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EFFECTS OF GAMMA IRRADIATION AND ETHYL METHANE SULPHONATE ON MORPHOMETRIC TRAITS AND PREVALENCE OF COMMON VIRAL DISEASES AND WHITEFLIES IN CASSAVA

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) is an important staple and food security crop for millions of people in Africa. However, its nutritional value is limited; yet its productivity is constrained by several pests and diseases. Induced mutagenesis is one approach with the potential to overcome such biotic stresses. The objective of this study was to assess the variability in morphometric traits and prevalence of common viral diseases and whiteflies in cassava to different doses and concentrations of gamma irradiation and ethyl methane sulphonate (EMS) treatments. The effects were assessed on seed germination and growth of stakes, as well as foliar viral disease symptoms and whitefly counts. Radio-sensitivity tests revealed LD_{50} for sprouting as 37.6*Gy* for γ-irradiation and 0.08% for EMS treatment. There was notable decrease in sprout, epicotyl length, shoot height, petiole length and number of leaf lobes, with increasing γ-ray doses and EMS concentrations. Contrastingly, total chlorophyll content increased with increasing doses of γ-rays and EMS concentrations. Basing on foliar whitefly counts and disease symptoms, there was a general increase in susceptibility to whitefly infestation and cassava mosaic disease (CMD) incidence. Significantly varying levels of resistance or tolerance to whiteflies and CMD were observed among plantlets derived from irradiated and EMS treated stems, compared to the controls. These findings lay a foundation for more future research on breeding for various traits, including disease resistance in cassava using induced mutagenesis approach.

Key Words: Mutagenesis, radio-sensitivity, whitefly

RÉSUMÉ

Le manioc (*Manihot esculenta* Crantz) est une culture de base et de la sécurité alimentaire importante pour des millions de personnes en Afrique. Cependant, sa valeur nutritionnelle est limitée; pourtant, sa productivité est limitée par plusieurs ravageurs et maladies. La mutagenèse induite est une approche ayant le potentiel de surmonter de tels stress biotiques. L'objectif de cette étude était d'évaluer la variabilité des traits morphométriques et la prévalence des maladies virales courantes et des aleurodes du manioc en raison de l'exposition à différentes doses et concentrations des traitements par l' irradiation gamma et éthyl méthane sulfonate (EMS). Les effets ont été évalués sur la germination des graines et la croissance des tuteurs, ainsi que sur les symptômes de la maladie virale foliaire et le nombre des aleurodes. Les tests de radiosensibilité ont révélé que la DL50 pour la germination était de 37,6 Gy pour l'irradiation et de 0,08 % pour le traitement EMS. Il y avait une diminution notable de la pousse, de la longueur de l'épicotyle, de la hauteur des pousses, de la longueur du pétiole et du nombre des lobes foliaires, avec l'augmentation des doses de rayons et des concentrations EMS. En revanche, la teneur totale en chlorophylle augmentait avec l'augmentation des doses de rayons et des concentrations EMS. Sur la base des dénombrements foliaires des aleurodes et des symptômes de la maladie, il y avait une susceptibilité généralement accrue à l'infestation des aleurodes et à l'incidence de la maladie de la mosaïque du manioc (CMD). Des niveaux significativement variables de résistance ou de tolérance aux aleurodes et à la CMD ont été observés parmi les plantules dérivées de tiges irradiées et traitées par EMS, par rapport aux témoins. Ces résultats jettent les bases des autres recherches futures sur la sélection pour les divers caractères, y compris la résistance aux maladies du manioc en utilisant une approche de mutagenèse induite.

Mots Clés : Mutagenèse, radiosensibilité, aleurode

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) provides a rich source of carbohydrates for nearly one million people in sub-Saharan Africa (Nassar and Ortiz, 2010), majority of whom are poor. However, it is reported to have a very limited nutritional value (Nassar and Ortiz, 2010; FAO, 2010); yet, its breeding and productivity are constrained by long propagation cycles (Jennings and Iglesias, 2002; Kawuki *et al*., 2011); which facilitate infection and transmission of systemic viral diseases (Ogwok *et al*., 2015).

Over the years, cassava improvement programmes have focused on use of conventional methods to breed for varieties with useful traits (Jennings and Iglesias, 2002). Conventional breeding, however, is laborious and time consuming, as it involves a long procedure from parental choice to cultivar release (Shimelis and Laing, 2012). Recently, efforts have been made to transfer genes from related or other species into the crop *via* genetic engineering or transformation (Taylor*et al*., 2004; Ogwok *et al*., 2015). Although this technique is specific and time saving, there is public outcry against the technology, besides the regulatory constraints in several countries in Africa (Adenele *et al*., 2012), including Uganda.

