ	5	2	2	8	1	5	2	4	2
3	SJ	5	2	2	8	1	5	2	4	2
4	SJ	5	2	2	8	1	5	2	4	2
5	SJ	5	2	2	8	1	5	2	4	2
6	SJ	5	2	2	8	1	5	2	4	2
7	SJ	5	2	2	8	1	5	2	3	2
8	SJ	5	2	2	8	1	5	2	2	1
9	SJ	5	2	2	8	1	5	2	4	2
10	SJ	5	2	2	8	1	5	2	4	2
11	SJ	5	2	2	8	1	5	2	4	2
12	SJ	5	2	2	8	1	5	2	4	2
13	SJ	5	2	2	8	1	5	2	5	2
14	SJ	5	2	2	8	1	5	2	4	2
15	SJ	5	2	2	8	1	5	2	4	2
16	CC1	5	3	2	7	1	5	2	4	2
17	CC1	5	3	2	7	1	5	2	3	2
18	CC1	5	3	2	7	1	5	2	4	2
19	CC1	5	3	2	4	1	5	2	3	2
20	SJ	5	2	2	8	1	5	2	3	2
21	SJ	5	2	2	8	1	5	2	4	2
22	SJ	5	2	2	8	1	5	2	1	2
23	SJ	5	2	2	8	1	5	2	2	2
24	SJ	5	2	2	8	1	5	2	1	2
25	SJ	5	2	2	8	1	5	2	1	2

Observations on qualitative traits of *in vitro* mutagen treated plantlets of cassava at 9 MAP

Plant no.	Genotype	Prominence of foliar scar	Colour of stem cortex	Colour of epidermis	Colour of stem exterior	Growth habit of stem	Length of stipule	Stipule margin	CMV	CLS
26	SJ	5	2	2	8	1	5	2	0	2
27	SJ	5	2	2	8	1	5	2	0	2
28	SJ	5	2	2	8	1	5	2	4	2
29	SJ	5	2	2	8	1	5	2	1	2
30	SJ	5	2	2	8	1	5	2	1	2
31	SJ	5	2	2	8	1	5	2	4	2
32	SJ	5	2	2	8	1	5	2	5	2
33	SJ	5	2	2	8	1	5	2	4	2
34	CC1	5	3	2	7	1	5	2	3	2
35	CC1	5	3	2	7	1	5	2	4	2
36	CC1	5	3	2	7	1	5	2	4	2
37	CC1	5	3	2	7	1	5	2	4	2
38	CC1	5	3	2	7	1	5	2	3	2
39	CC1	5	3	2	7	1	5	2	4	2
40	CC1	5	3	2	7	1	5	2	4	2
41	CC1	5	3	2	7	1	5	2	4	2
42	CC1	5	3	2	5	1	5	2	4	2
43	CC1	5	3	2	7	1	5	2	4	2
44	CC1	5	3	2	7	1	5	2	3	2
	SJ*	5	2	2	8	1	5	2	4	2
	CC1*	5	3	2	7	1	5	2	3	2

*Mode score of control plants

Observations on qualitative traits of *in vitro* mutagen treated plantlets of cassava at 9 MAP

Plant no.	Genotype	Extend of root peduncle	Root constriction	Root shape	External colour of root	Colour of root pulp	Colour of root cortex	Texture of root epidermis
3	SJ	3	2	1	4	1	3	5
4	SJ	0	3	1	4	1	3	5
5	SJ	3	0	1	4	1	3	5
6	SJ	3	1	1	4	1	3	5
7	SJ	0	1	1	3	1	3	5
10	SJ	3	2	1	4	1	4	5
11	SJ	3	2	1	4	1	3	5
12	SJ	3	2	3	4	1	3	5
13	SJ	3	1	1	4	1	3	5
14	SJ	5	2	1	4	1	3	5
15	SJ	3	1	3	4	1	3	5
21	SJ	3	1	3	2	1	4	5
23	SJ	0	1	1	4	1	3	5
24	SJ	3	1	1	4	1	3	5
28	SJ	3	2	1	4	1	3	5
29	SJ	3	1	1	4	1	3	5
30	SJ	3	1	3	4	1	3	5
31	SJ	3	2	1	4	1	3	5
32	SJ	3	2	1	4	1	3	5
33	SJ	3	2	1	4	1	3	5
34	CC1	3	2	1	4	1	3	5
35	CC1	0	2	1	4	1	3	5
36	CC1	5	1	3	4	1	3	5
37	CC1	3	1	1	4	1	3	5
41	CC1	0	1	1	4	2	3	5
42	CC1	0	1	1	3	1	3	5
43	CC1	0	1	1	3	1	3	5
44	CC1	0	2	4	3	1	3	5
	SJ*	3	2	1	4	1	3	5
	CC1*	0	1	1	4	1	3	5

