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African Journal of Plant Science

Full Length Research Paper

Spatial variation in the micro-minerals and β-carotene concentration in four varieties of plantain

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Nutrition plays a vital role in growth, development and the prevention of possible nutrient-deficiency diseases that may cause permanent health risks in the later stages of life. Due to this, the concentrations of iron, zinc, copper, manganese and β-carotene in four varieties of plantain were investigated in this study. The relationship between the micro-minerals in the fruit, the sampling location and soil were also studied. Plantain fruit samples were collected from farms in the Ashanti Region of Ghana. The samples were oven-dried and digested using the Kjeldahl method for micro-minerals analysis with atomic absorption spectrophotometer. The β-carotene content was determined using HPLC. The data obtained was subjected to analysis of variance to determine differences in the concentrations. The micro-minerals and β-carotene concentrations in the varieties of plantain did not vary significantly. Cluster analysis showed that some of the samples had almost the same nutrient concentrations though they were not of the same variety.

Key words: Anaemia, β-carotene, hidden hunger, micronutrients, nutrition, plantain, food security.

INTRODUCTION

The quest to fight malnutrition is a global concern which needs all hands to resolve. Malnutrition results from the inability to obtain the correct nutrients in the right proportion in diet. This may have a number of implications especially in developing countries where poor nutrition is predominant (Stein, 2010; Kalu and Etim, 2018; Mantadakis et al., 2020). It is important to note that inadequate intake of iron (Fe) is the prevailing nutritional deficiency in the world and the main cause of anaemia (Akhtar, 2013; Biesalski, 2013; Mantadakis et al., 2020; Mattiello et al., 2020). Likewise, zinc (Zn) deficiency in diet leads to various growth and reproduction disorders and often results in death, especially in developing countries (Oliver and Gregory, 2015; Wessels, 2021).

Copper (Cu), is another micro-mineral that maintains nerve cells, immune system and make red blood cells in the body (Scheiber et al., 2014; Greenough et al., 2016; Morrell, et al., 2017; Sigdel and Janaswamy, 2020; Espinosa and Stein, 2021). It also helps in the formation of collagen and Fe absorption in the body and also plays a role in energy production (Ware, 2017; Moustarah and Mohiuddin, 2019). Although high level of Cu can affect brain function, its deficiency can also lead to Menker's, Wilson's and Alzheimer's disease (Morrell et al., 2017). Manganese (Mn) is also another micro-mineral needed for digestion, bone growth, immune system functioning,

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cellular energy regulation, reproduction and blood clotting (Aschner and Aschner, 2005; Sigdel and Janaswamy, 2020). It is a co-actor for the activation of a number of enzymatic reactions in the body such as amino acid, lipid and carbohydrate metabolism (Yoon et al., 2011; Ge et al., 2022). Studies have shown that the element is good for pregnant women but excessive accumulation of manganese in the nervous system may cause Parkinson's type syndrome called Manganism (Aschner and Aschner, 2005).

It is established that the visual and immune systems are particularly dependent on vitamin A to function (Kennedy et al., 2003; Biesalski and Nohr, 2004; Huang et al., 2018) and a deficiency may lead to blindness in humans. Although this vitamin is freely available in animal products like milk, egg and fish, over 40% of children under age five years suffer from vitamin A deficiency (Black et al., 2013). This could be due to the fact that animal foods are expensive and therefore not affordable to the poor in developing countries. However, there is a precursor of vitamin A in plant products which may help improve the situation of vitamin A deficiency in poor countries and also create jobs for local farmers if they cultivate the plants that have precursors of vitamin A. According to Biesalski (2013), 80% of vitamin A in developing countries is from provitamin A which is found in plants. Vitamin A deficiency is considered as a major global health problem (Akhtar, 2013; Biesalski, 2013; Timoneda et al., 2018). Lack of vitamin A in a diet leads to an increased risk of eye disease and death from serious infections causing increased mortality rate among children and women in under-developed countries (Biesalski, 2013). This deficiency, if not corrected could lead to blindness (Lewallen and Courtright, 2001; Biesalski, 2013; Timoneda et al., 2018).

Plantain is among staple foods that are grown in many parts of the world (Tenkouano et al., 2019). It is an important source of nutrients in diets of people from Latin America, Africa and South-east Asia (Abiodun-Solanke and Falade, 2010). Plantain is a popular dietary staple food due to its versatility and good nutritional value. The fruit has high carbohydrate content (31 g/100 g) and low-fat content (0.4 g/100 g). They are good sources of vitamins and minerals, particularly iron (24 mg/kg), potassium (9.5 mg/ kg), calcium (715 mg/kg), vitamin A, phosphorus, zinc, sodium, and magnesium (Okareh et al., 2015). It has significant quantities of ascorbic acid, thiamin, pyridoxine, riboflavin and niacin, dietary fiber and resistant starch which helps to reduce the blood sugar level (Ayodele and Erema, 2011). The potassium in plantain plays a major role in regulating blood pressure (Fernandes and Rodrigues, 2007; Houston, 2011).

It had been established that, soil structure and physicochemical properties have great impact on plant growth and development (Wolkowski, 1990; Oldfield et al., 2019) as well as nutrient stored for consumption by consumers. The need to ensure high crop production and food security has intensified cropland management and food production which consequently results in soil degradation (Oldfield et al., 2019). The situation in Ghana is not different from the global picture where the government is embarking on "planting for Food and Jobs" to ensure food security. In so doing, "planting for Food and Jobs" uses fertilizer to enhance soil fertility. Potential contribution of building soil organic matter (SOM) as a means to increase crop production and minimize the environmental impact on agriculture has not yet been broadly quantified (Adhikari and Hartemink, 2016; Chabbi et al., 2017; Oldfield et al., 2019).

In Ghana where this study was conducted, plantain plays an important role in national food security. It also provides income to local farmers. Although farmers cultivate different varieties, they do not consider the nutritional content of the varieties cultivated. They also do not pay attention to planting location, cropland management, soil nutrient and nutrient quality of the crop consumed. Therefore, this study sought to investigate the concentrations of Fe, Zn, Cu, Mn and β-carotene in four varieties of plantain cultivated and consumed in Ghana. It also sought to investigate the relationship between trace and macro-elements in soil and plantain crop from different communities.

MATERIALS AND METHODS

Sampling of plantain

Four varieties of plantain, *Musa paradisiaca* var. "Apem", *M. paradisiaca* var. "Apantu", *M. paradisiaca* var. "Asamienu" and *M. paradisiaca* var. "Oniaba" and the soils in which they were planted were collected from different fields in Ashanti Region using purposive sampling for analysis of nutrients. These crops were sampled from Adudwan, Atonsuagya, Benim, Juaben, Kofiase, Krobo, Mampong, Nintin, New Koforidua and Obuasi in the Ashanti Region of Ghana.

Analysis of micronutrients

To analyse the micro-minerals (that is, Fe, Zn, Mn, Cu), the plantain samples were peeled, cut into pieces (ca. 1 cm × 1 cm), packaged in brown envelopes and oven dried at 60°C to remove moisture until a constant weight was obtained. The dried samples were then milled using a Kenwood blender, packaged and labeled for analysis. The samples were digested using the sulphuric acid-hydrogen peroxide method (Allen et al., 1974; Lowther, 1980). Thus, 0.10 g of oven-dried samples was placed into a 100 ml Kjeldahl flask. A volume of 4.4 ml of digestion reagent comprising of a mixture of 350 ml hydrogen peroxide, 0.42 g selenium powder, 14 g lithium sulphate and 420 ml sulphuric acid was added and heated gently at 80 to 90°C. The temperature was gradually increased to 150 to 200°C and held for 2 h until the digest was clear. The samples were then left on the plate for 30 min to cool. The digest was topped up to 50 ml with distilled water for further analysis. The filtrate from the digestion was used for Fe, Zn, Mn and Cu analysis using a 200 series Atomic Absorption Spectrophotometer (AAS) (Agilent Technologies, Santa Clara, CA). The digestion and analysis were repeated three times per sample and the mean value used for statistical analysis.

The micro-minerals in soil were extracted using diethylene triamine pentaacetic acid (DTPA) following Lindsay and Norvell (1978). An amount of soil sample (10 g) was placed in a 50 ml graduated conical centrifuge tube and 20 ml of DTPA extracting solution was added to it. The centrifuge tube and its content were shaken for 2 h on a shaker. The contents were then filtered. The extract obtained was used for estimation of different micronutrients using the AAS following (Motsara and Roy, 2008). To estimate the concentration of a specific micro-element, the element-specific hollow cathode lamp was selected and mounted on the AAS. When the flame was started, the instrument was set at zero using a blank solution. The standard solutions of different concentrations were aspirated and a standard concentration curve was plotted for the element. The samples were then aspirated and the concentration of the samples were read and recorded.

β-carotene analysis

The carotenoids in the plantain samples were extracted following Rodriguez-Amaya and Kimura (2004). Five grams (5 g) of the sample was weighed into a mortar (Fisher Scientific, Leicestershire, England) and 0.5 g of pyrogallol (Merck, Darmstadt, Germany) was added and mixed. Furthermore, 20 ml of ice-cold acetone (VWR, Leicestershire, England) was added to the mixture and left for 5 min. The extract was filtered using Whatman® filter paper (GE Healthcare Bio-Sciences, Pittsburgh, PA) after which the residue was washed twice with acetone till it became colourless. A total of 50 ml of acetone was used for the extraction. The residue was discarded and the filtrate washed in 15 ml of petroleum ether (Merck) in a 500 ml-separating funnel (Fisher Scientific). Deionized water was added slowly (by the side of the funnel to avoid emulsification of the carotenoid) to wash the acetone used for the extraction. The washing was repeated 6 to 9 times until all the acetone was washed out. The carotenoid extract dissolved in the petroleum ether was filtered through 2 g of anhydrous sodium sulphate (Merck). The volume of the filtrate was then recorded and the extract dried on nitrogen gas.

