Electronic Journal of Plant Breeding, 9 (2) : 433-449 (June 2018) DOI: 10.5958/0975-928X.2018.00054.6
ISSN 0975-928X

Research Article

In vitro mutagenesis creates distinct morphological variants in cassava (*Manihot esculenta* Crantz.): a characterisation study

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(Received: 15 Jun 2017; Revised: 17 May 2018; Accepted:31 May 2018)

Abstract
The present investigation was conducted to evaluate the variability created due to induction of *in vitro* mutagenesis in cassava. The calli and somatic embryos from the leaf explants of superior cassava genotypes Sree Jaya and CC1 established in Murashigue and Schoog (MS) media supplemented with 8 mg L^{-1} picloram were subjected to treatment with Ethyl Methyl Sulphonate (EMS), 0.6-1.2 per cent at 0.1 per cent interval. Forty-four plants survived out of sixty-eight regenerated plants kept for hardening under pad and fan greenhouse followed by rain shelter. Irrespective of the dosage field evaluation of these plants showed variations in morphological traits with respect to their respective controls at 3, 6 and 9 months after planting (MAP). These plants can be clonally propagated and the genetics of the observed variations in quantitative traits of these plants can be validated under different conditions.

Keywords
In vitro, hardening, callus, somatic embryo, mutation

Intoduction

Cassava (*Manihot esculanta* Crantz.) is a nutritionally important root crop grown in tropics with global production expanding by an average of 1.2 per cent annually (Ford, 2015). As cassava seeds are dormant and germinate very slowly, setts which are uniform with respect to different traits are used for propagation. However, this leads to the accumulation of viral and bacterial diseases Nassar and Ortiz (2007). Creation of variability for improvement of cassava through hybridisation is severely constrained by low fertility, low hybrid seed set and poor germination rate (Nassar, 2007). Hence, the breeder has to adopt alternate strategies like induction of mutation. *In vitro* techniques have been standardized in cassava by Magaia (2015) and he reported that the frequency of obtaining desired variability is more when mutation is done *in vitro*. Hence, an attempt was made to create variability in cassava through *in vitro* mutagenesis and characterise the treated plants.

Materials and Methods

The newly sprouted shoots of four to five centimetres were collected and properly labelled. Leaf bits with veins of about 1 × 1cm from unfolded to partially unfolded leaves of cassava genotypes, Sree Jaya and CC1 were inoculated in MS medium supplemented with 8 mg L^{-1} picloram. The Friable embryogenic callus and somatic embryos at torpido stage were then treated with chemical mutagen Ethyl Methyl Sulphonate (EMS) at 0.6, 0.7, 0.8, 0.9, 1.0, 1.1 and 1.2 per cent. The treated calli when attained friable stage was used for induction of SEs which was germinated in medium, MS + 8 mg L^{-1} picloram Magaia(2015).

When the *in vitro* derived plants attained at least 3 cm height with 3 to 5 roots, primary hardening was done, under pad and fan green house for three months. The Plants were grown in plastic pots of 15 cm height and 5 cm radius in Soilrite™ (commercial product consisting on perlite, Irish peat moss and vermiculite) at the proportion of 1:1. The temperature and humidity inside the chamber were maintained at 24.0- 27.0°C and 80-85 per cent, respectively. A water-soluble fertilizer concentrate Greencare TM ($N:P_2O_5:K_2O$ - 30:10:10), secondary and micronutrients like Boron, Calcium, Copper, Iron, Magnesium, Manganese, Molybdenum, Sulphur, Zinc) was applied as foliar spray at a concentration of 0.01 per cent. 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 on daily basis Magaia, (2015). Secondary hardening was done under rain shelter (temperature and relative humidity during hardening ranged from 27-32 °C and 60-90 per cent, respectively) for three months. The hardened plants were transplanted into large pots (60 cm × 40 cm) which were placed in rain shelter for one week, later transferred to the field

under shade for another one week before exposing to open condition (Fig. 1).

After one month's growth in the pots, the plants were field planted at 0.75 m × 0.75 m with untreated plants as checks in September 2015 following the standard cultivation practice (KAU, 2011). The crop was harvested 9 months after planting. Observations were recorded at 3, 6 and 9 MAP as per the descriptor Fukuda (2010).

Results and Discussion

The fundamental advantage of hardening in pad and fan greenhouse is the higher survival rates due to constant maintenance of temperature conditions and humidity Magaia *et al.*(2015). At two months after planting, more number of leaves was seen in Sree Jaya. At the time of planting taller plants were observed in Sree Jaya. However, plants with long roots were exhibited by CC1. After two months of planting, taller plants were observed in mutagen treated CC1 plants. 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 might have resulted in more absorption of nutrients leading to a faster growth. More number of mutagen-treated plants of Sree Jaya had more stem girth at two months after planting while most of the CC1 plants did not show a considerable increase in stem girth. In a study of *in vitro* mutation in cassava, it was reported that treatment of callus with the mutagens resulted in reduction in plantlet height, number of shoots, leaves and roots as well as length of the roots (Magaia, 2015).

Hardening as per the guidelines of International Institute of Tropical Agriculture (IITA) and Centro International Agricultural Tropics (CIAT) guarantees only 35-50 per cent survival of cassava in Mozambique and Zimbabwe Iglesias *et a.*(1996). In our study under pad and fan greenhouse condition, there was 64.7 per cent survival of *in vitro* mutagen treated plantlets and all the plants survived after secondary hardening.

Cassava descriptor (IITA) was used for both characterization and performance evaluation of field established *in vitro* mutagen treated plants. Since each mutant plant was capable of becoming a new clone, the data was recorded on an individual basis (Lebort, 2009). The results are presented in table 1 and 2.