Although there are few publications on mutation breeding in cassava, particularly in sub-Saharan Africa, induced mutagenesis offers a huge potential to overcome breeding challenges that face the crop. Since the discovery of radiations, including gamma ray radiation, over 3,000 mutant crop varieties comprising cereals (1541), ornamentals (709), legumes (432), oil seeds (145) and others (312), have been produced through induced mutagenesis (FAO and IAEA, 2011). Of these varieties, only two cassava mutants have been reported. These include varieties "Tebankye" and "Fuxuan 01" in Ghana and China, respectively (Maluszynski *et al*., 2000; Yan *et al*., 2013). The former exhibited improved attributes of large sized starch granules in the tubers, cooking quality and tolerance to African cassava mosaic virus disease (ACMVD). Also, there have been reports on successful generation of putative cassava mutant lines, with higher yields and starch content, from gamma-irradiated stakes (Khumaida *et al*., 2015 and 2017) and distinct variations in root phenotypes in a mutant population derived from irradiated botanical seeds at International Centre for Tropical Agriculture (CIAT), Cali, Colombia. In most of these studies, physical mutagens, especially gamma irradiation, have been used for mutation induction.

In Uganda, we have set up an improvement programme for cassava to develop mutants which are high yielding, with resistance to the common viral diseases, cassava brown streak disease (CBSD) and cassava mosaic disease (CMD); as well as whitefly infestation in farmer preferred varieties. Both diseases are devastating, causing severe yield losses (FAO, 2011), with CBSD being the most damaging (Legg *et al*., 2011) as it causes root rot and almost 100% yield loss due to the necrosis it causes to the starch storage tissues (Hillocks and Thresh, 1998). Both CBSD and CMD are transmitted by the whitefly (*Bemisia tabaci*) (Maruthi *et al*., 2005), which is a common cassava pest, but also by mechanical means (Lister, 1959) and through the exchange of infected cassava cuttings among farmers.

Induced mutations are of considerable value in creating variability in agronomically important traits that can be exploited in plant breeding programmes (Liu *et al*., 2004). Therefore, the objective of this study was to assess the variability in morphometric traits and prevalence of common viral diseases and whiteflies in cassava to different doses and concentrations of gamma irradiation and ethyl methane sulphonate (EMS) treatments.

MATERIALS AND METHODS

Experimental site and plant materials. The experiment was carried out once in the screen house and tissue culture laboratory of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC). Three cassava accessions were selected for this study, two from BNARI farm and one from National Crops Resources Research Institute (NaCRRI), Namulonge in Uganda. The BNARI accessions (Bankye Borodie and BNARI-UK) were stakes; while the NaCRRI accession (NASE 14) were seeds (because stakes could not be carried to Ghana due to phytosanitary restrictions). These accessions were chosen because they are among the farmer-preferred varieties for good yields in Ghana and Uganda. However, the BNARI accessions succumb to cassava mosaic disease (CMD); while the NaCRRI accession is resistant to CMD and only tolerant to CBSD. For the BNARI accessions, we deliberately used materials with visible CMD symptoms so as to be able to evaluate the effect of the mutagenic treatment on the disease prevalence using foliar symptoms. A total of 135 lignified stakes, with about 3-5 nodes each were prepared from each accession; while 240 dry seeds of NASE 14 were prepared for mutagenic treatment.

Gamma irradiation. To determine the sensitivity of the selected varieties to gamma rays, 15 stakes of each accession were irradiated at 0, 15, 20, 30 or 45*Gy* using ^{60}Co gamma source, at the Radiation Technology Centre (RTC) of GAEC prior to planting. The irradiated stakes were immediately planted in poly bags filled with garden soil, and placed in a plant barn.

The experiment was set up in a split plot design with two factors (Bankye Borodie and BNARI-UK varieties and mutagens) and three replicates. The main factor was the gamma dose and the sub-plot was the variety. Five pots were set up per sub-plot and one stake planted vertically in each pot. The stakes were periodically watered throughout the entire period of the experiment.

The effect of gamma irradiation on NASE 14 seeds was determined by irradiating 45 seeds of NASE 14 at 0, 100, 200, 300 or 400*Gy*. The irradiated seeds were immediately planted in poly bags, filled with garden soil in a screen house at BNARI (43 ± 1°C, under natural light). The pots were initially kept covered with black polyethylene sheet, to create dark conditions for augmented seed germination. The experiment was set up in a completely randomised design, in three replicates, with seeds of same gamma dose in the same plot. Four pots were planted per plot and three seeds were planted in each pot.

Ethylmethane sulphonate (EMS) treatment. The effect of EMS on morphometric features and disease prevalence in cassava plantlets derived from cassava seeds and stakes were evaluated by immersing the seeds and stakes in 0.05, 0.1, and 0.15% (v/v) EMS solutions, including the controls. Fresh solutions of EMS (Sigma-Aldrich, USA) were prepared in 20% dimethyl sulfoxide (DMSO). In each solution, 15 cuttings of each variety were immersed and kept at room temperature under a fume chamber. After 24 hours, the EMS solutions were decanted, stakes rinsed in tap water and immediately planted in polybags filled with garden soil. The experiment was set up in a split-plot design as previously described. The main plot was the EMS concentration and the sub-plot was the variety. Five pots were set up per concentration in three replicates. One stake was planted vertically in each pot. The stakes were periodically watered using a watering can.