The dried sample was reconstituted with 1 ml hexane, vortexed and transferred into a 20 ml vail (Agilent) for HPLC analysis using an Agilent 1100 series HPLC system. The analyses were performed on a tracer excel 120 ODS-A column (25 cm × 0.46 cm, 5µm particle size, Teknokroma, Barcelona, Spain) at a temperature of 25°C. The mobile phase was a mixture of methanol, hexane and methyl ether amine (Merck) in a ratio of (90: 10: 0.01 v/v/v). The flow rate of the mobile phase was 1 ml/min. The total analysis time per sample was 35 min. Hexane was analysed as a blank in order to identify chromatograms coming from the samples. The chromatogram of β -carotene was identified by comparing the retention times of chromatograms from the samples with that of a beta-carotene standard (Merck).

For the purposes of quantification, calibration curve was obtained using solutions containing a carrot standard at a concentration of 0.17 µg/ml in hexane, 3 injections per concentration. The concentration of beta-carotene in each sample was calculated based on the linear relationship between the peak area of the standard and that of the sample.

Determination of macro-elements

Determination of phosphorous (P), potassium (K) and sodium (Na)

Digest from the sulphuric acid–hydrogen peroxide method was used for the determination of potassium, phosphorus and sodium. Potassium and Sodium concentrations were determined using the flame photometer whiles Phosphoros was determined using the spectrophotometer.

Phosphorus

Phosphorus was determined using the ascorbic acid method

Colour forming reagent referred to as reagents ‘A’ and ‘B’ were used for the test. Twelve grams (12 g) of ammonium molybdate in 250 ml distilled water, 0.2908 g of potassium antimony tartarate in 100 ml distilled water and l L of 2.5 M H_2SO_4 constituted Reagent A. The three solutions were mixed together in a 2 L volumetric flask and made up to volume with distilled water. Reagent B was prepared by dissolving 1.056 g of ascorbic acid to every 200 ml of reagent A. Ten milliliters (10 ml) aliquot of the samples were pipetted into 25 ml volumetric flasks. 10 ml of distilled water was pipetted into each of the working standards to give sample and standard the same background solution.

Ten milliliters (10 ml) of distilled water was added to the standards as well as the samples after which 4 ml of reagent B was added and their volumes made up to 25 ml with distilled water and mixed thoroughly. The flasks were allowed to stand for 15 min for colour development after which the absorbance of the standards and samples were determined using a spectrophotometer at a wavelength of 882 nm.

Determination sulphate (SO_4)

The turbidimetric method was used. 5 ml of the sample was measured out of 100 ml extracted sample from the volumetric flask. 10 ml of sodium acetate buffer solution was added to the sample and 1.0 g of barium chloride ($BaCl_2$) was added to the extract and diluted with distilled water to 25 ml. The absorbent was read using AAS (Shimaduze, UV-1800 Series, Japan) at a wavelength 440 nm.

Statistical analysis

The data obtained from the micronutrient and β-carotene analysis were compared using analysis of variance followed by Turkey's test where statistical differences were obtained (Minitab version 17; Minitab Inc., State College, PA). The cluster analysis using Euclidean distance was performed with a pulled data of β-carotene concentration, total carotene concentration in 100 g, wet weight of the plantain samples; dry weight of the plantain samples, percentage moisture content (%), ash content and the concentrations of phosphorus, potassium, sodium, iron, copper and zinc in the samples.

RESULTS AND DISCUSSION

Concentration of micronutrient in plantain

Micronutrient deficiency is a major health challenge to humans due to the damage associated with it in the later ages of individuals. This is so because Fe is needed for blood formation while Zn serves as a co-enzyme for a number of biochemical reactions in the body. β-carotene is also said to be one of the provitamin A percussors obtained from plants. The consumption of β-carotene helps to improve eye sight. A deficiency in β-carotene may lead to eye disease or blindness (Lewallen and Courtright, 2001; Biesalski, 2013; Dewett et al., 2021).

The concentration of each of the micro-minerals, Fe, Zn, Mn, Cu studied was not significantly different in all

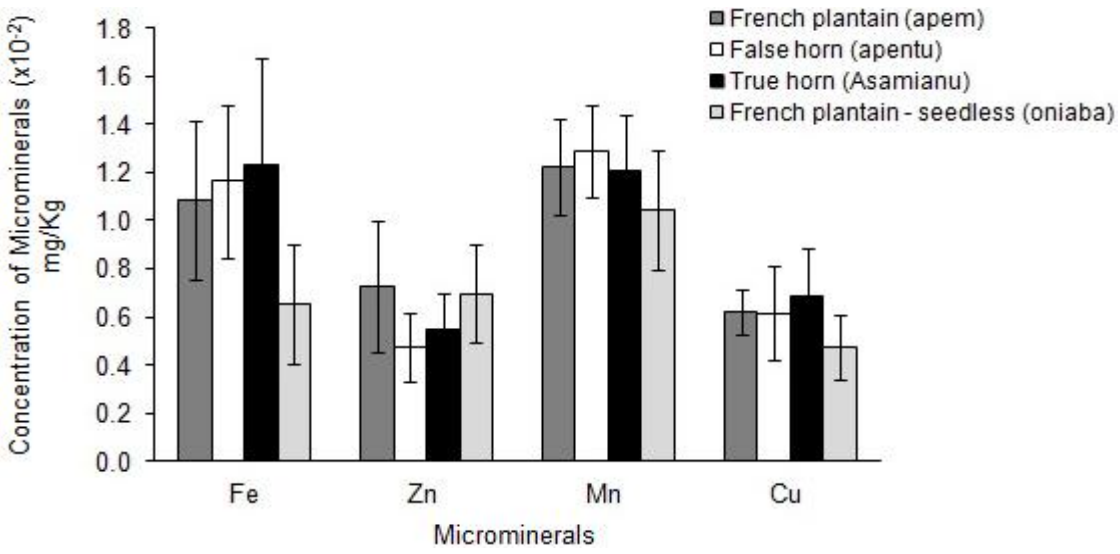

Figure 1. Mean ± concentration in mg/Kg of Fe, Zn, Mn and Cu in different varieties of Plantain. No significant differences were observed at P ≤ 0.05 (ANOVA, Minitab).
Source: Author

four varieties of plantain (Fe: F = 0.95, d.f. = 3, P = 0.428; Zn: F = 0.15, d.f. = 3, P = 0.927; Mn: F = 0.35, d.f. = 3, P = 0.789; Cu: F = 0.28, d.f. = 3, P = 0.841; Figure 1).

Plantains do well in a wide geographical area such as the tropics and serve as a staple food for some developing and tropical countries in the world (Englberger et al., 2006; Adepoju et al., 2012). The results from the analyses of the micronutrient concentrations of four varieties showed that French plantain, false horn, and true horn had higher concentrations of Fe than the French plantain-seedless although not statistically significant. This implies that the variety does not have any effect on the micronutrients obtained by the individual and that all four varieties can be used to manage iron deficiency. Findings from this study support the report by Okareh et al. (2015) that plantain is rich in nutrients such as Fe. The Mn concentrations in the four varieties were also high. The findings of the study were similar to the results obtained by Odenigbo et al. (2013) that reported that plantains are a rich source of Fe and vitamin A. Iron and Mn are micronutrients that help improve blood hemoglobin level for proper metabolic functioning of the body and enhance growth. This implies that the addition of plantain in the diet of pregnant women and nursing mother helps to reduce the potential risk of micronutrient deficiency. This explains why in Ghana the Ashantis give plantain-rich foods such as ‘fufu’, ‘ampesi’ and mashed plantain popularly called ‘etɔ’ in the twi language to pregnant women and breastfeeding mothers.

Analysis of beta-carotene content in plantain

The concentrations of beta-carotene in all four varieties of plantain were not significantly different (F = 1.17, d.f. = 3, P = 0.33; Figure 2).

β-carotene is one of the antioxidants obtained from plant food that helps in the prevention and management of eye problems. Plantain could contribute to the nutritional quality of the people in Ghana and help manage micro nutrient deficiency problems considering the micronutrients in the fruit. This is because carotenoids in the plant help in the production of provitamin A which is needed for biochemical processes in humans. Provitamin A carotenoids, most importantly beta-carotene, followed by alpha-carotene, are those which are converted into vitamin A in the body and help protect humans against infection, night blindness and eye disease (Newilah et al., 2009; Biesalski, 2013; Huang, 2018). The addition of these carotenoids in diet will help reduce the risk of degenerative diseases such as cancer, cardiovascular disease, cataracts and muscular degeneration (Silalahi, 2002; Bhatt and Patel, 2020; Chaudhary et al., 2020).

There was higher concentration of β-carotene in “Apem” (French plantain), “Apantu” (False orn), and “Oniaba” (seedless French plantain) varieties of *Musa paradisiaca* than “Asamianu” (True horn) although the variations in concentrations were not significantly different. This implies that the variety of plantain eaten by an individual does not affect the amount of vitamin A obtained from the food.

Cluster analysis

The results of the cluster analysis showed four major clusters at 66.67% similarity in nutrient concentration.