There are reports on variation in apical leaf colour from light green to light purple and also from purple-green to light green Joseph *et al.(*2004); Magaia(2015). The trait is controlled by genes in two loci and occurs contiguously in linkage group 2and explains 93 per cent of the phenotypic variation in cassava Rabbi *et al.*, (2014).

In the present study the colour of apical leaf in control plants of Sree Jaya was purplish green (score 7) and that of CC1 was light green (score 3). Out of the mutagen treated plants, irrespective of dose, 9 Sree Jaya plants scored 3 (light green). Variants in addition to the colours scored in the descriptor were also observed. One plant each of mutagen-treated Sree Jaya showed greenish purple, dark green with purple margin and light green with purple margin (Fig.2). 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 (score 3) apical leaves (Plate 3). All CC1 plants treated with 0.6 per cent EMS produced purplish green (score 7) apical leaves. One each of 0.9 per cent EMS treated CC1 plants produced dark green (score 5) and purple (score 9) leaves (Fig. 3). The gradation of purple colour varied from deep purple, greenish purple to purplish green. The pigmentation of various tissues is the most conspicuous morphological trait distinguishing different varieties of cassava Rabbi *et al.(*2014). Hence, the variation observed is an indication of role of mutation in cassava genotypes Sree Jaya and CC1.

Green leaf retention is a trait that increase cassava yields. Cassava adapts to water shortage by reducing its leaf canopy to reduce water use Connor and Cock, (1981); El-Sharkawy and Cock (1987). Leaf shedding is an effective mechanism as a response to moisture stress. Visual scoring for leaf retention was done using a scale of 1-5 by taking average leaf retention as benchmark Fukuda *et al.(* 2010). A plant with average leaf retention is the one with leaves covering about half of the plant.

Control plants of Sree Jaya showed better than average leaf retention while CC1 had less than average leaf retention. Among mutagen treated plants nine plants 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. Even though the crop was under moisture stress, the plants showed variation in leaf retention and the plants with outstanding green leaf retention indicated the presence of more chlorophyll leading to more photosynthetic efficiency and might have a yield advantage over other types under moisture stress.

The shape of leaf lobe is an identifying feature of any genotype. Sree Jaya had lanceolate (score 5) leaf lobe and CC1 had ovoid (score 1) leaf lobe. Two mutagen treated CC1 plants showed variation in the shape of central leaflet *i.e.*, lanceolate (Fig. 4). A similar variation was reported in mutagen treated cassava plantlets at hardening Fukuda(2010).

In mutant lines of cassava, the colour of leaf petiole varied from light-green to light-purple and also from purple-green to light green Magaia,(2015). In the present study, control plants of Sree Jaya had a red petiole (score 7) and CC1 had a purple petiole (score 9). In CC1 all *in vitro* treated plants had red coloured petiole except for two plants, (score 7); one had deep purple (score 9) and the other had a reddish-green petiole (score 3) (Fig.5).

The anthocyanin pigmentation of the leaf petiole is associated with a single locus which explained 75 per cent of the phenotypic variation in cassava Rabbi *et al.* (2014). Hence, this trait also could be used to distinguish potential variants in cassava.

Two types of leaf orientation were observed in the plants evaluated. Majority of the plants had leaf inclined downwards (score 3). However, four plants of Sree Jaya treated with different doses of EMS had horizontal leaves (score 5) (Fig. 6). In cassava, it was observed that upper leaves move towards vertical orientation at night and change to moderate angles during daylight (Williams and Ghazali, 1969). Hence, the four plants observed to have horizontal leaf orientation in the present study need not be a typical character to distinguish between genotypes of cassava.

The study revealed that stem exterior colour is a character controlled by a single locus with dominant effect Rabbi *et al.* (2014). Hence, variations observed in this trait can be considered as true variations in the population. Control plants of Sree Jaya scored 8 (grey) and CC1 scored 7 (silver) for the colour of stem exterior. One treated plant of CC1 had golden (score 5) coloured stem exterior. Similarly, one plant of CC1 treated with EMS had greeny-yellowish (score 5) stem colour (Fig. 7).

Plants were scored for Cassava Mosaic Disease (CMD) and *Cercospora* Leaf Spot (CLS). All plants showed moderate (score 3) to severe (score 5) CMD symptoms. CLS was also observed as angular leaf spots. However, one plant of Sree Jaya (plant 8) treated with 0.3 per cent EMS, produced no symptom for CLS (score 1) and only mild chlorotic pattern (score 2) of the leaf towards the end of crop season for CMD. Hence, this plant needs to be evaluated further for confirming the disease reaction.

Plants of Sree Jaya had pedunculate root (score 3) while CC1 had sessile root (score 0). Among *in vitro* plants, one plant each of Sree Jaya and CC1 treated with EMS had sessile root. Also, one plant each of Sree Jaya and CC1 treated with different doses of EMS had mixed root (score 5) (Fig.8, Fig.9). Cassava plants having roots with well-developed peduncle have to be identified and selected as they are suitable for better storage and those genotypes having roots with short peduncle are difficult to separate from the main stem (Lebort, 2009).

Cassava cultivars having compact, cylindrical, or conical roots are suitable for better storage (Lebort, 2009). Plants with cylindrical or conical roots have to be selected because irregular roots of cassava are difficult to harvest and peel by hand which results in heavy loss of usable root materials Hahn *et al.(*1988). Control plants of Sree Jaya and CC1 plants had conical roots (score 1). Most of the mutagen treated plants of both Sree Jaya and CC1 genotypes had conical roots while four plants of treated Sree Jaya produced cylindrical roots (score 2). Irregular roots (score 3) were also observed in one mutagen treated plant of CC1 (Fig.10, Fig.11). This variation can be attributed to genetic factors as well as to the incidence of CMD, soil moisture stress *etc.*

Control plants had dark brown root external colour (score 4). One plant of Sree Jaya treated with 1.2 per cent EMS resulted in the yellow root (score 2). Also, one plant of Sree Jaya treated with 0.3 per cent EMS resulted in the light brown root (Score 3) (Fig. 12). Three colour variations have been observed were light brown, dark brown and white and the majority of the plants had dark brown root external colour.

