Effect Of Gamma Irradiation Mutagenesis On Diversity Of Cassava Tissue Culture Plantlets

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ABSTRACT

Cassava, *Manihot esculenta Crantz* (*Euphorbiaceae*), is one of the leading food and feed plants of the world and stands as the 3rd largest source of carbohydrates for human consumption after rice and maize. However, the cassava sub-sector has not realized its full growth in terms of commercialization and utilization. It is evidenced that the tuber crop can enhance the food base of the poor, increase their income and mitigate poverty among rural households but challenge of diseases and pest hamper its performance. Mutation and efficiency enhancing techniques have been shown to improve resistance in crops through increased diversity from which desired selections can be made. This study was aimed at development of tissue culture generated mutant populations of cassava mosaic virus (CMV) resistant cassava through gamma irradiation and in-vitro selection. Tissue cultured plantlets of farmer and consumer preferred but CMV susceptible cassava variety KM-1 were irradiated with gamma rays at 15Gy and the mutagenised population subcultured 4 times to M_1V_3 to eliminate chimera. Selected putative mutants were screened for effect of mutagenesis, in comparison with the parent variety. The assessed parameters included plant height through the first growth period, number of nodes, leaf morphology (number of leaf lobes) and colour of petiole. The data was collected after every fortnight for 2 months. Results showed that the mutant selections differred among themselves as well as from the parent variety in the assessed paramenters. The lobes for the parent variety were 5 while for the mutant population these raged from 4 to 9 and the differences were found to be significant. There was no significant difference in the petiole colours

within the mutagenised population nor compared to the parent, while branching pattern was found to be different. Appreciable difference in terms of growth parameters recorded can be attributed to mutation that occurred among the different clones of the irradiated materials. The positive differences can be exploited to identify those with CMD resistance and positive agronomic traits.

Key words:
Mutation, cassava, tissue culture, variation, in vitro

INTRODUCTION

Cassava *Manihot esculenta Crantz* (*Euphorbiaceae*) is one of the most important starchy crops worldwide, providing staple food for up to more than 700 million people in the tropics and subtropics (Hershey, 2017). It is the 3rd largest source of carbohydrates for human consumption after rice and maize. Sustainable agricultural production is imperative to curb food insecurity, poverty alleviation, and impact the livelihoods of the smallholder farmers in the production areas (Ojijo et al., 2016; Donkor et al., 2017). According to the recent findings by FAOSTAT, (2020) Sub Saharan Africa produces 61.1% of the world's cassava. However, the cassava sub-sector has not realized its full growth in terms of commercialization and utilization. It is evidenced that the tuber crop can enhance the food base of the poor, increase their income and mitigate poverty among rural households (FAOSTAT, 2018). This is because the crop has high yield potential with minimal input investments. However, challenge of diseases and pest hamper its performance (Mbanjo et al., 2021). Mutation and efficiency enhancing techniques have been shown to improve resistance in crops and nutritional quality (Ahmar et al., 2020). Mutagenesis is the formation of mutations in DNA molecules. There are a variety of mutations that can occur in DNA, such as changes in the DNA sequence or rearrangement of the chromosomes. Mutations may also occur as a result of environmental exposure to genotoxins (chemicals that alter the structure of DNA). Mutagenesis is of concern because it may lead to irreversible effects that can affect fitness of organisms, which in turn may affect population-level processes. Induced mutation is a breeding method by which genetic make-up of a given variety can be changed without crossing with another variety. Using this method a variety retains all its original attributes but is upgraded in one or two changed characteristics. Mutation induction and selection of desired traits

in combination with in vitro and molecular techniques offer several advantages over conventional methods, particularly when the objective is to change one or two characters in an otherwise well adapted and high yielding clone. In vitro techniques allow mutagenic treatment of large numbers and multiplication of the selected genotypes in a small space and short duration under disease free conditions, with a minimal risk of loss of variants through accidents during propagation.

Disease resistance, self-compatibility are desired improvement of existing cultivars in a shorter period than it usually takes to produce a new cultivar by conventional breeding. The first attempt of using induced mutation date back to early 20th century (Pirovano, 1924) when several mutations in grape, pear, carnation and dahlia by exposing pollen to x-rays and other “electromagnetic field" were achieved.