* Mode score of control plants

Appendix V

Observations on quantitative traits of *in vitro* mutagen treated plants of cassava at 6 and 9 MAP

Plant no.	Genotype	Length of leaf lobe	Width of leaf lobe	Ratio of leaf lobe	Petiole length (cm)	Distance between leaf scar (cm)	Stem girth (cm)	Height of plant (cm)	No. of storage roots per plant	No. of commercial roots per plant	Thickness of tuber (cm)	Tuber length (cm)	Average cortex thickness (mm)	Fresh tuber weight (kg)	Above ground weight (kg)	Harvest index (HI)	Dry matter (DM) (%)	Starch (%)
		6 MAP				9 MAP												
1	SJ	19.15	4.65	4.12	26.25	2.60	1.30	52.50	0	0	-	-	-	-	0.47	-	-	-
2	SJ	18.05	4.25	4.25	27.20	3.25	1.20	72.50	0	0	-	-	-	-	0.51	-	-	-
3	SJ	18.25	4.20	4.35	27.55	4.65	1.56	92.05	1	1	4.12	27.10	2.1	0.40	1.12	0.36	46.38	27.00
4	SJ	18.15	3.75	4.84	29.90	3.90	1.33	245.00	4	1	4.30	20.30	1.3	0.26	0.64	0.41	28.96	14.67
5	SJ	17.90	4.00	4.48	28.55	4.15	1.42	236.00	2	0	4.26	15.40	1.0	0.33	0.49	0.67	21.05	9.06
6	SJ	18.40	4.60	4.00	29.75	4.10	1.83	234.50	3	1	4.92	23.40	1.1	0.49	0.96	0.51	27.38	13.55
7	SJ	18.10	3.65	4.96	31.80	3.30	2.17	324.00	5	0	6.30	13.20	1.4	0.82	1.17	0.70	28.96	14.67
8	SJ	19.35	4.55	4.25	20.6	2.75	1.51	105.00	0	0	-	-	-	-	0.36	-	-	-
9	SJ	16.35	3.90	4.19	23.50	1.95	1.42	121.00	2	0	-	-	-	-	0.55	-	-	-
10	SJ	16.65	4.10	4.06	24.55	1.85	1.22	174.00	3	0	3.90	10.20	2.1	0.09	0.34	0.26	36.88	20.27
11	SJ	15.45	4.25	3.64	24.00	3.50	1.31	183.00	3	2	3.90	25.40	1.1	0.18	0.33	0.55	36.88	20.27
12	SJ	20.50	4.90	4.18	30.60	2.45	1.62	200.00	2	2	3.90	19.20	1.1	0.40	0.73	0.55	24.22	11.31
13	SJ	19.35	4.15	4.66	33.00	7.60	1.61	136.50	2	2	4.43	25.40	1.2	0.44	1.11	0.40	24.22	11.31
14	SJ	23.05	5.35	4.31	36.60	5.10	1.76	500.00	4	1	6.21	28.10	1.6	0.60	1.37	0.44	27.38	13.55
15	SJ	23.15	5.60	4.13	34.20	4.80	1.72	556.00	1	1	5.71	24.30	1.8	0.57	1.72	0.33	25.80	12.43
16	CC1	17.40	4.30	4.05	23.55	5.20	1.24	174.00	0	0	-	-	-	-	0.32	-	-	-
17	CC1	16.30	3.70	4.41	23.35	3.75	1.61	185.00	0	0	-	-	-	-	0.33	-	-	-
18	CC1	17.15	4.05	4.23	23.20	4.95	1.33	128.50	0	0	-	-	-	-	0.41	-	-	-
19	CC1	15.55	3.70	4.20	21.55	1.60	1.28	110.00	0	0	-	-	-	-	0.39	-	-	-

Observations on quantitative traits of *in vitro* mutagen treated plants of cassava at 6 and 9 MAP