One among 0.9 per cent EMS treated plants of CC1 showed cream (score 2) colour of root pulp in contrast to white coloured root pulp (Fig.13). Three classes of colour for root pulp *viz.*, cream, white and yellow with majority falling under white have been observed (Magaia, 2015). The colour intensity of the cassava root and the carotene concentration were positively correlated Iglesias *et al.,(*1996). Hence, the cream coloured ones may contain carotene and may be nutritionally superior. The colour of root pulp is controlled by both major and minor genes Paninah *et al.(*2014) and hence, both additive and non-additive gene actions operate. Similarly, a few genes control the production of carotenoids, mostly β-carotene in cassava storage roots Iglesias *et al.(* 1996).

Control plants and most of the mutagen treated plants had pink coloured root cortex (score 3). 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 (Fig.14). Hence, to get an accurate measure of colour, Royal Horticultural Society (RHS) Colour Chart published by the Royal Horticultural Society in 1966 was used to code the colour. Based on the colour chart, control plant had light yellowish pink (RHS66 29C) cortex. The mutagen treated plants showed gradation in pink colouration like pale yellow (RHS66 18C), light pink (RHS66 49C), moderate pink (RHS66 35D) and purplish pink (RHS66 55B). Four classes for root cortex colour were observed *viz.*, pink, yellow, cream and purple with half of them being pink (Magaia, 2015). Hence, cortex pigmentation is a conspicuous morphological trait distinguishing different varieties of cassava. In communities that prefer mealy varieties for boil-and-eat, tubers with pinkish inner skin are given a premium value Rabbi *et al.(*2014).

In vitro mutagenesis is a potential tool in the hands of plant breeders to create variability especially in vegetatively propagated crops. Evaluation of qualitative traits of *in vitro* mutagen treated cassava at various stages of growth revealed presence of variation with respect to pigmentation of various parts of the plant, shape of leaf and tuber, leaf orientation and presence of root peduncle. The pigmentation with respect to colour

of apical leaves, petiole colour and external stem colour can be used as morphological markers to distinguish variants in cassava. Irrespective of dose, quantum of variability expressed in the *in vitro* mutated plants of cassava implies *in vitro* mutagenesis as an efficient method for creating variability. The genetics of variations observed has to be further validated under different conditions. All the plants evaluated can be advanced to next generation of evaluation (M1V1), with replications, to identify stable mutations and the potential mutants.

Acknowledgement
Financial assistance by Kerala Agricultural University and the technical assistance by Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University are is gratefully acknowledged.

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Electronic Journal of Plant Breeding, 9 (2) : 433-449 (June 2018) DOI: 10.5958/0975-928X.2018.00054.6
ISSN 0975-928X

Table 1. Observations on qualitative traits of *in vitro* mutagen treated plantlets of cassava at 2 and 6 months after planting (MAP)

Plant no.	Genotype	Treatment	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 months after planting							**Six months after planting**					
1	SJ	0.3 % EMS	3	0	5	5	7	5	5	3	7	3
2	SJ	0.3 % EMS	3	0	5	5	7	5	7	3	7	5
3	SJ	0.3 % EMS	3	0	5	5	7	5	7	3	7	5
4	SJ	0.3 % EMS	7	0	4	5	7	5	7	3	7	5
5	SJ	0.3 % EMS	7	0	5	5	7	5	7	3	7	5
6	SJ	0.3 % EMS	7	0	5	5	7	5	7	3	7	5
7	SJ	0.3 % EMS	7	0	5	5	7	5	7	3	7	5
8	SJ	0.3 % EMS	7	0	5	5	7	5	7	3	7	5
9	SJ	0.3 % EMS	7	0	5	5	7	5	5	3	7	5
10	SJ	0.3 % EMS	7	0	4	5	7	5	7	3	7	5
11	SJ	0.3 % EMS	7	0	4	5	7	5	7	3	7	5
12	SJ	0.3 % EMS	3	0	5	5	7	5	7	3	7	5
13	SJ	0.6 % EMS	7	0	5	5	7	5	7	3	7	3
14	SJ	0.6 % EMS	3	0	5	5	7	5	7	3	7	5
15	SJ	0.6 % EMS	3	0	5	5	7	5	7	3	7	3
16	CC1	0.6 % EMS	7	0	3	1	7	5	7	3	7	5
17	CC1	0.6 % EMS	7	0	3	1	7	5	7	3	7	5
18	CC1	0.6 % EMS	7	0	5	5	7	5	7	3	7	5
19	CC1	0.6 % EMS	7	0	5	5	7	5	7	3	7	5
20	SJ	1.2 % EMS	7	0	4	5	7	5	7	3	7	5
21	SJ	1.2 % EMS	7	0	4	5	7	5	7	3	7	5
22	SJ	1.2 % EMS	7	0	4	5	7	5	7	3	7	5