In Kenya mutation breeding has not been adopted widely as a method of breeding. However, it has been used in few crops like cowpea (Pathak et al 1996) wheat (Kinyua et al., 2000), potato (Chepkoech et al., 2020) and dolichos (Kamau et al., 2011).

During the last thirty years worldwide, more than 1800 mutant varieties of plants have been released many of which were induced with radiation (IAEA, 2005). Cassava breeders have shifted their attention to other value-added traits that are easier to breed such as nutritional quality (Ceballos et al. 2016, Ceballos and Hershey 2016). In this study effect of mutagenesis by irradiation of in vitro cassava plantlets was used to improve cassava mosaic disease resistance in local Cassava variety.

MATERIALS AND METHODS

The in vitro plantlets were developed from cassava variety KME1, sourced from Kenya Agricultural Research Institute (formally KARI)

ESTABLISHMENT OF IN VITRO PLANTLETS

The in vitro culture is often used as model system in the study of various physiological, bio chemicals, genetic and structural problems related to plants. There are several ways to promote the in vitro regeneration of a selected plant material relying on the initial material Torres, (1989):

Mother plants were grown in the greenhouse for two to three months where then they were used as sources for explants. The stems were excised from the mother plants and the leaves removed. Stem cuttings 2 to 3 cms long each were treated with a wide

spectrum acaricide at 0.5% for 10 minutes. The acaricide was then rinsed away by washing the stems in running water and then immersed in containers with water before surface disinfection.

To start surface disinfection of the stem cuttings, water was removed from the container, 96% alcohol added and let to stand for two seconds. Next, alcohol was removed and 2.5% solution of calcium hypochlorite (brought to pH 8 by addition of HCl) immediately added. A few drops of a dispersing-adhering agent tween 20 (4 drops/l of solution) was also added. The bottle was then placed in the laminar flow transfer chamber.

After 15 minutes under sterile conditions, the hypochlorite was eliminated, by washing three times with sterile water. Under these conditions, single nodes were excised and placed in culture tube with media.

The plantlets were grown under long day conditions (16 hours of light at 3000 lux) and at temperatures ranging from 25° C. Each node developed into a plantlet occupying the full length of the test tube. The plantlets were ready for subculture after six weeks. Growth media used was Murasige and Skooge 1962 formulation basal (MS) medium supplemented with Thiamine, Myoinosital, Benzyl-Amino- Purine, NAA, GA3, Sucrose and B5, agar was used as a jelling agent.

MUTATION INDUCTION

The irradiation was carried out at the International Atomic Energy Agency laboratory at Seibersdorf in Austria. Nodes from cassava plantlets growing in vitro were excised and placed on Petri dishes. These petridishes with nodes were then inserted into the irradiation chamber where they were exposed to Gamma rays from Co_{60} source at a dose rate of 15Gy. The irradiated nodal cuttings were then transferred into jars containing MS nutrients media and kept in the growth room under long day conditions (16 hours of light 3,000 lux) and at temperatures of about 25° C till they regenerated plantlets at M1V1. Four subcultures were done to M1V3 to dissolve chimeras. Each subculture was maintained as families of specific initial plantlet. Plantlets with fully developed root system were potted in poly-bag containing autoclaved mixture of sand, forest soil and manure at a ratio of 1:2:1.The potted materials were then grown in the green house. Watering was done when necessary.

EVALUATION OF MUTAGENISED SEEDLINGS POPULATION IN THE GREEN HOUSE

Ported putative seedlings were evaluated in the green house at Egerton University Njoro. The putative mutants were planted in a Complete Randomised Design (CRD) replicated three times in the green house where the subcultures marked into specific plantlets formed the replications. The un- irradiated parental seedlings were used as the control. Plots were made of 2 rows of 3 plants each (6 plants). Weeding and watering was done when appropriate. Data Collected included plant height, Number of nodes, leaf morphology (number of leaf lobes) and colour of petiole. The data was collected after every fortnight.

DATA COLLECTION

Data was collected at intervals of two weeks for a period of two months in the green house. The same selections were planted in the field and examined 4, 6 and 9 months after transplanting. The experiment was laid out in Radomised Complete Block Design (RCBD). Data taken was on plant height, branching, petiole colour leaf lobes.