Similarly, the effect of chemical mutagenesis on the seeds was evaluated by soaking the seeds in distilled water for 24 hours; followed by treating them in EMS solutions as above. The treated seeds were immediately planted in poly bags filled with garden soil in the screen house as above. Four pots were set up per concentration. And two seeds were planted in each pot. The experiment was set up in a completely randomised design in three replicates.

Meristem culture initiation of the putative mutants. Apical shoot tips were obtained from the sprouts of gamma irradiated and EMS treated cassava stakes, washed with soapy water and then rinsed under running tap water for 30 minutes. The shoot tips were then surface-sterilised by immersing in 10% chlorox solution for five minutes, then in 70% ethanol for three minutes. In each case, the shoot tips were vigorously agitated under the laminar flow hood, and thereafter rinsed twice with sterile distilled water.

Apical meristems were excised from the sterilised shoot tips under a stereomicroscope (Leica 2000, China), using a sterile scalpel and pair of forceps, and inoculated onto 15 ml shoot initiation medium containing Murashige and Skoog medium (MS) (Duchefa biochemie, Nertherlands), supplemented with 30gL^{-1} sucrose, and 3.5gL^{-1} phytagel as gelling agent. The pH of media had been adjusted to 5.8 before autoclaving at 121ºC for 15 minutes. The cultures were incubated at 26 ± 1ºC under a 16/8 hr (day/night) photoperiod.

CMD incidence and severity in the putative mutants. Foliar CMD incidence and symptom severity were scored at 14 and 36 days after planting (DAP). CMD incidence was scored basing on the presence (score 1) or absence (score 0) of foliar symptoms, whereas disease severity was scored based on a 5-point scale as previously described by Njock (1994); where a score of 1 means asymptomatic condition and a score 5 imply over 50% of the leaf show foliar symptoms.

Data collection and analysis. Different kinds of datasets on morphometric characters and foliar disease symptoms were generated in the barn, screen house and laboratories. Most of these were collected using a "Field Book App" (Rife and Poland, 2014). These included sprout or germination counts, foliar CMD incidence and severity, whitefly count, epicotyl length, shoot height, petiole length, number of leaf lobes, chlorophyll content and meristem initiation response of mutants, as well as still pictures at different stages. Height and length were measured using a meter rule, whereas chlorophyll content was determined using a CCM-200 plus Chlorophyll content meter (Optisciences, USA).

The datasets were processed using Excel computer software package; and thereafter, subjected to analysis of variance (ANOVA) using GenStat statistical software, edition 12. The Least Significant Differences (LSD) was used to compare means at the 5% significance level.

RESULTS AND DISCUSSION

Lethal dose for gamma irradiation and EMS treatment. For gamma irradiation and EMS treatment, results revealed LD_{50} of 38*Gy* and 0.08%, respectively, for BNARI-UK accession, and 37*Gy* and 0.27% for Bankye Borodie (BB) accession (Fig. 1). In previous studies, LD_{50} for gamma rays and EMS concentration for sprouting of cassava

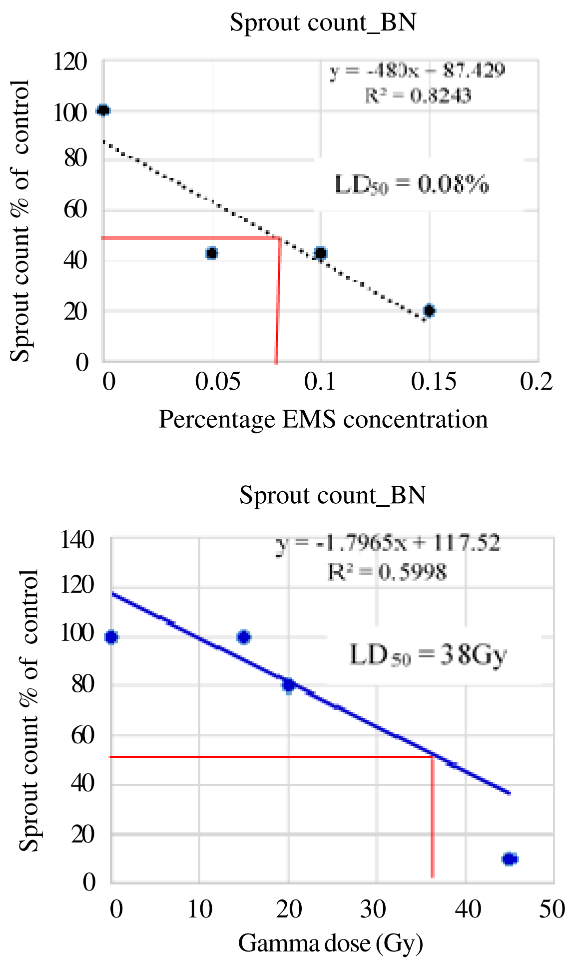

Figure 1. LD_{50} for sprout of cassava stakes of EMS-treated and γ-irradiated stakes in one BNARI (BN) accession.