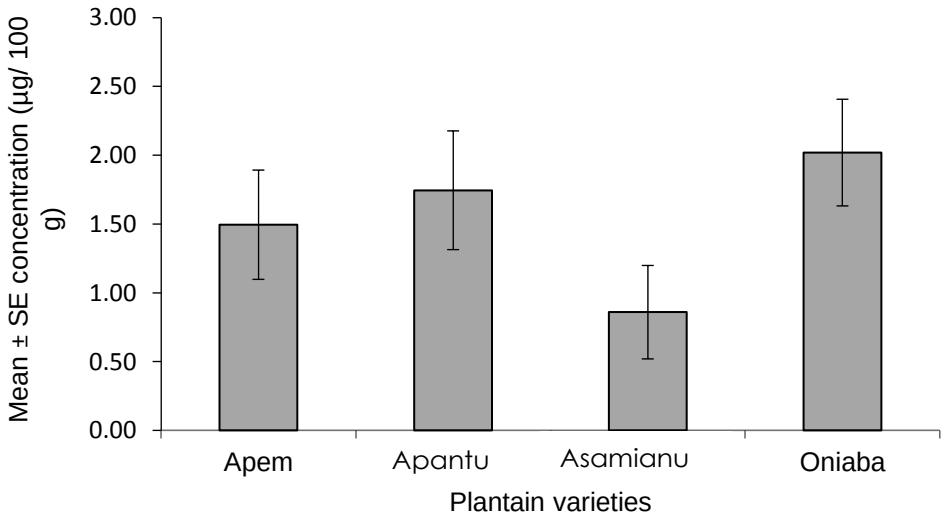

Figure 2. Mean ± concentration in µg/100g of β-carotene in the Apem (French plantain), Apantu (False horn), Asamianu (True horn) and Oniaba (seedless French plantain) varieties of *Musa paradisiaca*. No statistical differences were observed at a P-value ≤ 0.05 (ANOVA, Minitab).
Source: Author

The clusters with the four major groups from the dendrogram had samples from the four different varieties of plantain fruits used for the study. Samples AM 3, ON 2, and EM 17 clustered at 99% similarity while AAU 1, EM8, EM 10 and EM3 clustered with a similarity of 99.51%. In another cluster, AU 11, EM 5 and ON 3 clustered at a similarity of 99.81 closeness in nutrient concentration. On the other hand, sample ON 11 did not cluster with any other sample from the same or different variety after the 61% similarity (Figure 3).

Cluster analysis showed almost perfect similarity between AU11, EM 5 and ON3. This implies that these samples from the different varieties picked from different locations contained almost the same nutrient. It can also be deduced from the results that AG-AM-1 and AG-ON-1 which were different varieties had similar nutrient profile. AG-AM-1 clustered in group 2 and AG-ON-1 clustered in group 4 from the dendrogram produced.

The significant differences observed in the concentrations of Fe, Cu, Zn, P, Na and S in the crop samples from the different locations where the plantain crops were sampled shows that the place of planting has an influence on the concentration of nutrient obtained by the plant. The results support the report of the soil structure. Moreover, physicochemical characters determine the soil nutrients made available to plant (Wolkowski, 1990; Oldfield et al., 2019). There was also a significant difference between the soil pH and electrical conductivity. This eventually influences the nutrient quality of the crop produced by plants.

The results in Table 1 show the concentrations of micro-elements (Fe, Cu and Zn) in plantain crop sampled from different locations in Ashanti Region. There were significant differences in the concentrations of Fe, Cu and Zn in plantain crops from the different locations (Table 1).

Table 2 show the mean concentration of macro elements (N, P. Na and S) in the plantain crops sampled from the different communities. There were significant differences in the concentrations of N, P, Na and S in the plantain crop from the different locations (Table 2).

The results of correlation of primary minerals in the soil and the plantain crops show significant negative correlation between potassium concentrations in the crop against nitrate, pH in the soil and electrical conductivity in the soil (Table 3). There was negative correlation between total nitrogen in the crop against nitrate, pH in the soil and electrical conductivity. pH and electrical conductivity in the soil against P in the crop also showed negative correlation. There was statistically significant correlation between total nitrogen in the crop and K concentration in the crop and pH in the soil and electrical conductivity. There was highly significant correlation between S in the soil and S in the crop (Table 4). The same was observed in pH in the soil and electrical conductivity. Sulphur in the soil showed insignificant negative correlation with sodium in the crop. Sodium in the crop also showed insignificant negative correlation with pH in the soil and electrical conductivity.

Table 5 shows the Pearson's correlation of the micro-elements in the crop and soil. Iron in the soil showed negative non-significant correlation with iron in the crop, copper in both the soil and the crop, zinc in both the soil and the crop and pH. Though iron in the soil showed negative correlation with electrical conductivity in the soil, it was highly significant. Copper in the crop also showed negative non-significant correlation with Zn and pH in the

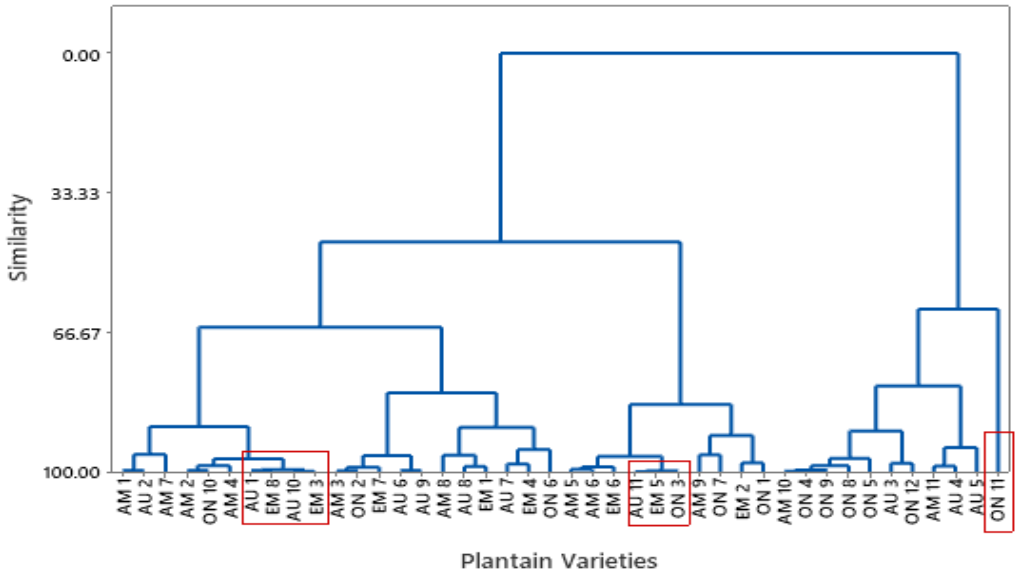

Figure 3. Single-joining tree of individual plants of plantain using Euclidean distances from nutrient concentration.
Source: Author

Table 1. Mean (± standard error) concentrations of micro-minerals in plantain crops harvested from different locations.

Location	Fe	Cu	Zn
Adudwan	1.05±0.06^{e}	1.48±0.33^{b,c}	0.18±0.02^{b}
Atonsuagya	1.56±0.06^{c,d}	0.36±0.07^{d}	0.1717±0.02^{b}
Benim	2.00±0.19^{b}	0.61±0.09^{c,d}	0.26±0.07^{b}
Juaben	1.885±0.140^{b,c}	0.2992±0.036^{d}	0.20±0.02^{b}
Kofiase	1.77±0.05^{b,c,d}	0.38±0.06^{d}	0.14±0.01^{b}
Krobo	3.12±0.24^{a}	2.58±0.44^{a}	0.46±0.06^{a}
Mampong	2.93±0.20^{a}	1.9±0.27^{a,b}	0.53±0.06^{a}
Nintin	1.44±0.21^{d}	1.13±0.08^{b,c,d}	0.42±0.10^{a}
New Koforidua	0.99±0.07^{e}	1.62±0.55^{a,b,c}	0.17±0.01^{b}
Obuasi	0.94±0.04^{e}	1.22±0.11^{b,c,d}	0.133±0.01^{b}

Means with different letters attached in columns indicate statistical difference at P< 0.05.
Source: Author

Table 2. Mean (± standard error) concentrations of macronutrients in plantain crops from different locations.

Location	N	P	K	Na	S
Adudwan	0.29±0.01^{e}	0.05±0.01^{c,d}	22.34±0.65^{b,c,d}	10.21±0.29^{a,b,c}	3.32±0.11^{a}
Atonsuagya	0.28±0.04^{d,e}	0.05±0.01^{c,d}	21.20±0.34^{c,d}	9.86±0.19^{a,b,c}	2.93±0.35^{a}
Benim	0.53±0.02^{a,b,c}	0.02±0.002^{e}	21.93±0.16^{b,c,d}	9.86±0.29^{a,b,c}	3.61±0.14^{a}
Juaben	0.53±0.03^{a,b}	0.06±0.01^{b,c,d}	24.20±1.34^{a,b}	10.86±0.60^{a}	1.76±0.61^{b}
Kofiase	0.42±0.02^{c}	0.07±0.01^{a,b}	20.43±0.57^{d}	9.43±0.26^{c}	0.36±0.06^{c}
Krobo	0.41±0.01^{b,c,d,e}	0.07±0.003^{a,b}	22.47±0.28^{a,b,c,d}	9.57±7.94^{a,b,c}	0.31±0.07^{c}
Mampong	0.40±0.01^{c}	0.07±0.002^{a,b,c}	22.47±0.29^{b,c,d}	9.57±5.36^{b,c}	0.13±0.02^{c}
Nintin	0.45±0.02^{b,c}	0.06±0.002^{b,c,d}	23.37±0.61^{a,b,c}	10.28±0.39^{a,b,c}	0.32±0.06^{c}
New Koforidua	0.48±0.02^{b,c}	0.05±0.003^{d}	22.05±0.34^{b,c,d}	9.80±0.16^{b,c}	0.33±0.03^{c}
Obuasi	0.64±0.03^{a}	0.08±0.01^{a}	25.13±1.40^{a}	10.57±0.68^{a,b}	0.30±0.02^{c}

Means with different letters attached in columns indicate statistical difference at P< 0.05.
Source: Author

Table 3. Correlations of primary minerals in plantain crop and their accumulation in the soil.