23	SJ	1.2 % EMS	7	0	4	5	7	5	7	3	7	5
24	SJ	1.2 % EMS	7	0	5	5	7	5	7	3	7	5
25	SJ	1.2 % EMS	7	0	5	5	7	5	7	3	7	3
26	SJ	1.2 % EMS	7	0	5	5	7	5	7	3	7	5
27	SJ	1.2 % EMS	7	0	5	5	7	5	7	3	7	5
28	SJ	1.2 % EMS	7	0	4	5	7	5	7	3	7	5
29	SJ	1.2 % EMS	7	0	5	5	7	5	7	3	7	5
30	SJ	1.2 % EMS	7	0	4	5	7	5	7	3	7	5
31	SJ	1.2 % EMS	3	0	5	5	7	5	7	3	7	5
32	SJ	1.2 % EMS	3	0	5	5	7	5	7	3	7	5
33	SJ	1.2 % EMS	3	0	5	5	7	5	7	3	7	5
34	CC1	0.9 % EMS	5	0	5	5	7	3	7	3	7	5
35	CC1	0.9 % EMS	3	0	5	5	7	3	7	3	7	5
36	CC1	0.9 % EMS	7	0	4	5	7	3	7	3	7	5
37	CC1	0.9 % EMS	3	0	5	5	7	3	5	3	7	5
38	CC1	0.9 % EMS	7	0	4	5	7	3	7	3	7	5
39	CC1	0.9 % EMS	7	0	4	5	7	3	7	3	7	5
40	CC1	0.9 % EMS	7	0	4	5	7	3	7	3	7	5
41	CC1	0.9 % EMS	3	0	5	1	3	3	7	3	7	5
42	CC1	0.9 % EMS	3	0	5	1	7	3	7	3	7	5
43	CC1	0.9 % EMS	3	0	5	1	7	3	7	3	7	5
44	CC1	0.9 % EMS	9	0	5	1	7	3	7	3	7	5
	SJ*	Control	7	0	4	5	7	5	7	3	7	5
	CC1*	Control	3	0	2	1	9	3	7	3	7	5

Table 2. Observations on qualitative traits of *in vitro* mutagen treated plantlets of cassava at 9 MAP

Plant no.	Genotype	Treatment	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	0.3 % EMS	5	2	2	8	1	5	2	4	2
2	SJ	0.3 % EMS	5	2	2	8	1	5	2	4	2
3	SJ	0.3 % EMS	5	2	2	8	1	5	2	4	2
4	SJ	0.3 % EMS	5	2	2	8	1	5	2	4	2
5	SJ	0.3 % EMS	5	2	2	8	1	5	2	4	2
6	SJ	0.3 % EMS	5	2	2	8	1	5	2	4	2
7	SJ	0.3 % EMS	5	2	2	8	1	5	2	3	2
8	SJ	0.3 % EMS	5	2	2	8	1	5	2	2	1
9	SJ	0.3 % EMS	5	2	2	8	1	5	2	4	2
10	SJ	0.3 % EMS	5	2	2	8	1	5	2	4	2
11	SJ	0.3 % EMS	5	2	2	8	1	5	2	4	2
12	SJ	0.3 % EMS	5	2	2	8	1	5	2	4	2
13	SJ	0.6 % EMS	5	2	2	8	1	5	2	5	2
14	SJ	0.6 % EMS	5	2	2	8	1	5	2	4	2
15	SJ	0.6 % EMS	5	2	2	8	1	5	2	4	2
16	CC1	0.6 % EMS	5	3	2	7	1	5	2	4	2
17	CC1	0.6 % EMS	5	3	2	7	1	5	2	3	2
18	CC1	0.6 % EMS	5	3	2	7	1	5	2	4	2
19	CC1	0.6 % EMS	5	3	2	4	1	5	2	3	2
20	SJ	1.2 % EMS	5	2	2	8	1	5	2	3	2
21	SJ	1.2 % EMS	5	2	2	8	1	5	2	4	2
22	SJ	1.2 % EMS	5	2	2	8	1	5	2	1	2
23	SJ	1.2 % EMS	5	2	2	8	1	5	2	2	2

24	SJ	1.2 % EMS	5	2	2	8	1	5	2	1	2
25	SJ	1.2 % EMS	5	2	2	8	1	5	2	1	2
26	SJ	1.2 % EMS	5	2	2	8	1	5	2	0	2
27	SJ	1.2 % EMS	5	2	2	8	1	5	2	0	2
28	SJ	1.2 % EMS	5	2	2	8	1	5	2	4	2
29	SJ	1.2 % EMS	5	2	2	8	1	5	2	1	2
30	SJ	1.2 % EMS	5	2	2	8	1	5	2	1	2
31	SJ	1.2 % EMS	5	2	2	8	1	5	2	4	2
32	SJ	1.2 % EMS	5	2	2	8	1	5	2	5	2
33	SJ	1.2 % EMS	5	2	2	8	1	5	2	4	2
34	CC1	0.9 % EMS	5	3	2	7	1	5	2	3	2
35	CC1	0.9 % EMS	5	3	2	7	1	5	2	4	2
36	CC1	0.9 % EMS	5	3	2	7	1	5	2	4	2
37	CC1	0.9 % EMS	5	3	2	7	1	5	2	4	2
38	CC1	0.9 % EMS	5	3	2	7	1	5	2	3	2
39	CC1	0.9 % EMS	5	3	2	7	1	5	2	4	2
40	CC1	0.9 % EMS	5	3	2	7	1	5	2	4	2
41	CC1	0.9 % EMS	5	3	2	7	1	5	2	4	2
42	CC1	0.9 % EMS	5	3	2	5	1	5	2	4	2
43	CC1	0.9 % EMS	5	3	2	7	1	5	2	4	2
44	CC1	0.9 % EMS	5	3	2	7	1	5	2	3	2
	SJ*	Control	5	2	2	8	1	5	2	4	2
	CC1*	Control	5	3	2	7	1	5	2	3	2