Plant no.	Genotype	Length of leaf lobe	Width of leaf lobe	Ratio of leaf lobe	Petiole length (cm)	Distance between leaf scar (cm)	Stem girth (cm)	Height of plant (cm)	No. of storage roots per plant	No. of commercial roots per plant	Thickness of tuber (cm)	Tuber length (cm)	Average cortex thickness (mm)	Fresh tuber weight (kg)	Above ground weight (kg)	Harvest index (HI)	Dry matter (DM) (%)	Starch (%)
		6 MAP			9 MAP													
20	SJ	16.45	3.75	4.39	31.05	2.55	1.13	310.00	3	0	-	-	-	-	0.45	-	-	-
21	SJ	17.55	3.90	4.50	28.20	5.70	1.12	220.00	4	2	5.41	13.40	2.2	0.13	0.62	0.21	28.96	14.67
22	SJ	17.65	3.95	4.47	23.40	5.45	1.36	221.00	1	0	-	-	-	-	0.53	-	-	-
23	SJ	15.70	4.50	3.49	23.45	4.25	1.32	188.33	3	1	4.31	14.30	1.2	0.22	0.79	0.28	32.13	16.91
24	SJ	16.90	4.90	3.45	23.45	2.75	1.25	167.00	1	0					0.54			
25	SJ	17.70	4.40	4.02	25.35	3.50	1.21	207.00	1	0	3.24	12.30	1.8	0.11	0.35	0.31	32.13	16.91
26	SJ	17.60	4.65	3.78	24.85	3.35	1.64	194.00	0	0	-	-	-	-	0.39	-	-	-
27	SJ	16.50	4.60	3.59	22.35	1.30	2.00	140.00	0	3	-	-	-	-	0.48	-	-	-
28	SJ	16.95	3.90	4.35	27.75	3.45	1.82	255.00	4	1	3.80	21.30	1.1	0.45	0.68	0.66	27.38	13.55
29	SJ	17.70	3.90	4.54	25.40	3.35	1.64	262.00	1	0	3.70	15.20	1.5	0.4	0.66	0.61	24.22	11.31
30	SJ	18.70	4.30	4.35	23.40	3.65	1.02	210.00	1	0	4.20	25.40	1.7	0.17	0.35	0.49	25.80	12.43
31	SJ	23.35	4.40	5.31	33.90	3.85	2.1	260.00	4	0	4.60	16.40	1.2	1.19	1.12	1.16	49.54	29.24
32	SJ	15.15	3.40	4.46	25.05	3.05	2.73	277.00	6	2	6.30	29.40	1.7	1.16	1.03	1.13	24.22	11.31
33	SJ	19.10	4.90	3.9	25.55	1.70	2.62	289.00	4	2	6.40	30.10	1.5	0.35	0.95	0.37	36.88	20.27
34	CC1	17.70	4.00	4.43	29.35	4.85	1.68	141.00	2	1	6.22	26.3	1.9	0.60	1.77	0.34	21.05	9.06
35	CC1	15.70	3.45	4.55	24.4	6.10	1.32	159.33	2	1	2.31	18.1	0.8	0.22	0.45	0.49	16.30	5.70
36	CC1	15.70	3.15	4.98	22.55	5.45	1.45	313.00	4	1	3.24	19.5	1.2	0.32	0.60	0.53	32.13	16.91
37	CC1	17.90	4.25	4.21	24.3	3.95	1.11	215.00	2	0	4.21	12.3	1.3	0.12	0.33	0.36	30.55	15.79
38	CC1	16.30	4.05	4.02	22.65	3.90	1.05	176.00	3	0	-	-	-	-	0.35	-	-	-
39	CC1	18.15	5.05	3.59	26.65	2.65	1.86	185.00	0	0	3.80	25.3	1.7	0.70	0.29	0.24	16.30	5.70

Observations on quantitative traits of *in vitro* mutagen treated plants of cassava at 6 and 9 MAP

Plant no.	Genotype	Length of leaf lobe	Width of leaf lobe	Ratio of leaf lobe	Petiole length (cm)	Distance between leaf scar (cm)	Stem girth (cm)	Height of plant (cm)	No. of storage roots per plant	No. of commercial roots per plant	Thickness of tuber (cm)	Tuber length (cm)	Average cortex thickness (mm)	Fresh tuber weight (kg)	Above ground weight (kg)	Harvest index (HI)	Dry matter (DM) (%)	Starch (%)
		6 MAP				**9 MAP**												
40	CC1	16.90	4.05	4.17	23.05	2.45	1.44	143.50	0	0	-	-	-	-	0.34	-	-	-
41	CC1	23.20	5.45	4.26	24.55	1.75	1.71	172.50	2	0	4.90	23.1	2.0	0.40	0.73	0.55	24.22	11.31
42	CC1	16.80	3.6	4.67	29.5	2.90	1.9	209.00	2	0	4.60	18.4	1.2	0.54	0.46	1.17	22.63	10.18
43	CC1	17.85	4.15	4.3	30.65	3.95	1.31	166.00	4	0	4.20	15.2	1.9	0.35	0.26	1.35	21.05	9.06
44	CC1	16.50	4.15	3.98	29.25	3.45	2.86	161.50	4	0	7.10	34.2	1.7	1.05	0.85	1.24	24.22	11.31
	SJ*	16.77	4.48	3.86	23.27	3.60	1.57	185.00	2	0	4.87	19.63	1.5	1.44	1.47	0.98	23.16	10.56
	CC1*	18.20	5.15	3.22	24	3.90	1.06	196.00	3	0	3.90	17.3	1.6	0.32	0.40	0.30	30.55	15.79