Minerals	Concentration						pH (of soil)
	Total N (in crop)	P (in soil)	P (in crop)	K (in soil)	K (in crop)	NO_3 (in soil)	
P (in soil)	0.047						
P (in crop)	0.131	0.269					
K (in soil)	0.120	0.343	0.321				
K (in crop)	0.661*	0.002	0.384	0.179			
NO_3 (in soil)	-0.152	0.235	0.016	0.224	-0.061		
pH (of soil)	-0.250	0.423	-0.476	0.165	-0.312	0.551	
E.C (ms) soil	-0.159	0.550	-0.084	0.196	-0.259	0.300	0.810*

Source: Author

Table 4. Correlations of secondary minerals in plantain crop and their accumulation in the soil.

Minerals	Concentration				pH (of soil)
	S (in soil)	S (in crop)	Na (in crop)	Na (in soil)	
S (in crop)	0.763*				
Na (in crop)	-0.020	0.175			
Na (in soil)	0.110	0.284	0.058		
pH (in soil)	0.303	0.287	-0.162	0.243	
E.C. (ms) soil	0.295	0.050	-0.235	0.395	0.810*

Source: Author

Table 5. Correlations of micronutrients in the soil and their accumulation in plantain crop.

Minerals	Concentration						pH (of soil)
	Fe (in soil)	Fe (in crop)	Cu (in soil)	Cu (in crop)	Zn (in soil)	Zn (in crop)	
Fe (in crop)	-0.591						
Cu (in soil)	-0.515	0.185					
Cu (in crop)	0.073	0.406	-0.190				
Zn (in soil)	-0.179	0.276	0.335	-0.249			
Zn (in crop)	-0.286	0.772*	0.210	0.617	0.194		
pH (in soil)	-0.627	0.372	0.678*	-0.018	0.356	0.373	
E.C. (ms) soil	-0.645*	0.502	0.548	0.175	0.457	0.266	0.810*

Source: Author

soil. There was positive significant correlation between copper in the soil and pH and electrical conductivity and pH.

Conclusion

All the varieties of plantain studied were rich in Fe, Zn and β-carotene. It is important for people to diversify their diets to include plantain dishes to help improve nutrition and minimize future health challenges which could occur due to micronutrient deficiency. The variety consumed does not have significant effect on the nutrient obtained by consumers. The planting location and quality soil has significant effect on the quality of nutrient obtained by the crop. It is therefore important to consider the planting location and soil quality to ensure quality nutrition and food security.

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CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Evaluation of maize germplasm for resistance to maize chlorotic mottle virus and sugarcane mosaic virus: The casual agents of maize lethal necrosis disease

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In Kenya, at national policy level to the individual household level, food security is synonymous to maize productivity and availability. However, the productivity of maize is affected majorly by maize lethal necrosis disease (MLND) that was first reported in Kenya in 2011. MLND results from co-infection between maize chlorotic mottle virus (MCMV) and any cereal-infecting viruses in Potyviridae family particularly sugarcane mosaic virus (SCMV). Majority of maize germplasm are susceptible to MLND. This study was therefore carried out to identify potential germplasm for breeding for MLND resistance. A total of 38 maize germplasm (5 temperate lines with inherent resistance to maize-infecting viral diseases, 32 assorted tropical lines and one Kenyan hybrid) were artificially inoculated with MCMV and SCMV in the green house at the University of Nairobi Field Station and screened for two seasons between April 2020 and October 2021. Based on the Area Under Disease Progress Curve (AUDPC) and final severity score, germplasm KS23-6, 18, KS23-5 and 19 were identified as the most promising sources of MCMV resistance with disease severity scores of 2, 2.3, 2.3 and 3, respectively while germplasm 50, 19, and 22 were identified as source of SCMV resistance with scores of 2.0, 2.3 and 3, respectively. These germplasms could serve as potential donors for introgression of the resistance genes into locally adapted maize background to combat yield losses due to MLND.

Key words: Maize lethal necrosis disease, sugarcane mosaic virus, maize chlorotic mottle virus, resistance, maize germplasm.

INTRODUCTION

Maize (*Zea mays*) contributes significantly to food security in Kenya; with 90% of the country population depending on maize as the main staple food and source of income (Eunice et al., 2021). Per capita consumption of maize in Kenya is between 98 to 100 kg (Onono et al., 2013). Maize occupies 2.1 million ha which is 40% of the total crop area and the annual yield was 3.39 million tons in 2016 (Mwatuni et al., 2020) and 3.8 million tons in 2021

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(FAOSTAT, 2022). Maize production suffers from abiotic and biotic stress. Abiotic stresses include low rainfall and infertile soils (Simtowe et al., 2020) while biotic stress include diseases and insect pest such as aphids, thrips and fall armyworm (De Groote et al., 2020). Diseases such as Gray leaf spot, Common smut, Northern leaf blight, Maize streak virus and Head smut are endemic in major maize growing regions (Charles et al., 2019). Emergence of maize lethal necrosis disease (MLND) in 2011 saw the most devastating effect on maize production in Kenya (Wangai et al., 2011; Marenya et al., 2018; Jafari et al., 2020; Redinbaugh and Stewart, 2018; Wamaitha et al., 2018). MLND is commonly caused by synergistic interaction between maize chlorotic mottle virus (MCMV) and sugarcane mosaic virus (SCMV) (Adams et al., 2014; Mwatuni et al., 2020). In addition, other potyviruses such as maize dwarf mosaic virus (MDMV), wheat streak mosaic virus (WSMV) (Mahuku et al., 2015; Masanga et al., 2020), and Johnson grass mosaic virus (JGMV) (Stewart et al., 2017) can associate with MCMV to induce MLND. Symptoms associated with MLND and its causative viruses include chlorotic specks on young leaves, leave necrosis, shortening of internodes, premature dying of the husks and few grains filling at maturity stage (Mahuku et al., 2015).

MCMV is the only member in genus *Machlomovirus* of the family Tombusviridae (Zhang et al., 2011). It has been reported in Peru (Nault et al., 1978), USA, Argentina, Brazil (Braidwood et al., 2018), and China (Wang et al., 2017). In Kenya, MCMV was first reported in Bomet (Wangai et al., 2012) and later in all maize growing regions of the country. Presence of MCMV in the region resulted in the outbreak of devastating MLND leading to almost 100% yield losses of maize (Lukanda et al., 2014; Adams et al., 2012). MCMV is transmitted by onion thrips (*Thrips tabaci*), maize thrips (*Frankliniella williamsi*) (Mwando et al., 2018), and at least six beetle species (Isabirye and Rwomushana, 2016) and through seed but at very low rate (Kimani et al., 2021; Jensen, 1991).

SCMV is more prevalent worldwide (Masanga et al., 2020) and was first reported in USA in 1963 (Janson and Ellet, 1963). A study by Louie (1980) confirmed presence of SCMV in 20 of 33 districts surveyed in Kenya. It belongs to *Potyvirus* genus of Potyviridae family (Redinaugh and Stewart, 2018). SCMV is transmitted by aphids (Redinaugh and Stewart, 2018).

Management strategies for MLND include crop nutrition, weed control (Fatma et al., 2016), crop rotation (Frank et al., 2016), and use of certified seeds (Mwatuni et al., 2020). However, it is difficult to manage MLND using these strategies due to nature of its spread (Mudde et al., 2018). Breeding for resistance is the most effective and sustainable method to manage MLND (Beyeni et al., 2017; Awata et al., 2021). This study focused on identifying germplasm that are resistant to maize lethal necrosis disease causative viruses (MCMV and SCMV) that can be used as donor in breeding programs.

MATERIALS AND METHODS

Plant, experimental site and layout

A total of 38 maize germplasm (5 temperate lines with inherent resistance to maize-infecting viral diseases 32 assorted tropical lines and 1 Kenyan hybrid) were evaluated (Table 1). The experiment was conducted in a net house at the University of Nairobi, College of Agriculture and Veterinary Sciences' field station. The station is situated in Kabete, which lies at a longitude of 36° 44" East and latitude of 1° 15" South and about 1940 m above the sea level. The area experiences a bimodal rainfall averaging 1000 mm of rainfall per annum. The site's daily maximum temperature ranges between 13 and 27°C (Wasonga et al., 2015). Maize germplasms were screened for their responses to MCMV and SCMV for two seasons in 2020 and 2021. Completely randomized design (CRD) was used to set up the experiments with three replications. Three maize seeds per pot were planted in black polythene pots measuring 30 cm diameter and 30 cm height. Di-ammonium Phosphate (DAP) was applied at planting 5 g per pot. Watering was done four times a week.

Preparation of the virus inoculum and leaf inoculation

At three leave stage, maize seedlings were singly inoculated with MCMV and SCMV, respectively as described by Karanja et al. (2018) and Sitta et al. (2017). The inoculum was prepared from maize leaves showing classical MCMV and SCMV symptoms derived from virus collection at Kenya Agricultural and Livestock Research Organization (KALRO), Biotechnology center. Inoculation solution (0.1 M phosphate buffer) was constituted by dissolving 10.8 g of potassium phosphate monobasic, 4.8 g potassium phosphate dibasic, 1.26 g Na_2SO_3 and 1 g of Carborandum in 1 L of sterile distilled water (Sitta et al., 2017). Reagents were from SIGMA® Life Science. For each of the virus isolate, 200 g of infected leaves were obtained, homogenized and dissolved in 1 L of the inoculum buffer. The inoculum was applied on the leaves by hand rubbing. A second inoculation was done one week later (Tembo et al., 2021).