 DOI: 10.5958/0975-928X.2018.00054.6

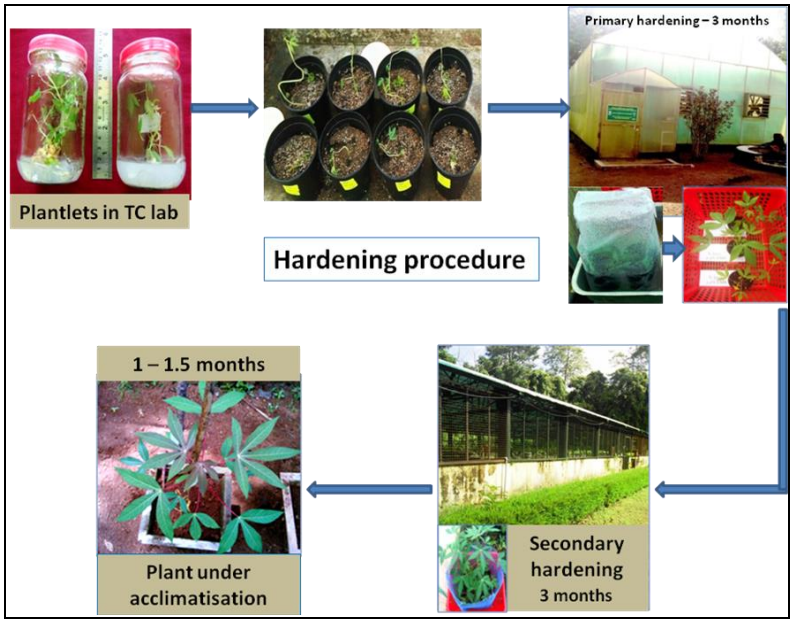

Fig. 1. Stages of hardening of *in vitro* derived plantlets in cassava

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ISSN 0975-928X
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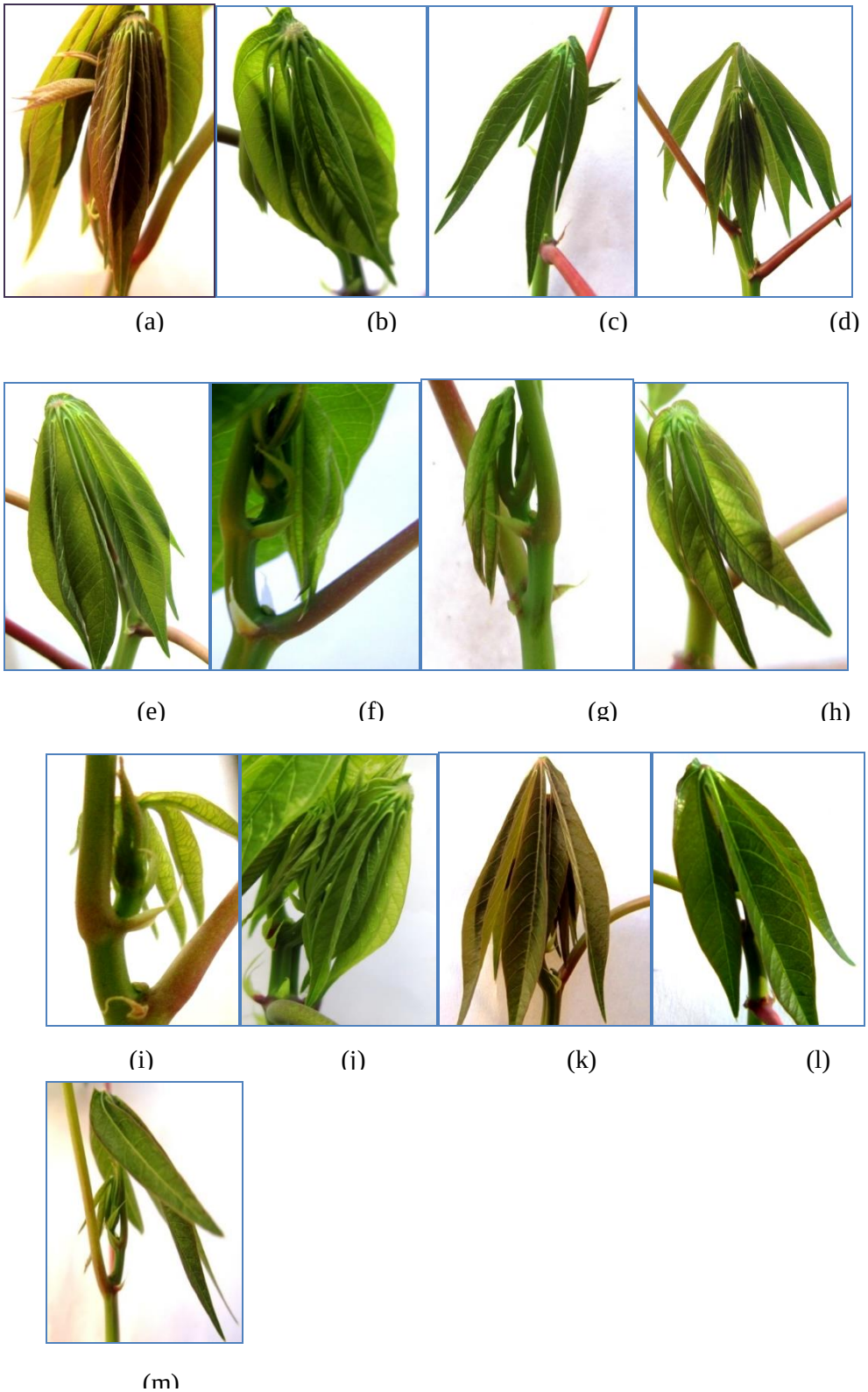

Fig. 2. Variation of apical leaf colour in Sree Jaya. a) Purplish green (Control), b) Light green (Plant no. 14), c) Light green (Plant no. 2), d) Light green (Plant no. 34), e) Light green (Plant no. 12), f) Light green (Plant no. 3), g) Light green (Plant no. 1), i) Light green (Plant no. 15), j) Light green (Plant no. 32), k) Light green (Plant no.33), l) Greenish purple (Plant no.4), m) Light green with purple margin (Plant no. 21), n) Dark green with purple margin (Plant no. 25)