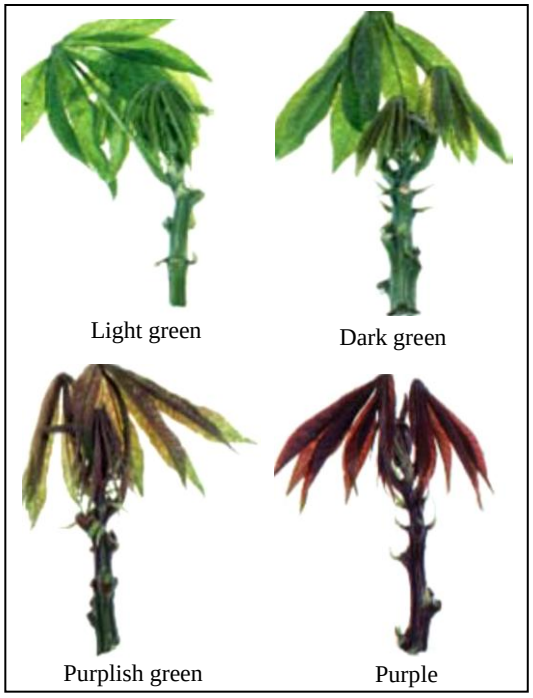
* Mean of control plants

Appendix VI
Cassava descriptor by Fukuda *et al.* (2010)

Descriptors to be scored at three months after planting

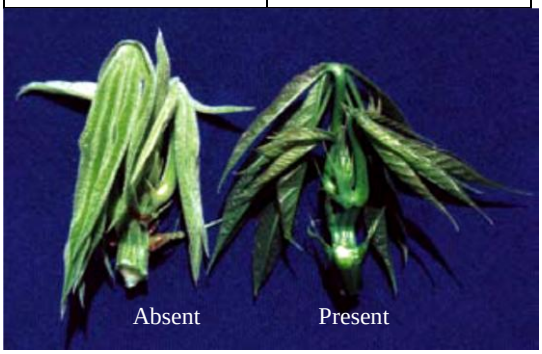
1. Colour of apical leaves

Score	Colour
3	Light green
5	Dark green
7	Purplish green
9	Purple



2. Pubescence on apical leaves

Score	Pubescence
0	Absent
1	Present



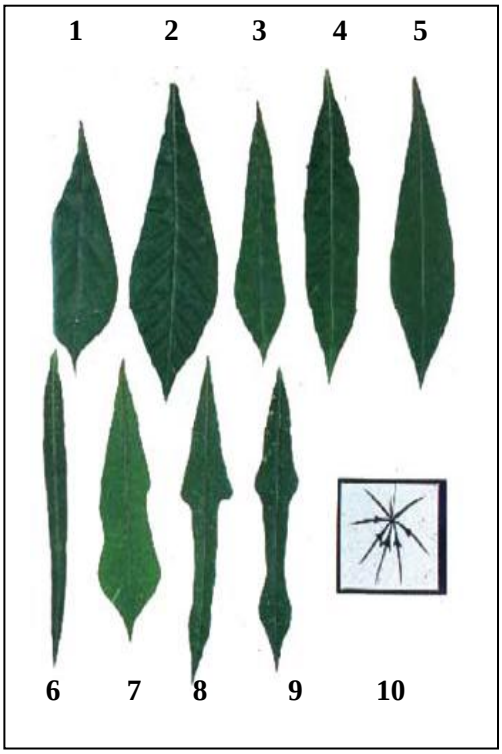
Descriptors to be scored at six months after planting

3. Leaf retention

Score	Retention
1	Very poor retention
2	Less than average retention
3	Average leaf retention
4	Better than average retention
5	Outstanding leaf retention

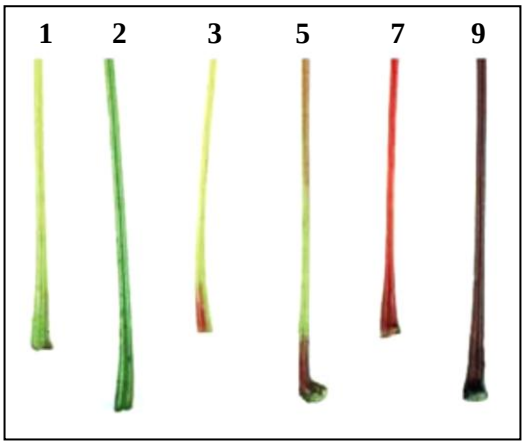
4. Shape of central leaflet

Score	Shape
1	Ovoid
2	Elliptic-lanceolate
3	Obovate-lanceolate
4	Oblong-lanceolate
5	Lanceolate
6	Straight or linear
7	Pandurate
8	Linear-piramidal
9	Linear-pandurate
10	Linear-hostatilobalate