Data collection/rating

Data was collected on disease severity as described by International Maize and Wheat Improvement Center (CIMMYT). Disease severity was based on visual subjective five-point scale of 1-5, where 5 represent very severe symptoms, 4 severe symptoms, 3 moderate symptoms, 2 mild symptoms and 1 no symptoms (Figure 1) (Karanja et al., 2018; Sitta et al., 2017). Data was collected for six weeks after the first inoculation.

Data analysis

Analysis of variance was done to determine variability between germplasm and between different weeks using GenStat 15th edition. The scores obtained on disease severity from the screen house over the 6 weeks were converted into AUDPC values using the

Table 1. Maize germplasm used during the study.

No.	Germplasm	Description	No.	Germplasm	Description
1	KS23-6	Temperate line	20	39	Assorted tropical line
2	OHVRS-C1	Temperate line	21	5	Assorted tropical line
3	ks23-5	Temperate line	22	52	Assorted tropical line
4	OH28	Temperate line	23	3	Assorted tropical line
5	OH7B	Temperate line	24	35	Assorted tropical line
6	19	Assorted tropical line	25	CO80	Assorted tropical line
7	22	Assorted tropical line	26	50	Assorted tropical line
8	7	Assorted tropical line	27	60	Assorted tropical line
9	24	Assorted tropical line	28	DUMA	Kenyan hybrid
10	19	Assorted tropical line	29	17	Assorted tropical line
11	34	Assorted tropical line	30	30	Assorted tropical line
12	CO79	Assorted tropical line	31	114	Assorted tropical line
13	25	Assorted tropical line	32	119	Assorted tropical line
14	36	Assorted tropical line	33	18	Assorted tropical line
15	32	Assorted tropical line	34	112	Assorted tropical line
16	8	Assorted tropical line	35	12	Assorted tropical line
17	51	Assorted tropical line	36	9	Assorted tropical line
18	122	Assorted tropical line	37	14	Assorted tropical line
19	58	Assorted tropical line	38	16	Assorted tropical line

Sources: Author

Figure 1. Disease severity scale.
Source: Karanja et al. (2018).

formula:

$$AUDPC = \sum_{i=1} n-1[(t_{i+1} - t_i)(y_i + y_{i+1}) / 2]$$

where ‘‘t’’-time in days for each reading, ‘‘y’’-Disease score using the disease score (1-5), and ‘‘n’’-number of readings.

RESULTS

Response of maize germplasm to infection with MCMV

All the inoculated germplasm developed symptoms but disease severity differed significantly at $P < 0.01$ (Figure 2 and Table 2). Susceptible germplasm showed disease symptoms one week after the first inoculation. Leaf symptoms began as chlorotic strips running parallel to the veins that later joined to produce elongated chlorotic blotches (Figure 2B and C).

A t-test ($p<0.05$) confirmed there was no significant difference between season one and two for resistant germplasm and susceptible germplasm hence average of season one and season two was done for the resistance germplasm and susceptible germplasm. MCMV final severity scores of the 38 germplasm ranged from 2 to 4.3, the AUDPC ranged from 52.8 to 122.8 (Table 2). Five germplasm had a severity score of below 3. They

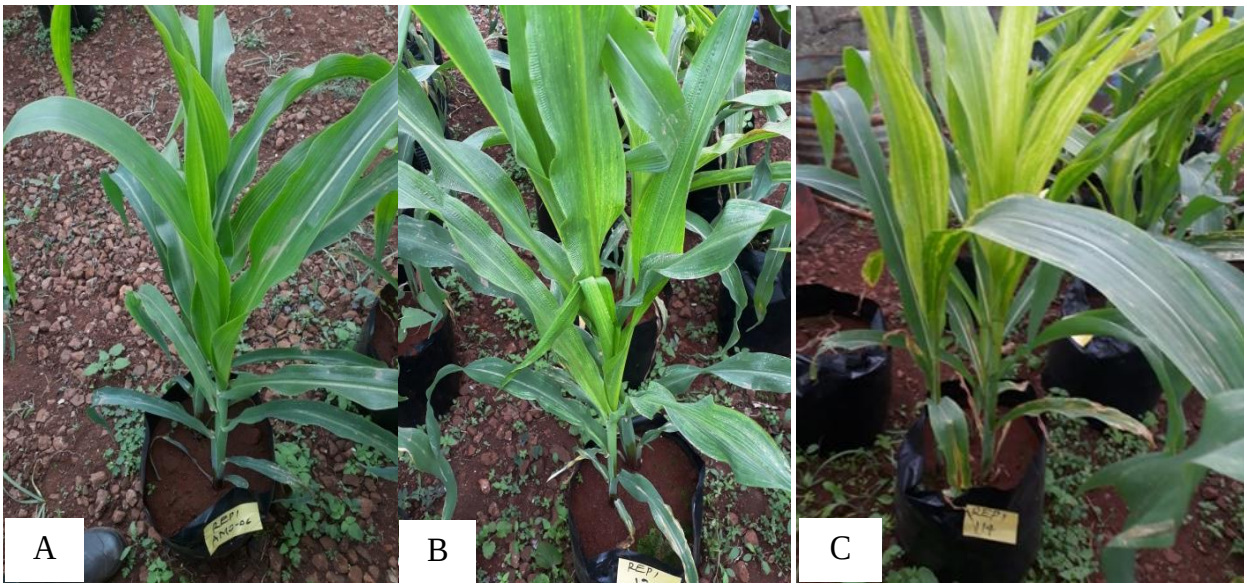

Figure 2. Variable *MCMV* disease severity observed during the trial. (A) Ks23-6 with low severity score of 1.5, (B) 19 with a medium severity of 3 and (C) 114 high severity score of 4.5.
Sources: Author

include germplasm Ks23-6 with a score of 2, germplasm 18, 9, 60, and Ks23-5 with a final score of 2.3 while 19 had a score of 3. Germplasm OH 28, 58, 14,122 and 58 had the highest scores of 4 or above (Table 2 and Figure 3). Germplasm 18 had the lowest AUDPC of 52.8 followed by Ks23-6 with 58.5, while 58 had the highest AUDPC of 122.8 (Table 2).

Response of maize germplasm to infection with SCMV

Infection was observed in all the plants inoculated with SCMV. Germplasm differed significantly at ($p< 0.001$) for resistance to SCMV.

Disease severity also differed over time at ($P<0.01$). The susceptible germplasm showed symptoms one week from the first inoculation. Final SCMV score ranged from 2.0 to 5 (Figure 4). Only two germplasm had a score of <2.5, that is, germplasm 50 with a score of 2.0 and germplasm 19 with 2.3. The germplasm 7, 22, and 48 had scores of 3 while germplasm 58 had the highest final score of 5.

Germplasm 19 and 7 showed low scores to both viruses. Germplasm 19 had a final severity score of 2.3 and AUDPC of 74.7 for SCMV trail and final severity score of 3.0 and AUDPC of 79.9 for MCMV trail while germplasm 7 had a final severity score of 3.0 and AUDPC of 79.9 for SCMV trail and final severity score of 2.7 and AUDPC of 66.7 for MCMV trail (Table 2, Figure 5).

DISCUSSION

Maize lethal necrosis disease is as a result of combined effect of MCMV and SCMV leading to yield losses of up to 100% (Gowda et al., 2015; Xia et al., 2016). Exposing plants to disease has been used to test and select germplasm for the presence of genes for resistance (Gowda et al., 2015). Previous work has reported that most elite inbred lines and commercial hybrids are susceptible to MCMV and MLND (Sitonik et al., 2019). This study partly agree with those previous report because among the studied germplasm, there was none that was immune to infection with either SCMV or MCMV. However there were significant differences in severity among different germplasm (Table 2). Earlier reports of work by Sitta et al. (2018), Karanja et al. (2018); Tembo et al. (2021) and Awata et al. (2021) where different germplasm were screened for MCMV, SCMV and MLND also reported development of symptoms on all screened germplasm but with different disease severity.

This study involved screening of 38 maize germplasm that are genetically diverse. Final severity/infection and AUDPC values were used as indicators of response of test germplasm to SCMV and MCMV (Tembo et al., 2021). There was significant difference between germplasm and between different scoring time/weeks at

Table 2. Weekly disease severity scores and the AUDPC of the germplasm studied.