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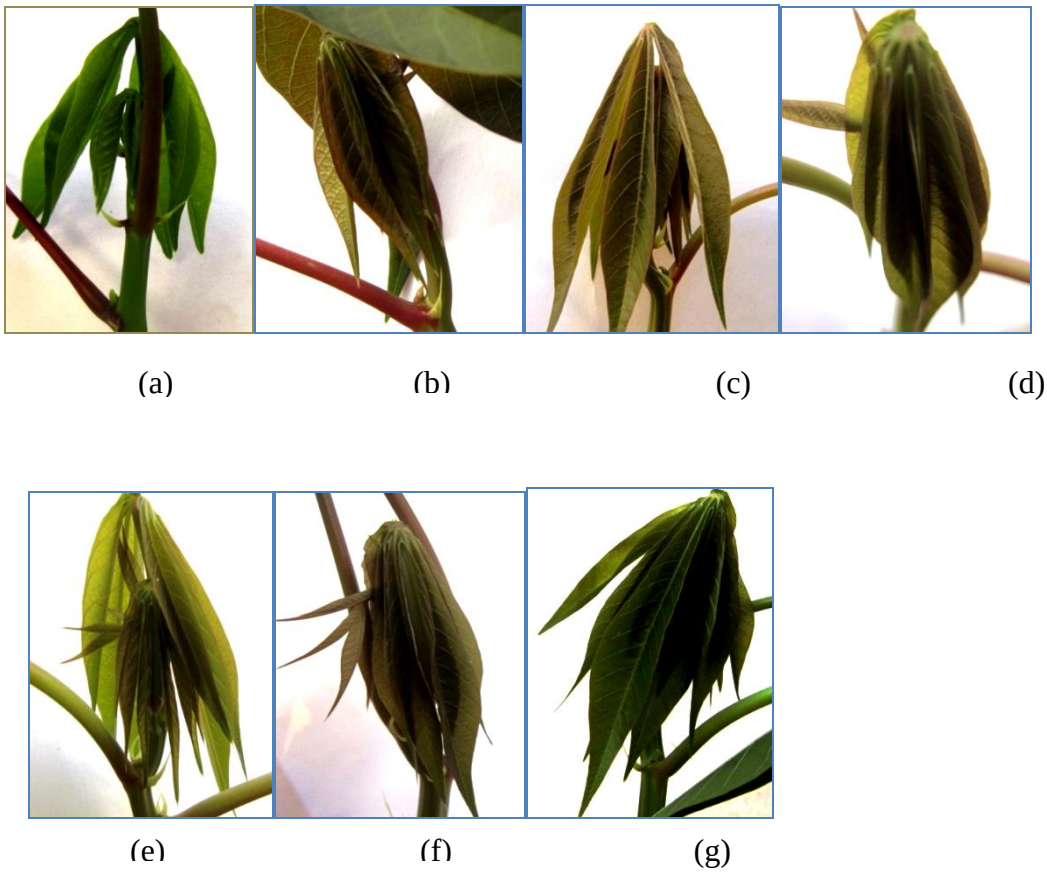

Fig. 3. Variation of apical leaf colour in CC1. a) Light green (Control), b) Greenish purple (Plant no. 38), c) Greenish purple (Plant no. 40), d) Greenish purple (Plant no. 39), e) Light green (Plant no. 36), f) Deep purple (Plant no. 44), g) Dark green (Plant no. 34)

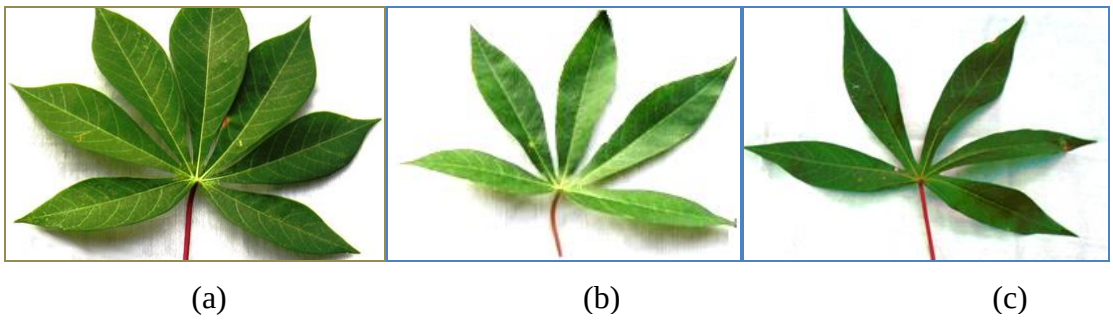

Fig. 4. Variation in shape of a central leaflet of CC1. a) Ovoid (Control), b) Lanceolate (Plant no. 18), c) Lanceolate (Plant no. 19)

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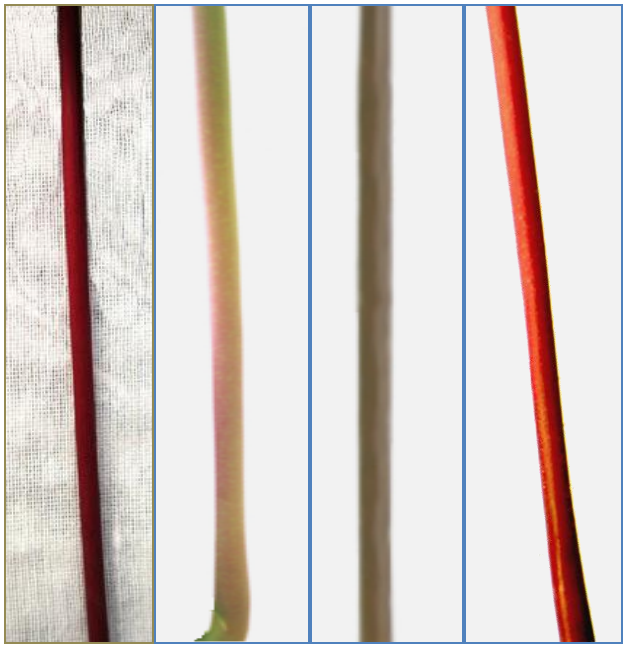