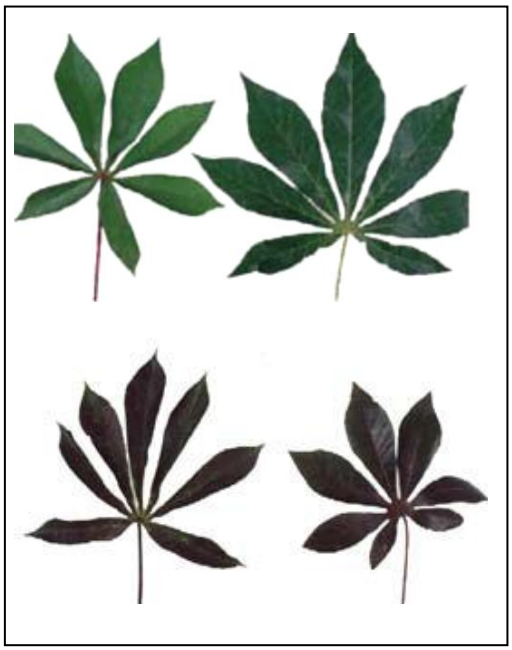
5. Petiole colour

Score	Colour
1	Yellowish-green
2	Green
3	Reddish-green
5	Greenish-red
7	Red
9	Purple



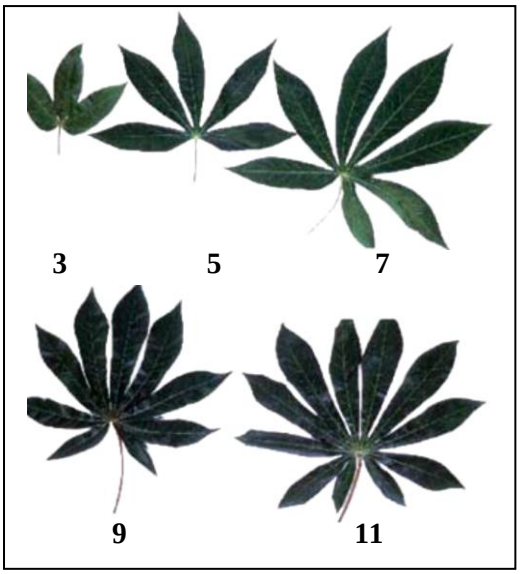
6. Leaf colour

Score	Retention
3	Light green
5	Dark green
7	Purple green
9	Purple



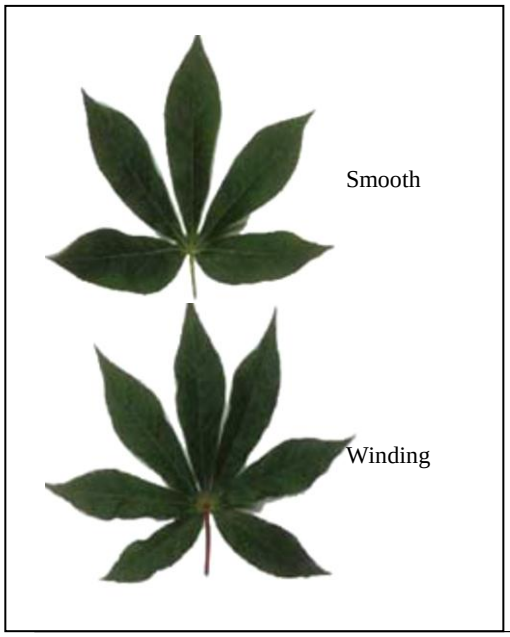
7. Number of leaf lobes

Score	Retention
3	Three lobes
5	Five lobes
7	Seven lobes
9	Nine lobes
11	Eleven lobes



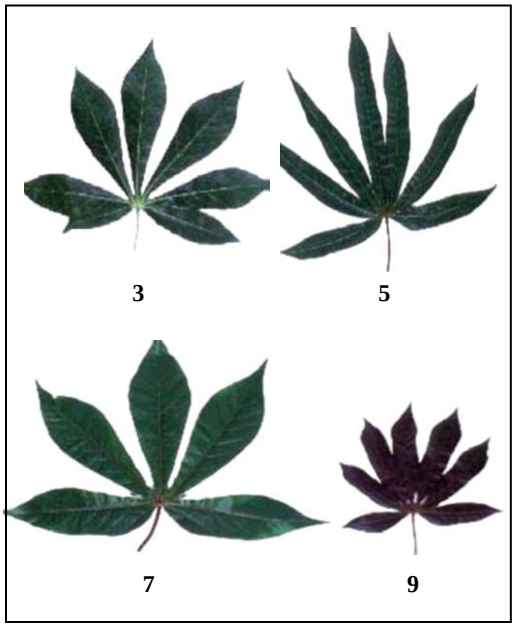
8. Lobe margins

Score	Retention
3	Smooth
7	Winding



9. Colour of leaf vein

Score	Colour
3	Green
5	Reddish-green in less than half of the lobe
7	Reddish-green in more than half of the lobe
9	All red