cuttings was 27.5*Gy* and 1.5% (~122Mm) respectively (Kangarasu *et al.*, 2014); and 32*Gy* (Amenorpe, 2004). Thus, these results suggest that higher irradiation doses and EMS concentrations than the optimal ones are inhibitory to sprouting of cassava stakes. Beyond the optimal doses/concentrations, irreparable chromosomal or entire genome damage and tissue death occurs. Also, at high gamma doses, growth is inhibited due to cell cycle arrest at growth phase 2 (G2) during mitotic division in somatic cells (Minisi *et al.*, 2013). Determination of LD_{50} is important because it helps to prevent excessive loss of actual experimental materials. At the gamma doses and EMS concentrations used in this study, it was not possible to determine lethal dose (LD_{50}) for seed germination. This is because the gamma doses and EMS concentrations used in this study were sub-optimal with less or no poisoning potential to the seeds. Thus, further studies are needed for this purpose.

Impact of mutagenesis on morphometric traits. There was a general decrease in sprout, petiole length and number of leaf lobes with increasing doses of gamma rays (Table 1a) and EMS concentration (Table 1b). On the contrary, there was an increase in total chlorophyll content with increasing doses of gamma irradiation; while for EMS treatments, the increase peaked at 0.1% and then decreased with further increase in concentration. Meanwhile, shoot height increased with gamma dose and decreased with increase in EMS concentration. Hamideldin and Hussin (2014) also reported similar effects of gamma irradiation on potato and attributed the cause to changes in DNA patterns within the potato varieties that were used. The impact of gamma irradiation was more obvious than that of EMS treatment (Fig. 2), and more peculiar morphometric characters were noticed in plantlets derived from gamma irradiated stakes (Fig. 3) with most of the characters observed at 20*Gy* treatment. Thus, characters at this gamma irradiation dose may be valuable in early assessment of effectiveness of irradiation to induce mutations and stimulation of traits of agronomic importance, including disease and pest resistance in the crop.

It is plausible that the more pronounced effects of gamma irradiation than EMS treatment is because the latter only causes point mutations, whereas the former causes chromosomal breakages and rearrangements or deletions (Bhat *et al.*, 2007); hence resulting in greater biological effect with easily recognisable phenotypic traits. Also, the failure of stakes to sprout at 45*Gy* may have been due to the highly toxic gamma irradiation levels that led to metabolic activity breakdown. Gamma rays are known to interact with atoms or molecules (e.g. water, proteins, lipids, ascorbic acid, carotenoids and enzymes such as proteases and peroxidase) (Hameed *et al.*, 2008) to produce free radicals in cells such as hydrogen and hydroxide ions, quinones and celluloses (Calucci *et al.*, 2003, which can damage important components of plant cells; hence affecting their morphology, anatomy, biochemistry and physiological characters (Ashraf *et al.*, 2003). For example, peroxidase enzyme activity was found to be higher in seedlings derived from irradiated chickpea seeds, which consequently inhibited their growth (Hameed *et al.*, 2008). Ultimately, the radicals cause inhibitory changes in plant structure and metabolism including growth (Kim *et al.*, 2004; Wi *et al.*, 2005).

Similarly, the effects of gamma irradiation and EMS treatment were observed on seed germination, epicotyl length, shoot heights and chlorophyll content (Table 2). The results showed no significant differences ($P>0.05$) in germination, shoot height and chlorophyll content in plants derived from both gamma-irradiated (Table 2a) and EMS-treated seeds (Table 2b), except for epicotyl length which varied significantly in irradiated seeds ($P=0.002$) and not significantly in EMS-treated seeds ($P=0.08$) (Table 2b). Relatedly, an

TABLE 1a. Cassava plant growth characteristics and chlorophyll content of putative mutants derived from gamma irradiated cassava stakes

Gamma dose (*Gy*)	Sprout (% of control)		Shoot height (% of control)		Petiole length (% of control)		Number of leaf lobes (% of control)		Chlorophyll content (% of control)	
	BB	BN	BB	BN	BB	BN	BB	BN	BB	BN
0 (Control)	100	100	100	100	100	100	100	100	100	100
15	100	100	129	101	105	97	100	86	105	110
20	100	71	121	128	88	93	86	71	121	116
30	100	86	99	115	64	74	71	71	120	119
45	0	14	0	0	0	0	0	0	0	0
Mean*	80	74	90	89	71	73	71	66	89	89
LSD	8.8	8.8	1.6	1.6	0.4	0.4	0.2	0.2	0.7	0.7