(a) (b) (c) (d)

Fig. 5. Variation in petiole colour of CC1. a) Dark green (Control), b) Greenish red (Plant no.41), c) Deep purple (Plant no.39), d) Red (Plant nos.16-19; 34-38; 40-44)

(d) (e)

Fig. 6. Variation in leaf orientation of Sree Jaya. a) Inclined downwards (Control), b) Horizontal (Plant no.1), c) Horizontal (Plant no.13), d) Horizontal (Plant no.15), e) Horizontal (Plant no.25)

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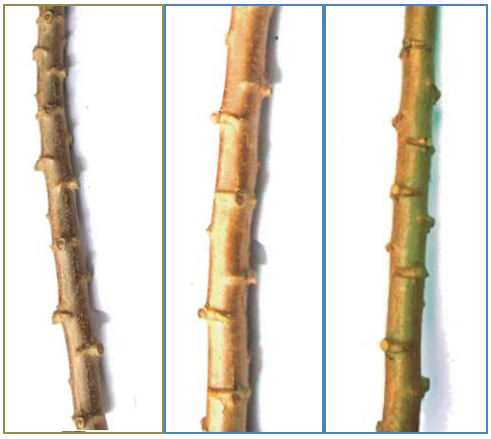

(a) (b) (c)

Fig. 7. Variation in external stem colour of CC1. a) Silver (Control), b) Golden (Plant no.42), c) Greeny yellowish (Plant no.19)

(a) (b) (c)

(d) (e)

Fig. 8. Variation in extends of root peduncle of Sree Jaya. a) Pedenculate (Control), b) Sessile (Plant no.4), c) Sessile (Plant no.23), d) Sessile (Plant no.7), e) Mixed (Plant no.14)

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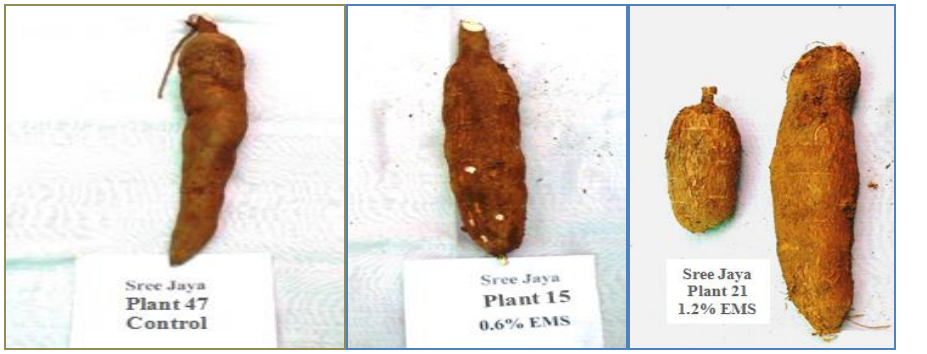
(a) (b) (c)

Fig. 9. Variation in extends of root peduncle of CC1. a) Sessile (Control), b) Pedenculate (Plant no.37), c) Mixed (Plant no.36)



(a) (b) (c)

(e) (f)

Fig. 10. Variation in tuber shape in Sree Jaya. a) Conical tuber (Control), b) Cylindrical tuber (Plant no.15), c) Cylindrical tuber (Plant no.21), d) Cylindrical tuber (Plant no.12), e) Cylindrical tuber (Plant no.30)

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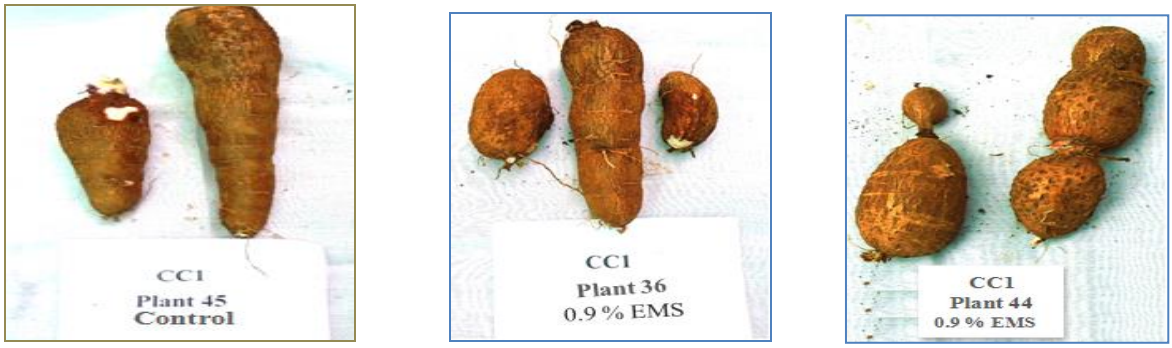
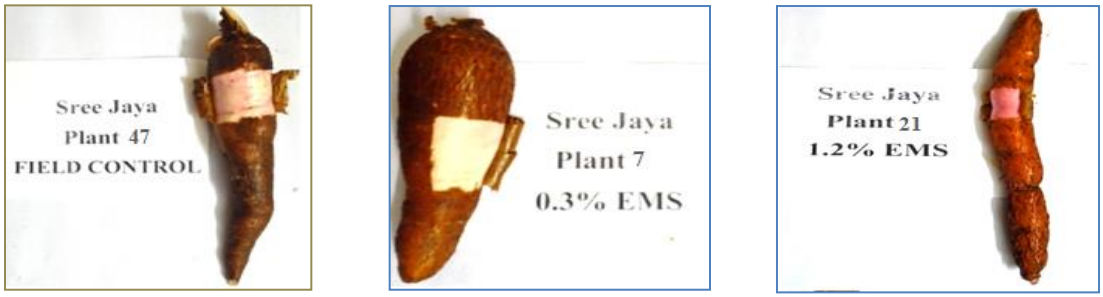