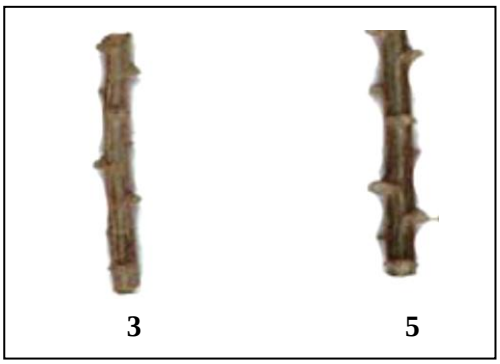
10. Orientation of petiole

Score	Orientation
1	Inclined upwards
3	Horizontal
5	Inclined downwards
7	Irregular

Descriptors to be scored at nine months after planting

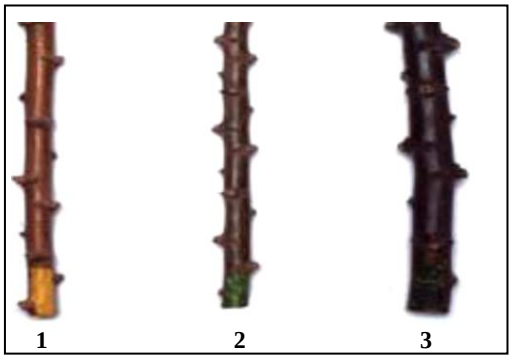
11. Prominence of foliar scars

Score	Prominence
3	Semi-prominent
5	Prominent



12. Colour of stem cortex

Score	Colour
1	Orange
2	Light green
3	Dark green



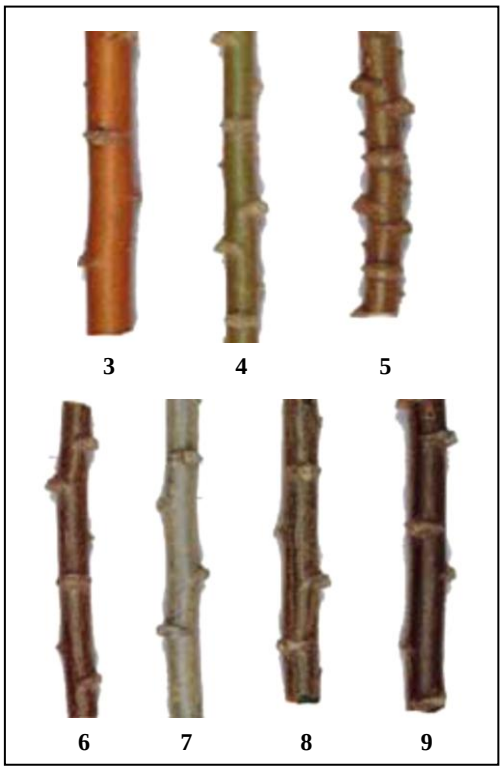
13. Color of stem epidermis

Score	Retention
1	Cream
2	Light brown
3	Dark brown
4	Orange

1 2 3 4

14. Color of stem exterior

Score	Retention
3	Orange
4	Greeny-yellowish
5	Golden
6	Light brown
7	Silver
8	Gray
9	Dark brown



3 4 5

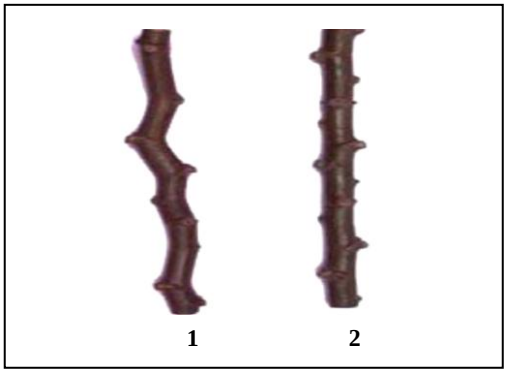
6 7 8 9

15. Distance between leaf scars
For conversion to qualitative data:

Score	Class	Measurement
3	Short	≤ (8 cm)
5	Medium	(8–15 cm)
7	Long	≥ (15 cm)