Growth parameters

Vertical height from the ground to the top of the canopy expressed in cm was recorded from three plants in each plot, while nodes were counted from the first node at the base of the plant to the last node at the shoots of the plant. All branches emerging from the base of the plant were counted. The colour of petiole was assessed from a mid-height position. Records were taken of the most frequency occurrence. A scale was developed that was used to measure the different shades of colour of petiole. The scale ranged from 1 to 4(1= yellow- green, 2= green, 3 =light pink and 4 =dark pink. Colour of petiole was determined from four randomly selected leaves.
The assessment of leaf type was done on five leaves and the predominant numberof lobes per leaf was taken as the representative of that plot.

RESULT AND DISCUSSION

For each of the determined growth parameters, analysis of variance (ANOVA) was computed, and where significance F-values ($P<0.05$) existed, means separation were done using the Least Significant Difference (LSD)

The results of this study are presented under the following subtopics: (i) Effect of mutation on the genetic variation of vitro cassava clones (ii) Effects of mutation on growth and morphological parameters of mutagenised cassava at seedling stage under green house condition.

EFFECT OF MUTATION ON GROWTH OF CASSAVA AT SEEDLING STAGE

Table 1 shows results of the different parameters assessed on the seedlings of putative cassava mutants grown at Njoro

Table: 1 Plant heights, Number of lobes, Number of nodes and petiole colour

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TREATMENT	HEIGHT	LOBES	NODES	P. COLOUR
1	84.00b	5.00a	20.00c	4.00a
2	54.66c	5.00a	24.00a	3.00b
3	31.00d	5.00a	12.00i	3.00b
4	113.00a	5.00a	23.00b	3.00b
5	90.66b	5.00a	18.00d	3.33ab
6	26.00de	3.00b	14.00g	4.00a
7	16.00e	5.00a	11.00j	3.33ab
8	24.00de	5.00a	16.00e	4.00a
9	25.00de	3.00b	16.00e	4.00a
11	23.66de	4.33ab	15.00f	3.66ab
control	24.33de	4.33ab	13.00h	3.00b
C.V	**14.37922**	**10.90275**	**20.1535**	**9.0523**
LSD	**11.355**	**0.8337**	**1.1791**	**0.010**

* Means with the same letter are not significantly different.

There are many different features of a plant that can be measured through observation to determine the extent of /healthy plant growth. Some of the features include Plant height, number of nodes/ length of internodes. Difference in the rates of growth between the mutants and control as well as within the mutagenised clone population were found to be significant. Some clones grew slower than others notably clones 8 and 4 from Table clone numbers were lower than the control by .There was significant difference in growth between clones 1, 2, 3, 5, 7, 9, 6 with control. There was no significant difference in the growth rate of following sets of clones (1, 2, 5) (3, 7, 9), (6, 10) and (4, 8) from the control.

Increase of plant height is likewise not uniform phenomenon. It is in many crops fundamentally due to an initial increase in internode length, sometimes, accompanied by an increase in the internode number. Moreover length of the cells is increased in some genotypes also their number per unit area (Weber and Gottschalk 1973), *Pisum sativum*.

Tallness is generally an undesirable trait, because the stability of the stem is negatively influenced. Therefore tall mutants are only in exceptional cases of agronomic interest.

EFFECT OF MUTATION ON PLANT GROWTH AT ADULT STAGE

There are many different features of a plant that can be measured through observation to determine the extent of /healthy plant growth

Difference in the rates of growth between the mutants and control were significant. Similarly, differences in the rates of growth within the mutagenised population were significant. Height as the growth the parameters used significantly varied within the mutant population and with the control. Some clones grew slower than others notably clones 8 and 4 from table clone numbers were lower than the control by .Similarly clone 1, 2, 3, 5, 7 and 9 grew faster than control. There was no significant difference in the growth rate in the following sets of clones (1, 2, 5), (3, 7, 9), (6, 10) and (4, 8) from the control. Clone 6 grew at the same rate as the control

Clones 3, 5, 7 and 9 showed no significant difference in their growth rates.

Similarly clones 1 and 2 had no significant difference in their growth.

EFFECT OF MUTATION ON ADULT PLANT MORPHOLOGY

PLANT BRANCHING

The organisation of the stem of a crop plays an important role with regard to the productivity of the plant. In some cases, genetic conditioned alteration of the shoot structure leads to an improvement. The degree of branching can influence the yielding properties of a crop positively. In other cases, the lack of lateral branches proves to be a favorable character.