*Means for sprout (P=0.006) and shoot height (P<0.001) were significantly different while for petiole length (P=0.3), leaf lobes (P=0.2) and chlorophyll content (P=0.5) were not significantly different at 5%. BB= Bankye Burodie and BN= BNARI-UK accession

TABLE 1b. Plant growth characteristics and chlorophyll content of putative mutant plants derived from EMS treated cassava stakes

EMS concentration (%)	Sprout (% of control)		Shoot height (% of control)		Petiole length (% of control)		Number of leaf lobes (% of control)		Chlorophyll content (% of control)	
	BB	BN	BB	BN	BB	BN	BB	BN	BB	BN
0 (Control)	100	100	100	100	100	100	100	100	100	100
0.05	67	33	79	118	106	118	86	86	98	73
0.1	83	33	87	82	95	86	100	71	103	113
0.15	67	33	64	61	88	104	86	71	90	98
Mean*	79	50	83	90	97	102	93	82	98	96
LSD(0.05)	16.3	16.3	2.0	2.0	0.8	0.8	0.3	0.3	0.8	0.8

*Means for sprout (P=0.9), petiole length (P=0.2) and leaf lobes (P=0.09) were not significantly different whereas means for shoot height (P=0.04) and chlorophyll content (P<0.001) was significantly different at 5% at different EMS concentrations. Petiole length stimulated at 0.05% EMS concentration, while chlorophyll content was stimulated at 0.1% EMS concentration

Figure 2. Effect of gamma irradiation on growth of cassava stakes of variety Bankye burodie (BB) at 21 days after planting. The plantlets represent effects of irradiation at 0, 15, 20, 30 and 45*Gy,* respectively.

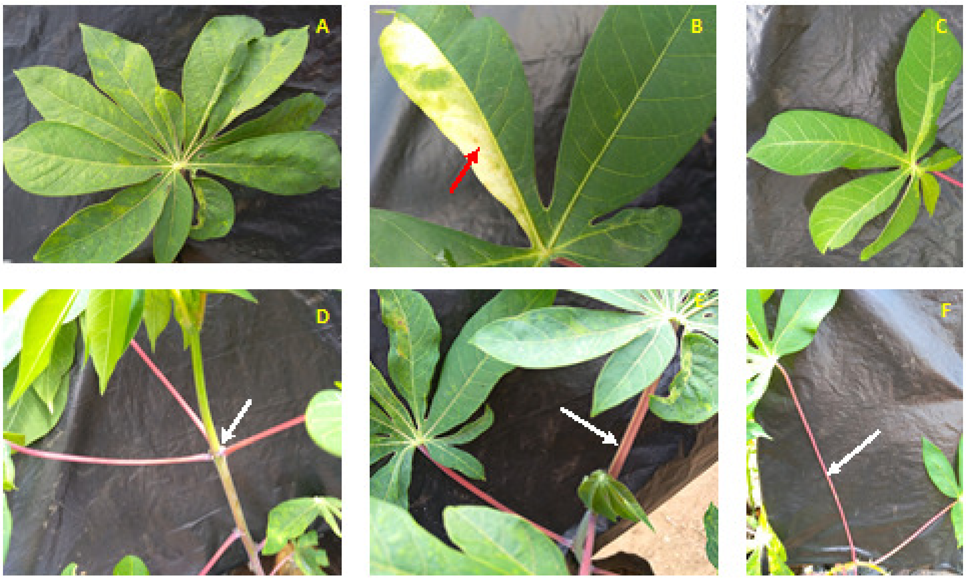

Figure 3. Peculiar characters noticed on cassava plantlets derived from gamma-irradiated stakes. The arrows show the peculiar characteristics exhibited in the plantlets. (A) Increased number of leaf lobes_20*Gy*, (B) Chlorophyll chimerism_15*Gy*, (C) Irregular leaf lobes_30*Gy*, (D) Whorled leaf arrangement_30*Gy*, (E) Thickened petiole_20*Gy*, (F) Elongated petiole_20*Gy*.