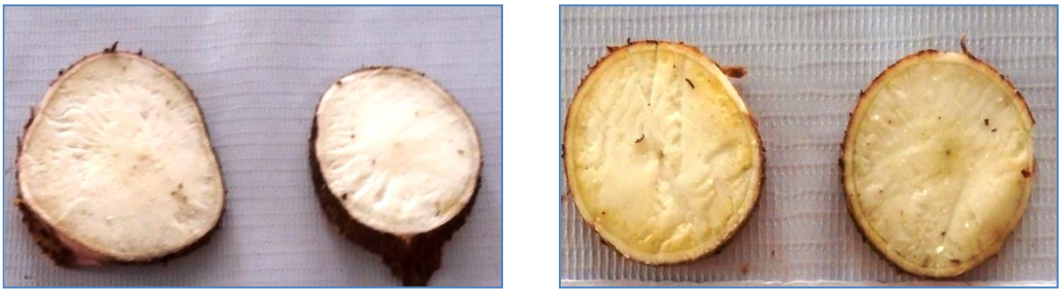
(a) (b) (c)

Fig. 11. Variation in tuber shape in CC1. a) Conical tuber (Control), b) Cylindrical tuber (Control), b) Cylindrical tuber (Plant no.36), c) Irregular tuber (Plant no.44)



(a) (b) (c)

Fig. 12. Variation in colour of external root epidermis of Sree Jaya. a) Dark brown (Control), b) Light brown (Plant no.7), c) Yellow (Plant no.21)



(a) (b)

Fig. 13. Variation in root pulp colour in CC1. a) White root pulp (Control), b) Cream root pulp

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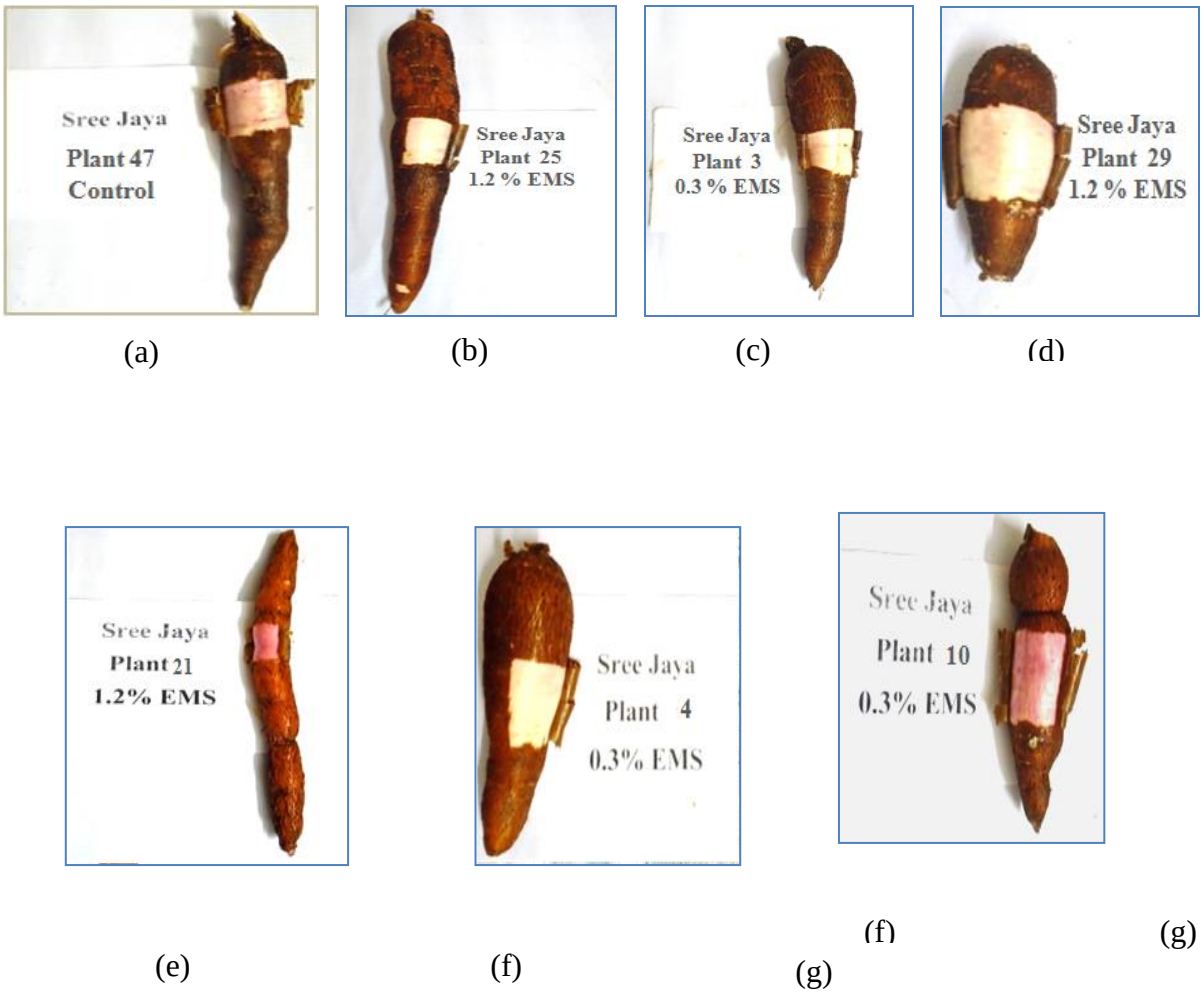

Fig. 14. Variation in root cortex colour of Sree Jaya. a) Light Yellowish Pink (Control), b) Light pink (Plant no.25), c) Light pink (Plant no.3), d) Light pink (Plant no.29), e) Purplish pink (Plant no.10), f) Purplish pink (Plant no.21), g) Pale Yellow (Plant no.4)