The population of cassava showed branching patterns that varied from the branching pattern of the control. The pattern also varied among the clones. There was no significant difference between clones 5 and clone 6 with control. Branching patterns

of clones 1, 2, 3, 4, 8, and 9 were not significant. They were however significantly different from the control.

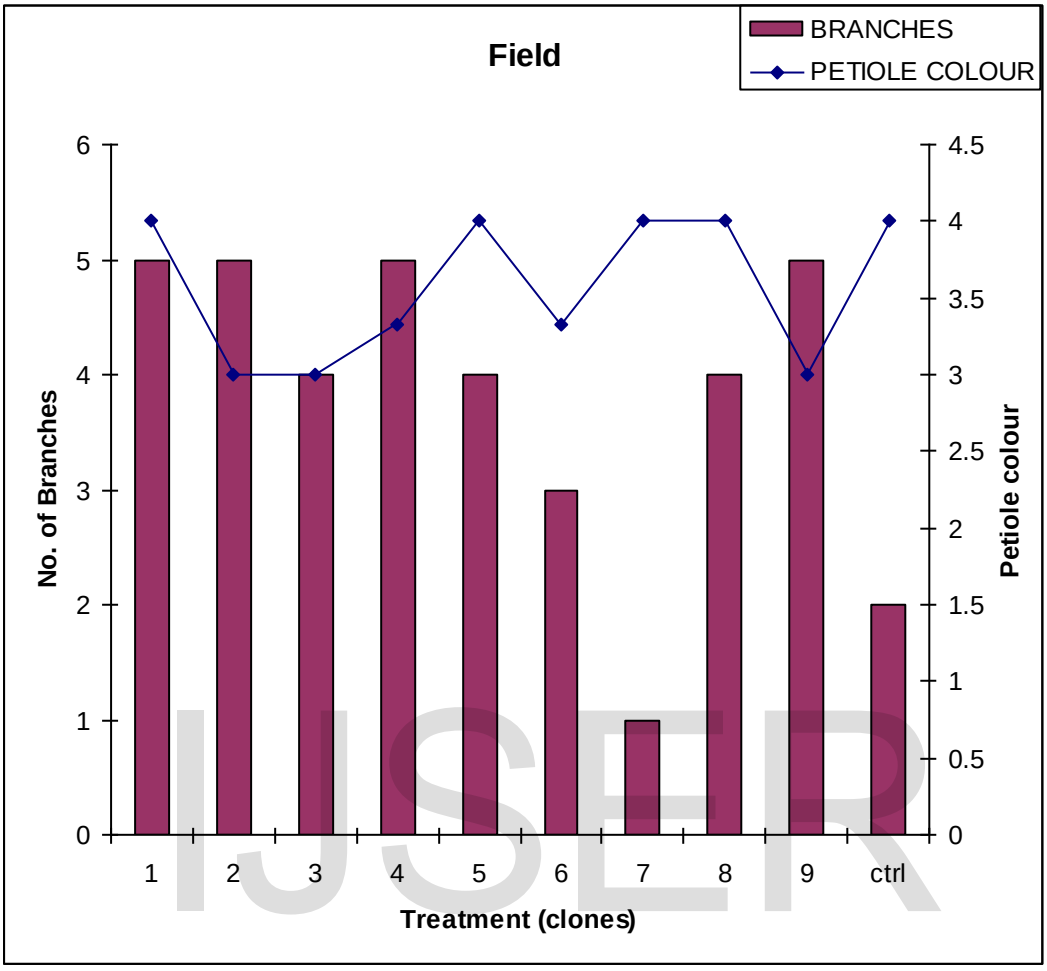

Figure 1: number of branches and petiole colour of selected mutant plantlets

Number of leaf lobes and Petiole colours

Leaf shape and orientation can be used as a phenotypic variability feature in a particular population. Homogeneous population would exhibit similar features.

The number of leaf lobes within the mutagenised population ranged between 4 lobes and 9 lobes. The control had 5 lobes. %0% of the population had lobes more than the control.

Clones 3, 4, 8and 9 had 5 lobes similar to the control

Clones 1, 2, 5, 6 and 7 had more lobes than the control with clone 5 having 9 lobes.

There was no significant different in colour of petiole within the mutagenised population.

CONCLUSION

Appreciable difference in terms of growth parameters recorded can be attributed to mutation that occurred among the different clones of the irradiated material and differences were noted signifying differences in tissue response to irradiation which can be utilized for selection of CMD resistance.

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