TABLE 2a. Cassava plant growth characteristics and chlorophyll content of putative mutant plants derived from irradiated seeds of NASE 14

Gamma dose (*Gy*)	Germination (% of control)	Epicotyl height (% of control)	Shoot height (% of control)	Chlorophyll content (% of control)
0	100	100	100	100
100	87	57	65	152
200	175	102	102	116
300	50	48	96	112
400	125	86	107	99
Mean	108	79	94	116
LSD(0.05)	25.1	1.1	6.5	3.2

TABLE 2b. Cassava plant growth characteristics and shoot height and chlorophyll content of cassava mutant plants from EMS treated seeds of NASE 14

EMS concentration (%)	Germination (% of control)	Epicotyl height (% of control)	Shoot height (% of control)	Chlorophyll content (% of control)
0	100	100	100	100
0.05	123	88	117	103
0.1	131	102	123	108
0.15	108	93	113	109
Mean	115	96	113	105
LSD(0.05)	25.8	1.2	3.6	2.9

inverse relationship between germination of gamma-irradiated chickpea seeds and irradiation doses was reported (Hameed *et al.*, 2008). This inhibitory effect of irradiation is likely to have been caused by the stress effect of free radicals formed upon irradiation of seeds (Rogozhin *et al.*, 2000).

On the contrary, seed germination decreased with increasing concentration of EMS in Basmati rice (Wattoo *et al.*, 2013).This was attributed to the lethality of EMS which may have caused a drop in auxin levels, chromosomal abrasions or due to decline in the uptake of assimilates. The EMS concentrations used in this study may not have been lethal enough to adversely affect the biochemistry or physiology of the germinating seeds.

Contrastingly, chlorophyll content increased at all gamma doses, except 400*Gy* (Table 2a) and EMS concentrations (Table 2b) compared to control. Singh (1971) and Idrees *et al.* (2007) obtained results with a similar trend on application of gamma irradiation. The increased chlorophyll content was due to a stimulatory effect on the development of meristematic cells that led to the synthesis and accumulation of auxin, which is believed to have an important role in chlorophyll accumulation. Meanwhile, results of increase in chlorophyll content with EMS concentration are in agreement with those reported by Junaid *et al.*, (2008), though the content decreased with further increase in EMS concentration. The increased chlorophyll content could have been due to a mutant type induced by EMS.

Therefore, the EMS concentrations used in this study were below the lethal limits and hence were beneficial in stimulating chlorophyll production.

The trends for epicotyl length and shoot heights were not consistent. However, for gamma irradiation, most stimulation was at 200*Gy*, while for EMS treatment this was at 0.1%. Decreased shoot length with increasing gamma dose in chickpea (Hameed *et al*., 2008) and EMS concentration in *Dracaena sanderiana* (Junaid *et al*., 2008) and *Coriandrum sativum* L. (Kumar and Pandey, 2019) were previously reported. The growth stimulatory effect of low gamma doses was ascribed to a change in the hormonal signaling network in plant cells or an increasing anti-oxidative capacity of the cells so as to easily overcome stresses (Minisi *et al*., 2013). Meanwhile, the stimulatory effect at lower EMS concentrations, congruent with the observations in this study, can be attributed to a stimulated cell division that caused the epicotyls to elongate.

Meristem culture initiation. Though analyses revealed no significant differences in response of meristem tips derived from irradiated and EMS-treated stakes (P=0.9 and P=0.8, respectively), meristem response decreased with increase in gamma dose and EMS concentration, except for one variety, BNARI-UK (BN), in which the response increased with EMS concentration (Fig. 4). Contrary to the results of the gamma-treated meristems in this study, Shin *et al*. (2011) reported easy regeneration of axillary buds into shoots on stems of gamma-irradiated sweet potato. The reduction in regeneration response in this study can be attributed to chromosomal damage in the cells by the high gamma doses, while the lower doses may have been stimulatory for high meristem responses. However, the results of the EMS treatment in this study are in accordance with Sadat and Hoveize (2012) who also observed failure of plant regeneration from sugarcane plantlets exposed to increasing concentrations of EMS. This was attributed to the poisonous effect of EMS on plant cells as the concentration increased. On the other hand, the increased meristem response of BINARI-UK with increase in EMS concentration may have been due to a stimulatory effect based on varietal/ genotypic differences. This, therefore means that EMS solutions may be efficiently used to speed up induction and regeneration of new plants (with mutations of agronomic importance) in cassava breeding programmes.

Whitefly infestation, CMD incidence and severity. Generally, the results revealed increased whitefly infestations and CMD severity in both cassava accessions, all of which were maximum at gamma dose 30*Gy* (Table 3a). However, the lowest whitefly infestation and CMD severity were recorded at 20*Gy* and 15*Gy*, respectively for accession BB, which could, therefore, be considered as tolerant.

Although literature on mutagenic induction of resistance to pests in plants is scanty, it can be postulated that the 20*Gy* gamma dosage caused changes in the DNA that resulted in antibiotic compounds such as phenolics and proteinase inhibitors that may have imparted some resistance to whitefly infestation (Jan *et al*., 2012). At 30*Gy*, the DNA was damaged leading to so severe stresses that broke down the defence mechanism to render the plants susceptible to infestation (Ouhibi *et al*., 2015).