No.	Genotype	SCMV Weekly Severity						AUDPC	No.	Genotype	MCMV Weekly Severity						AUDPC
		1	2	3	4	5	6				1	2	3	4	5	6	
1	50	1.0	1.0	1.0	1.0	1.5	2.0	42.0	1	KS23-6	1.2	1.3	1.5	1.8	1.8	2.0	58.5
2	19	1.0	1.3	2.5	2.8	2.3	2.3	74.7	2	18	1.2	1.2	1.2	1.6	1.9	2.3	52.8
3	7	1.2	1.7	2.3	2.5	2.8	3.0	79.9	3	ks23-5	1.1	1.1	1.7	2.1	2.2	2.3	61.1
4	22	1.0	1.2	1.3	1.7	2.5	3.0	60.7	4	9	1.3	1.3	1.5	1.5	1.8	2.3	59.8
5	48	1.0	1.2	1.5	2.7	3.0	3.0	72.3	5	60	1.5	1.5	1.7	1.7	1.7	2.3	59.8
6	34	1.0	1.2	2.2	2.3	2.8	3.0	73.5	6	19	1.2	1.5	2.2	2.7	3.0	3.0	60.3
7	24	1.0	1.2	2.7	3.0	3.2	3.0	84.0	7	22	1.3	1.5	1.5	1.8	2.2	3.5	65.9
8	25	1.0	1.5	2.8	3.0	3.2	3.2	88.1	8	7	1.7	1.5	1.7	1.8	2.3	2.7	66.5
9	5	1.0	1.3	2.5	2.5	3.3	3.3	81.4	9	24	1.3	1.5	1.5	1.8	2.3	3.3	66.5
10	15	1.0	1.2	2.0	2.5	3.0	3.3	75.8	10	19	1.5	1.5	1.7	1.8	2.3	3.0	67.1
11	16	1.0	1.5	2.5	2.8	3.0	3.3	84.0	11	34	1.5	1.5	1.5	2.0	2.5	2.8	67.7
12	OHVRS-C1	1.0	1.3	2.2	2.3	2.7	3.3	74.7	12	CO79	1.5	1.3	1.7	2.2	2.5	3.0	69.4
13	C080	1.3	1.7	2.5	3.0	3.5	3.3	91.0	13	25	1.5	1.7	1.7	2.2	2.5	3.0	71.8
14	3	1.0	1.3	2.7	2.7	3.2	3.5	84.6	14	36	1.5	1.5	1.5	2.2	2.8	3.2	72.3
15	36	1.2	1.7	2.8	3.2	3.3	3.5	93.3	15	32	1.3	1.3	1.5	2.0	3.3	3.5	72.6
16	60	1.0	1.3	2.0	2.5	2.8	3.5	76.4	16	8	1.3	1.5	1.5	2.2	3.0	3.3	73.5
17	119	1.0	1.5	2.5	2.8	3.3	3.5	86.9	17	51	1.3	1.5	1.8	2.2	2.7	3.5	74.1
18	KS23-6	1.0	1.0	1.8	2.2	2.7	3.5	69.4	18	39	1.5	1.5	1.7	2.2	3.0	3.2	74.7
19	OH7B	1.0	1.3	1.3	1.8	2.0	3.5	61.3	19	5	1.5	1.5	1.7	2.0	3.2	3.5	75.8
20	DUMA	1.0	1.2	2.7	3.2	3.5	3.5	89.3	20	52	1.3	1.5	1.5	2.0	3.5	3.5	76.4
21	18	1.0	1.5	2.5	2.8	3.3	3.7	87.5	21	3	1.5	1.3	1.5	2.2	3.5	3.7	77.6
22	30	1.0	1.2	1.8	2.0	3.2	3.7	73.5	22	35	1.2	1.3	1.3	2.5	3.5	3.7	77.6
23	35	1.0	1.5	2.3	3.2	3.2	3.7	87.5	23	CO80	1.5	1.5	1.5	2.5	3.2	3.3	77.6
24	39	1.0	1.7	2.5	2.8	3.2	3.7	87.5	24	50	1.3	1.3	1.8	2.7	3.7	3.7	84.0
25	9	1.0	1.2	1.5	2.8	3.7	3.8	81.1	25	OH7B	1.2	1.3	1.7	2.7	3.8	3.8	84.0
26	51	1.0	1.3	2.7	3.3	3.3	3.8	91.6	26	DUMA	1.2	1.2	1.7	3.5	3.5	3.5	85.4
27	CO79	1.0	1.3	3.0	3.3	3.5	3.8	95.1	27	17	1.0	1.5	1.5	1.5	3.0	3.0	66.5
28	8	1.0	1.3	2.8	3.5	4.0	4.0	99.2	28	30	1.2	1.3	1.8	3.2	3.5	4.0	86.9
29	12	1.0	1.5	2.3	3.3	3.5	4.0	91.0	29	114	1.3	1.5	2.0	2.5	3.8	3.8	86.9
30	14	1.0	1.8	3.0	3.8	3.5	4.0	101.5	30	119	1.5	1.5	1.5	3.2	3.7	3.8	87.5
31	52	1.0	1.3	2.2	3.0	3.7	4.0	88.7	31	OHVRS-C1	1.2	1.3	2.0	3.0	3.7	4.0	88.1
32	112	1.3	2.3	3.5	4.0	4.0	4.0	115.5	32	112	1.3	1.7	1.8	3.3	3.3	3.7	88.7

Table 2. Contd.

33	114	1.0	1.8	2.3	3.2	3.7	4.0	94.5	33	12	1.5	1.5	1.8	3.0	3.8	4.0	89.3
34	AMO-4	1.0	1.3	2.5	3.2	4.0	4.0	94.5	34	OH28	1.5	2.2	3.7	4.0	3.8	4.0	114.9
35	OH28/AMO-5	1.2	2.7	3.7	4.0	4.0	4.0	118.4	35	14	1.3	1.7	3.2	4.0	4.0	4.0	108.2
36	40	1.0	1.2	2.2	3.0	3.5	4.2	86.9	36	16	1.5	1.7	1.8	3.7	4.0	4.0	96.0
37	122	1.2	1.8	3.7	4.2	4.2	4.2	115.5	37	122	1.5	2.3	3.5	4.0	3.8	4.0	114.9
38	58	1.0	2.2	3.8	4.0	4.0	5.0	119.0	38	58	1.6	2.8	3.7	4.0	4.2	4.3	122.8

Sources: Author

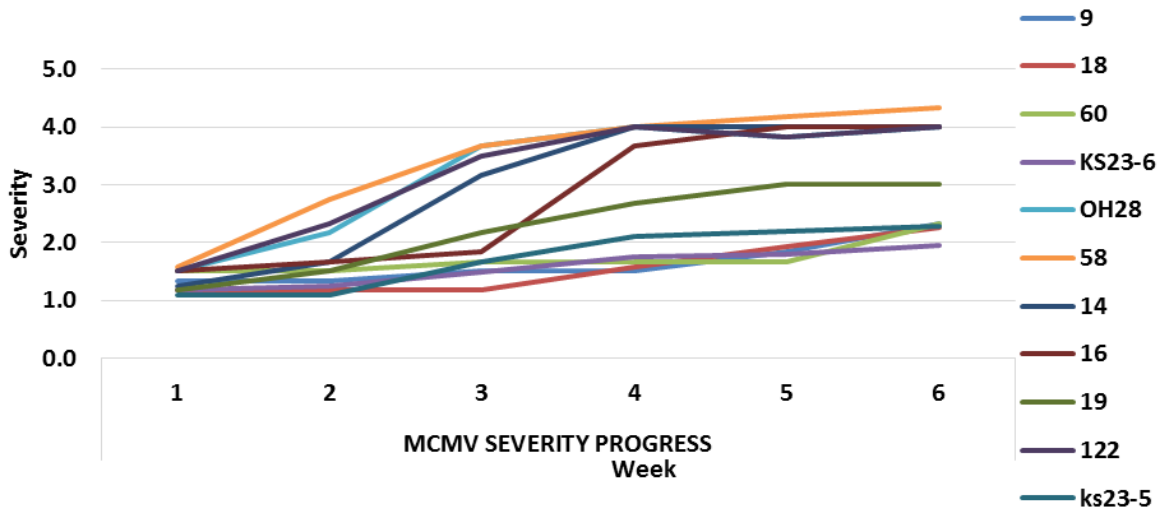

Figure 3. MCMV disease progress for the susceptible germplasm at the top and tolerant germplasm at the bottom derived from plotting disease severity scores over time (6 weeks).
Sources: Author

P<0.01; hence, the need for scoring at different time interval due to virus dynamics with time. High severity scores were recorded among the susceptible germplasm as the weeks progressed leading to high AUDPC. According to Karanja et al. (2020) and Sitta et al. (2017), germplasm can be classified as susceptible with a score 4 or above, tolerant with a score of 3 and resistance with a score of 2.

More than 80% of the studied germplasm were susceptible to SCMV and MCMV with scores of > 3.0, this puts emphasis on risk posed by MLND on

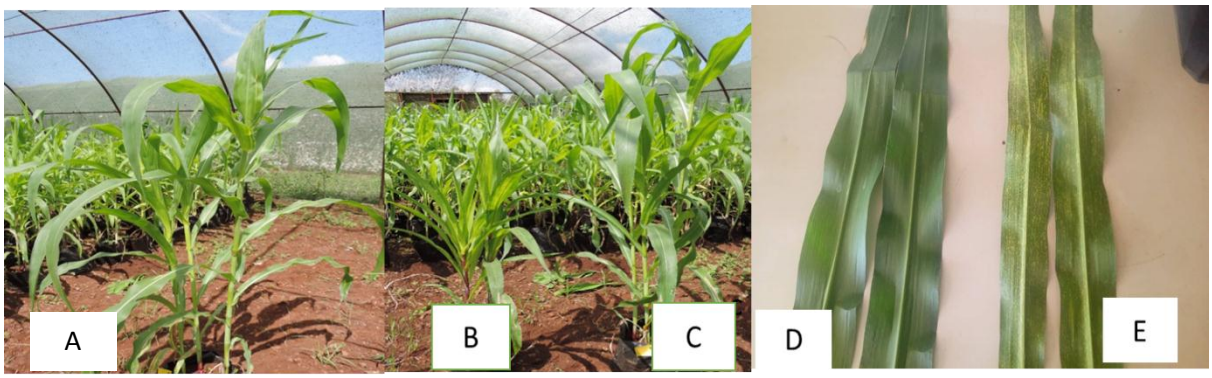

Figure 4. Variable *SCMV* disease severity observed during the trial. (A, C) Germplasm 50 with low severity score, (B) 58 high severity score, (D) leaves of germplasm 50 on the 6th week, (E) leaves of germplasm 58 at 6th week.
Sources: Author