16. Growth habit of stem

Score	Retention
1	Straight
2	Zig-zag



1 2

17. Length of stipules

Score	Colour
3	Short
5	Long

3 5

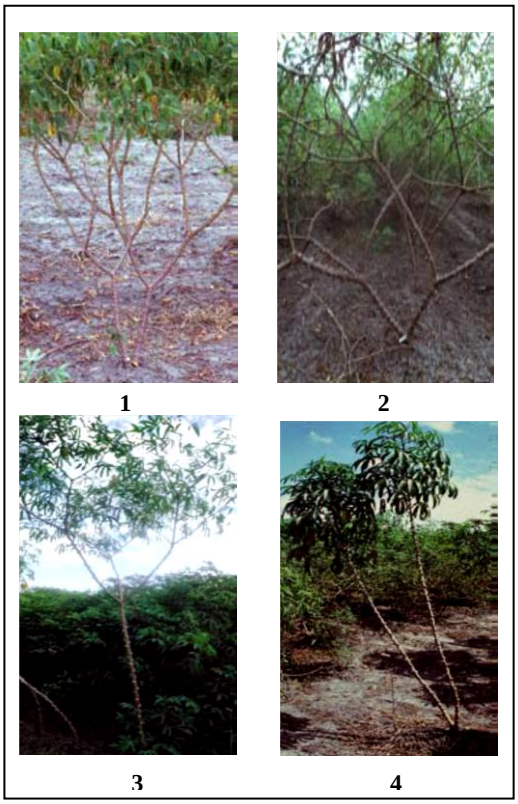
18. Stipule margin

Score	Colour
1	Entire
2	Split or forked

1 2

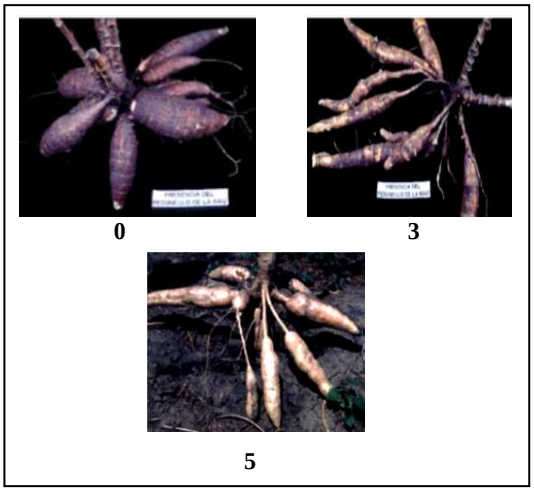
19. Shape of plant

Score	Shape
1	Compact
2	Open
3	Umbrella
4	Cylindrical



20. Extent of root peduncle

Score	Root peduncle
0	Sessile
3	Pedunculate
5	Mixed



21. Root constrictions

Score	Constrictions
1	Few to none
2	Some
3	Many

22. Root shape

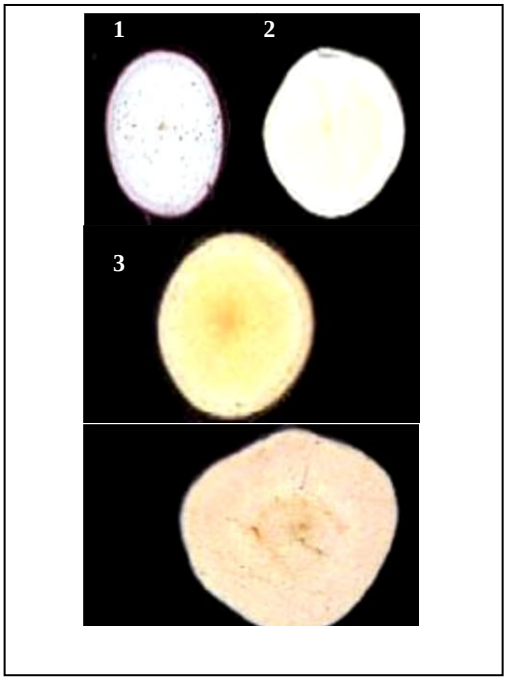
Score	Shape
1	Conical
2	Conical-cylindrical
3	Cylindrical
4	Irregular

23. External colorof storage root

Score	Colour
1	White or cream
2	Yellow
3	Light brown
4	Dark brown

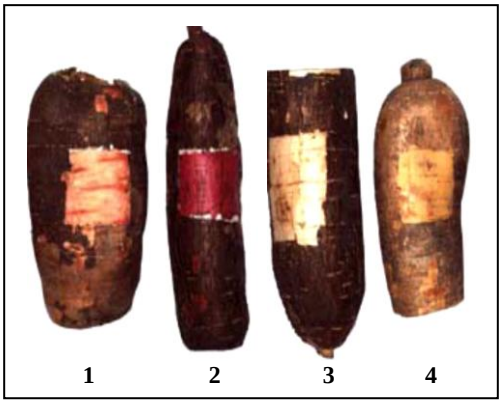
24. Color of root pulp (parenchyma)