The lower incidence and increased resistance to diseases induced by irradiation at lower gamma irradiation have been reported in pear fruits (*Pyrus pyrifolia* "Niitaka") (Jeong *et al*., 2017) and mutants of *Citrus reticulata* and *Oryza sativa* (Raina and Danish, 2018). Studies have further reported that lower gamma doses are more effective at inducing disease resistance than higher ones because the latter causes DNA damage, resulting in secondary adduct molecules involved in induction of defence mechanisms against diseases in plant tissues (Ouhibi *et al*., 2015).

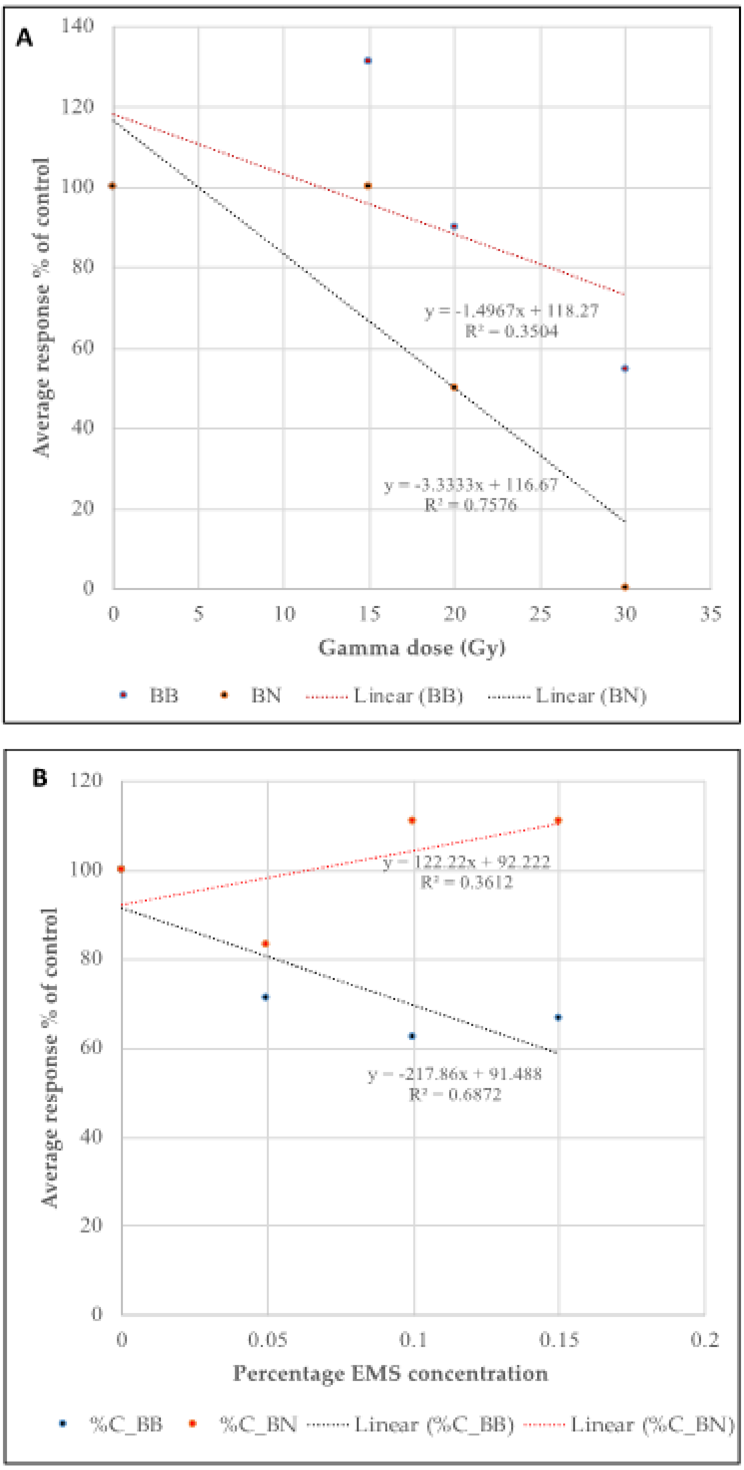

Figure 4. Cassava meristem culture response: (A) response decreased with increase in gamma dose; (B) response for BB decreased as that for BN increased with increase in EMS concentration.

TABLE 3a. Whitefly count, CMD incidence and severity in putative mutant plants derived from irradiated cassava stakes

Gamma dose (*Gy*)	Whitefly count		CMD incidence		CMD severity	
	BB	BN	BB	BN	BB	BN
0 (Control)	5.6	5.1	0.9	0.9	1.4	1.8
15	5.3	5.4	0.6	0.4	1.1	1.8
20	3.8	7.4	1.0	0.7	1.2	2.3
30	8.5	8.0	0.6	1.0	2.4	2.3
45	-	-	-	-	-	-
Mean*	5.7	5.9	0.8	0.7	1.5	2.0
LSD(0.05)	1.0	1.0	0.1	0.1	0.2	0.2

*Means for whitefly count (P=0.04), CMD incidence (P=0.006) and CMD severity (P=0.004) were all significantly different at 5%.