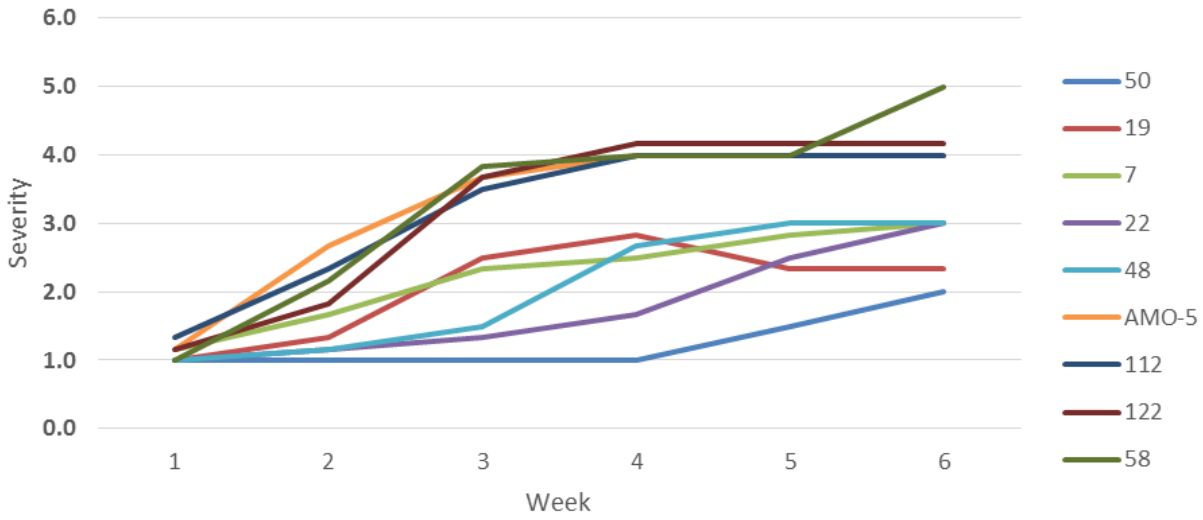

Figure 5. *SCMV* disease progress for the susceptible germplasm at the top and tolerant germplasm at the bottom derived from plotting disease severity scores over time (6 weeks).
Sources: Author

maize production and food security in the country. Germplasm 58, 28, 14, 122 and OH28 were the most susceptible to MCMV with a final score of ≥ 4 across the two seasons. Germplasm OH28, 112, 122 and 58 were the most susceptible to SCMV with severity score of ≥ 4 with the highest AUDPC of > 100. Three germplasms (OH28, 58, 122) were very susceptible to both viruses with the highest final score and AUDPC (Table 2), while germplasm 19 and 7 showed levels of resistance to both viruses. Paraschivu et al. (2013) reported a correspondence between germplasm AUDPC and susceptibility pointing that the most susceptible wheat germplasm had higher AUDPC values. This report is in agreements with the studies by Sitta et al. (2017) and Gowda et al. (2015) that reported high susceptibility of studied germplasm to MLND and causal agents.

Five germplasm showed tolerance to MCMV with a final score of < 3 across the six weeks and lowest AUDPC ranging from 58.5 to 61.1. Ks23-6 had the lowest score of 2 while germplasm 18,9,60, Ks23-5 had a score of 2.3 and germplasm 19 had a score 3.0. Evaluation of germplasm in response to SCMV suggest that germplasm 50 and19 are resistant with scores of below 2.5 while germplasm 7, 22 and 48 had a score of 3 meaning they are moderately tolerant. This study found that germplasm 7 and 19 may be having genes resistance

to both SCMV and MCMV with low severity scores and AUDPC in both trails (Table 2).

This study suggests that germplasm 18, 9, 60 and 19 may be carrying genes for MCMV resistant while germplasm 50 and 19 may be carrying genes resistant to SCMV. In addition, this study has confirmed that KS23-6 and KS23-5 are resistant to MCMV. KS23-6 and KS23-5 were identified as strong sources for MLND resistance and were developed by Kasetsart University in Thailand after crossing 26 inbred lines (Jones et al., 2018; Awata et al., 2021). Disease resistance is a mechanism developed by plants through evolution to survive attack by parasites. Quantitative trait loci (QTL) on chromosome six at 157 MB influences resistance to MCMV, as reported by Johns et al. (2018). It is inherited to the F2 population recessively. Two major genes Scmv1 and Scmv2 that confer resistance to Sugar cane mosaic virus have been mapped in various studies (Xia et al., 1999; Ingvardsen et al., 2010; Leng et al., 2017; Tao et al., 2013; Liu et al., 2009). More study on germplasm 18,9,60 needs to be carried out to confirm the presence of QTL that confers resistance to MCMV and for germplasm 50, 19, 22 and 48 to confirm presence of Scmv1 and Scmv2 responsible for SCMV resistance.

Conclusion

The results from this study show that the germplasm studied here are variable in response to MCMV and SCMV. The germplasm identified as tolerant in this research study could serve as potential donors to improve the adapted maize to combat MLND in the country. This will restore maize productivity and improve small scale farmer livelihood. Further studies should be done on the mode of inheritance of SCMV and MCMV resistance QTLs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Radio-Sensitivity of four selected rice (*Oryza sativa* L.) varieties in Kenya, as a pre-requisite for mutation breeding

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Radio-sensitivity test informs the optimal irradiation dose for use in mutation breeding. In this study, four rice varieties (Basmati 217, Basmati 370, ITA310, and Komboka) were irradiated with gamma-ray doses ranging from 0 to 500 Gy, to examine their morphological responses at the seedling stage, and estimate their optimal doses. Two experiments were carried out at varied time points between March and May 2022. In the first experiment, the irradiated seeds were pre-germinated before sowing in soil premixed with base fertilizer, while in the second experiment, irradiated seeds were imbibed by soaking in water for 24 h followed by direct sowing in soil. Observations were made on germination percentage, rate of seedling emergence, survival, and seedling height. The results indicated that sensitivity to gamma irradiation varied across varieties. Basmati 370 and Komboka were the most sensitive, ITA310 was moderate, while Basmati 217 was the least sensitive. At low doses of 50, 100, and 150 Gy, gamma irradiation enhanced seedling survival and height, but at higher doses above 300 Gy, they were significantly reduced. LD50 values ranged from 354 to 556 Gy, reduction dose 30 (RD30) ranged from 267 to 426 Gy, while reduction dose 50 (RD50) ranged from 335 to 531 Gy.

Key words: Mutation breeding, radio-sensitivity, LD50, RD30, RD50, gamma irradiation.

INTRODUCTION

Rice (*Oryza sativa* L.) is an important staple food for more than 50% of the world's population (FAO, 2012), with the bulk of the production globally based in Asia, which is also the largest consumer (FAO, 2018). In Africa, and more specifically in Kenya, there is overreliance on imports with only 20% of the consumed rice grown locally (FAO, 2021). There are many constraints toward increased rice productivity, majority of

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which can be dealt with by breeding of high productive cultivars, resistance to biotic, and abiotic stresses.

Mutation breeding is a powerful method of rapidly introducing novel genetic diversity from which favorable traits can be selected (Till et al., 2014). Mutation breeding scheme is based on induction of mutation, detection, fixation, development of mutant line and the release of new mutant cultivars (Chepkoech, 2018; FAO/IAEA, 2021). More than 3,200 mutants have been released with over 25% of the mutants being rice varieties (FAO/IAEA, 2021).

In Kenya, mutation breeding has been applied in a few crops including wheat, cassava, potato, pigeon peas and cowpeas (Chepkoech et al., 2020; FAO/IAEA, 2021; Kinyua and Okwaro, 2021; Njau et al., 2005). To introduce mutation breeding technique in Kenyan rice cultivars, there is a need to determine the appropriate doses for the local varieties. This can be achieved through radio-sensitivity study of the plant materials to be utilized in breeding (FAO/IAEA, 2018; Solim and Rahayu, 2021; Ulukapi and Ozmen, 2018). Radio-sensitivity differs depending on plant species, varieties, plant part and water content of the plant material to be irradiated (Kant et al., 2020; Solim and Rahayu, 2021; Toker et al., 2005). Effective mutations have been achieved at lethal dose 50% (LD50), where half of the materials irradiated die, and where 30% or 50% of growth reduction (RD30 and RD50) is achieved (Al-Azab, 2013; FAO/IAEA, 2018; Kant et al., 2020). At these optimal dosages there is maximal probability of getting beneficial mutations with minimal impact on the genome (Al-Azab, 2013; Kant et al., 2020).

This study was aimed at determining the optimal dosages of three most popular, locally adapted rice varieties in Kenya, Basmati 370, Basmati 217, ITA310 and a newly released variety, Komboka.

METHODOLOGY

Plant and treatment with mutagen

The genotypes used in the study were four locally adapted lowland rice varieties. These included Komboka, Basmati 370, Basmati 217 and ITA310 which were obtained from the Kenya Agricultural and Livestock Research Organization (KALRO), Mwea Research Center. Average moisture content of seeds prior to radiation was 11%.

Radio-sensitivity protocol by FAO/IAEA (2018) with a few modifications was adopted for the study. Two experiments were conducted at varied time points. In the first experiment, eight gamma ray dosages, that is, 0, 100, 150, 200, 250, 300, 400, and 500 Gy were used (dosages adopted from Lee et al., (2019) with a few modifications). Approximately 60 seeds were randomly drawn from the seed lots and placed in labeled brown paper envelopes to constitute 8 dosages and placed in the plastic jar on the irradiator.

In the second experiment, eleven portions each containing 60 seeds were randomly drawn from the same seed lots as the first and were treated with eleven gamma ray dosages from 0 to 500 Gy at 50 Gy intervals; 0, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 Gy.

The irradiation was conducted using ^{60}Co Gammacell 220 irradiator emitting 2.25 KGy/h belonging to the KALRO Biotechnology Research Institute, Muguga, Kenya.