Score	Colour
1	White
2	Cream
3	Yellow
4	Orange (no photo)
5	Pink



25. Color of root cortex

Score	Colour
1	White or cream
2	Yellow
3	Pink
4	Purple



26. Texture of root epidermis

Score	Texture
3	Smooth
5	Intermediate
7	Rough

27. Cortex thickness

Score	Thickness
1	Thin
2	Intermediate
3	Thick

IN VITRO MUTAGENESIS AND EVALUATION OF SOMATIC EMBRYO DERIVED PLANTLETS IN CASSAVA (*Manihot esculenta* Crantz.)

By
RIYA ANTONY
(2014-11-117)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University

Department of Plant Breeding and Genetics
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA
2016

Abstract

The present study '*In vitro* mutagenesis and evaluation of somatic embryo derived plantlets in cassava (*Manihot esculenta* Crantz.)' was conducted in the Department of Plant Breeding and Genetics, College of Horticulture during 2014-15. They study attempted to assess the variability existing among the *in vitro* derived plantlets of cassava genotypes Sree Jaya and CC1 as well as to generate further variability through *in vitro* mutagenesis.

With the aim to induce variability, *in vitro* mutagenesis of callus derived from cassava genotypes Sree Jaya and CC1 was attempted earlier in the department. This had resulted in 10 somatic embryo derived plantlets in primary hardening stage and 58 plantlets yet to be transferred for hardening.

The above plantlets formed the basic material for field evaluation undertaken in the present study. Sree Jaya and CC1 genotypes were planted as control. Out of the 58 plantlets transferred from the *in vitro* cultures to hardening, only 34 plantlets survived *i.e.,* the survival was found to be 58.62 per cent. All the ten plants that were already in the primary hardening stage survived.

Observations were recorded during field evaluation of the *in vitro* mutagen treated plants at three, six and nine months after planting as per the descriptor of cassava (Fukuda *et al.,* 2015). The plants varied with respect to qualitative characters like colour of apical leaves, leaf retention, shape of leaf lobe, petiole colour, leaf orientation, colour of stem exterior, extend of root peduncle, shape of tuber, root colour, colour of root pulp and colour of root cortex. Variability was also observed for quantitative characters like length and width of leaf lobe, length to width ratio of leaf lobe, petiole length, distance between leaf scars, height of plant, tuber weight per plant, tuber girth, stem girth, extend of root peduncle, starch content and dry matter content. Among the mutagen treated plants of Sree Jaya none of *in vitro* mutagen treated plants were

found to be superior with respect to tuber yield while in CC1 genotype, six plants yielded better than the control.

Sensory evaluation of the tubers produced by *in vitro* mutagen treated plants as well as control plants was done by twelve panelists to assess consumer perception. The tubers from plant 32 (Sree Jaya; 1.2 % EMS), followed by plant 31 (Sree Jaya; 1.2 % EMS) and plant 42 (CC1; 0.9 % EMS) were most preferred for various sensory attributes evaluated.

In vitro mutation being a potential method to induce variability, mutation of callus derived from genotypes was as attempted to create more variants. The callus cultures of Sree Jaya and CC1 genotypes were established as per the protocol standardised by Magaia, (2015). Friable embryogenic calli production was higher in the media MS + 8 mg L^{-1} 2,4-D + 1 mg L^{-1} NAA + 0.5 mg L^{-1} BAP using leaf explants. Calli were subjected to physical (γ irradiation at 30- 60 Gy at an interval of 10Gy) and chemical (EMS 0.1 - 0.9 % at an interval of 0.1%) mutagens as advocated by Magaia, (2015). However, regeneration of mutagen treated friable calli was not obtained in both genotypes.

Quantum of variability expressed in the *in vitro* mutated plants of cassava with respect quantitative traits shows the efficiency of *in vitro* mutagenesis in creating variability in cassava. *In vitro* mutagenesis is a potential tool in the hands of plant breeder to create variability especially in vegetative propagated crops. The evaluation of *in vitro* mutagen treated plants in the field showed wide variation with respect to most morphological as well as biometrical traits. All the plants evaluated can hence, be advanced to next generation of evaluation (M1V1) with replication to identify the mutants. From the results obtained on induction of *in vitro* mutation to create more variants in cassava, it is concluded that the friable callus in both the genotypes need to be cultured in alternate regeneration medium for successful regeneration of somatic embryos.