TABLE 3b. Whitefly count, CMD incidence and severity in putative mutant plants derived from EMS treated cassava stakes

EMS concentration (%)	Whitefly count		CMD incidence		CMD severity	
	BB	BN	BB	BN	BB	BN
0 (Control)	4.0	10.3	1.0	1.0	1.7	2.3
0.05	5.9	5.7	1.0	0.5	1.8	3.0
0.1	7.7	5.3	0.9	1.0	1.8	2.0
0.15	6.0	9.6	0.9	1.0	1.6	2.0
Mean	5.8	8.6	1.0	0.9	1.8	2.3
LSD(0.05)	1.7	1.7	0.1	0.1	0.3	0.3

Means for whitefly count (P=0.005) and CMD incidence (P<0.001) were significantly different while that for CMD severity (P=0.09) was not significantly different at 5%.

In the present study, irradiation at 30*Gy* seems to have been beyond optimal and caused severe damage to the DNA that resulted in severe stress in the plants, thus boosting their susceptibility to CMD infection.

On the other hand, whitefly infestation and CMD incidence varied significantly with EMS concentration and among accessions (Table 3b). Whitefly infestation was generally higher in the treatments than in the control and peaked at 0.1% EMS concentration in accession BB. This can be regarded as susceptible, while in accession BN the infestation was lower than control, peaking at 0.15% concentration. This can be inferred as tolerant. All accessions exhibited foliar symptoms of CMD at all EMS concentrations; however, reduced or tolerance levels (lowest incidence and severity) were noted at 0.05% and 0.1-0.15% in accession BN.

The low whitefly infestation and CMD infection levels could be a sign of acquired tolerance (Simons, 1971). This can be attributed to EMS treatment, which could have induced cell wall fortification or release of chemicals such as phytoalexins and antioxidants, or enhancement of the activity of disease resistance- related enzymes (Simons, 1971; Daayf *et al*., 2012); these may have aided in the reduction in whitefly and CMD prevalence. The different tolerances or susceptibilities to whitefly infestations and CMD infection, following gamma irradiation and EMS treatment study can be explained by the differences in resistance genes and/or genetic constitutions of the different cassava accessions (BB and BN) that were used.

It is worth to note that various mutant crops with improved and stable resistance to a number of diseases induced mainly by gamma irradiation have been reported (Maluszynski *et al*., 2000; Ahloowalia *et al*., 2004; Pathirana, 2014). In this regard, over 320 cultivars resistant to different diseases have been obtained either as direct mutants or derived from hybridisation with mutants, or through self- or cross-fertilisation (Kozjak and Megliè, 2016). For example, using gamma irradiation, disease resistance has been induced in crops such as rice against rice blast and rice yellow mottle virus; mungbean against yellow mosaic virus; soybean against myrothecium leaf spot, yellow mosaic virus and rust; and cotton against bacterial blight and cotton leaf curl virus (Pathirana, 2014). Similarly, chemical mutagenesis has been used to create resistance to pathogen and disease outbreak in crops such as cabbage, *Brassica oleracea* (Maluszynski *et al*., 2000) and soybean, *Glycine max* (Khan and Tyagi, 2013). Thus, the results obtained in this study give a great possibility for inducing durable resistance to common cassava viral diseases and pests as well as morphometric variability that may be of significance in cassava breeding.

CONCLUSION

The LD_{50} doses based on the sprouting of stakes after treatment with different doses of gamma rays and different concentration of EMS were 38*Gy* and 0.08%, and 37*Gy* and 0.27% for cassava accessions BNARI-UK (BN) and Bankye Borodie (BB), respectively. The LD_{50} mutagen doses determined for cassava accessions in this study will be useful in guiding determination of gamma dosage and EMS concentration to use in future cassava mutation breeding programmes. Gamma irradiation influenced morphometric traits more than EMS treatment and accession BB was generally more sensitive and responsive to both treatments than BN.

The different morphometric traits observed may be helpful in early assessment of effectiveness and potentiality of the mutagenic agent in a breeding programme. Although some of the resulting morphometric traits may be undesirable, they can be a source of enormous variation for practical utility in the generation of new crop varieties. This study demonstrates that gamma rays and EMS may be valuable mutagens in inducing resistance and/or tolerance to whitefly infestation and CMD infection, though exposure to low doses of gamma (15-20*Gy*) is a more promising approach than EMS treatment when using cassava stakes. Further investigation of effects of mutagenesis on viral disease and whitefly resistance as well as economically important agronomic traits under field conditions could yield cassava varieties with desired resistance, while identification of compounds, molecular markers and QTL associated with the induced resistance could provide deeper knowledge.

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