Planting of the M1 seeds

First experiment

The irradiated seeds were immediately pre-germinated according to Kega et al. (2015) and PHILRICE (2007) with some modifications. Seeds were soaked in water for 24 h then transferred and incubated in moist paper towels contained in plastic containers. Germination percentage was recorded on the 4th day after initiation of pre-germination step, when the radicle had emerged. The germinated seeds were sown in completely randomized design in 160-cell plastic seedling trays filled with soil collected from rice fields. Prior to seedlings transfer, the soil was mixed thoroughly with concentrated phosphate-base fertilizer (diammonium phosphate, DAP). Thereafter, the trays were placed on water pods in a greenhouse at the KALRO, Mwea Research Center.

Second experiment

The irradiated seeds were soaked in water for 24 h and immediately sown in soil media in order of increasing gamma ray dosage according to Mba et al. (2010), with three replications sown in different trays. Irrigation was done on daily basis. Planting of the imbibed irradiated seeds was carried out in a greenhouse at the KALRO, Mwea Research Center.

Data collection

Data collected on the study included germination percentage, rate of seedling emergence, survival rates, and seedling height according to Solim and Rahayu (2021) and Gupta et al. (2021). Germination percentage was recorded on the 4th day after initiation of the pre-germination step. Data on seedling emergence was recorded on the 7th day after sowing (7 DAS) in the first experiment and 11 DAS in the second experiment (due to some delays in emergence of Basmati 370 and Komboka varieties). Seedling survival rate and seedling height were recorded at 14 and 21 DAS in both experiments. LD50 was calculated from the emergence and survival rates, while the median reduction dose 50 (RD50) and reduction dose 30 (RD30) (Lee et al., 2019) were calculated by analyzing plant height at 21 DAS according to Gupta et al. (2021) and Kant et al. (2020).

Statistical analysis

The data was entered in Excel and the analysis of variance computed in R software. Tukey HSD test ($P<0.05$) was used in mean separation of the significant groups from the ANOVA table. Data on seedling germination percentage, rate of emergence, survival rate and seedling height were fitted in regression models using Curve Expert Professional software version 2.7.3 available at https://www.curveexpert.net/. On the regression models, seedling emergence, survival rate and seedling height were the dependent variables while the gamma ray dosage was the independent

ANOVA table in response to germination

SOV	Df	Mean Sq.
Dosage	7	15.33
Variety	3	1651.79***
Residual	21	21.43

Significant codes: '***'0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Mean separation of the significant group

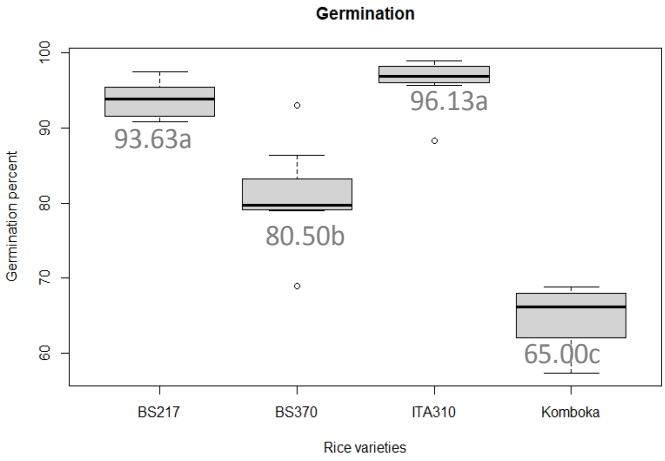

Figure 1. Analysis of variance for germination as recorded at the end of the pre-germination step, and mean separation of the significant group.
Source: Authors

variable. GraphPad prism 9 software version 9.3.1 (available at https://www.graphpad.com/) was utilized in combining data of the dependent and independent variables for the varied varieties in single plots.

RESULTS AND DISCUSSION

Germination percentage

In the first experiment, data on germination was recorded when the primary root or radicle had emerged from the cotyledon. This was done on the 4th day after initiation of pre-germination step. Analysis of variance in response to germination rate was computed in R software, followed by mean separation of the significant group using Tukey HSD at P<0.05. As depicted in the ANOVA Figure 1, gamma irradiation had no significance effect on germination, but there were significant varietal differences not associated with radiation. The results were consistent with previous studies on rice (Harding, 2012; Singh et al., 1998), common beans (Ulukapi and Ozmen, 2018) and groundnuts (Mondal et al., 2017), where the authors reported no significant effects of gamma irradiation on seedling germination.

As shown in Figure 1, ITA310 variety had the highest germination percentage, followed by Basmati 217 and Basmati 370 in that order, with Komboka having the lowest germination percentage.

Germination percentages for ITA310 (96.13%) and Basmati 217 (93.63%) were not significantly different, but were significantly different from those of Basmati 370 (80.50%) and Komboka (65%). In addition, Basmati 370 and Komboka showed significantly different germination percentages.

Effect of varied gamma ray dosage on seedling emergence

The data on the rate of seedling emergence was subjected to analysis of variance per variety. From the ANOVA Table 1, gamma radiation caused significant effect on the rate of seedling emergence in both experiments and in varieties.

Mean separation for the significant group was carried out using Tukey HSD at P<0.05 on R software. As shown in Table 2, high gamma ray dosages from 400 Gy caused significant reduction on the rate of seedling emergence. Variation in radiation sensitivity among varieties was evident. In the first experiment, seedlings emanating from seeds irradiated at 400 and 500 Gy showed significant reduction in seedling emergence for Komboka and Basmati 370, while for Basmati 217 and ITA310 varieties, significant reduction in emergence was achieved at 500 Gy. In the second experiment, there was significant reduction in emergence on Basmati 370 and ITA310 mutants at high dosage of 500 Gy, but this was not the case for Basmati 217. Our results were consistent with similar studies in rice by Cheema and Atta (2003), where they reported no significant effect of radiation on seedlings emergence at lower dosages. At high dosage, gamma radiation causes generation of free radicals in the irradiated material, causing metabolic disorders that ultimately cause growth retardation of the seedlings

Table 1. Analysis of variance table for the rate of seedling emergence at 7 and 11 DAS for the 1st and 2nd experiments

SOV	Df	Experiment 1	
		Mean Sq. (Basmati 217)	Mean Sq. (ITA310)
Dosage	7	0.0286476**	0.045190 *
Rep	2	0.0000542	0.001904
Residual	14	0.0064065	0.014276
		Mean Sq. (Basmati 370)	**Mean Sq. (Komboka)**
Dosage	7	0.067109***	0.214021***
Rep	2	0.007600	0.004175
Residual	13	0.005539	0.012833

		Experiment 2			
		Mean Sq. (Basmati 370)	Mean Sq. (ITA310)	Mean Sq. (Basmati 217)	Mean Sq. (Komboka)
Dosage	10	0.063136***	0.0171667**	0.0213788*	0.083894***
Rep	2	0.008864	0.0023485	0.0037121	0.027500
Residual	20	0.012364	0.0040985	0.0073788	0.011417

Significant codes: '***'0.001, '**' 0.01, '*' 0.05 '.' 0.1 ' ' 1.
Source: Authors

Table 2. Effect of gamma radiation on seedling emergence (data collected 7 DAS and 11 DAS for the first and second experiments).

Gamma ray dosage	The rate of seedling emergence per variety in response to gamma irradiation							
	Basmati 217		Basmati 370		ITA310		Komboka	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
0	0.9333^{a}	0.7833^{ab}	0.9833^{a}	0.8333^{a}	0.7433^{a}	0.9333^{a}	0.7900^{a}	0.2000^{bcd}
50	-	0.8333^{ab}	-	0.8833^{a}	-	0.8667^{a}	-	0.3333^{abcd}
100	0.8667^{ab}	0.9500^{a}	0.9733^{a}	0.8333^{a}	0.8133^{a}	0.9333^{a}	0.8900^{a}	0.4500^{ab}
150	0.9300^{a}	0.8500^{a}	1.0000^{a}	0.7333^{ab}	0.7133^{ab}	0.8833^{a}	0.8100^{a}	0.4833^{ab}
200	0.9800^{a}	0.8000^{ab}	0.9167^{a}	0.8167^{a}	0.7667^{a}	0.9333^{a}	0.7900^{a}	0.4333^{ab}
250	0.8867^{a}	0.8333^{ab}	0.9567^{a}	0.8000^{a}	0.6900^{ab}	0.9000^{a}	0.8966^{a}	0.4500^{ab}
300	0.9467^{a}	0.8333^{ab}	0.9233^{a}	0.7500^{a}	0.7567^{a}	0.9500^{a}	0.7333^{a}	0.4167^{abc}
350	-	0.8500^{a}	-	0.5667^{ab}	-	0.9333^{a}	-	0.5333^{a}
400	0.8433^{ab}	0.8167^{ab}	0.6567^{b}	0.6333^{ab}	0.5867^{ab}	0.8500^{ab}	0.2900^{b}	0.5000^{ab}
450	-	0.7667^{ab}	-	0.5833^{ab}	-	0.8500^{ab}	-	0.1000^{cd}
500	0.6667^{b}	0.6000^{b}	0.5700^{b}	0.4167^{b}	0.4333^{b}	0.6833^{b}	0.1150^{b}	0.0500^{d}

Source: Authors

(Kumar et al., 2013). It has also been attributed to reduced enzyme activity and inhibition of auxins in seeds exposed to high gamma ray doses (Kant et al., 2020; Kumar et al., 2013). In the context of this study, it is possible that even though the seeds germinated, the seedlings vigor was greatly depressed by the inhibitory effects of deleterious mutations at higher gamma ray dosages causing low emergence.

In the second experiment, very low rates of emergence were observed for Komboka variety, with only 20% of the sowed seeds emerged from the non-irradiated group. For this variety, the rate of seedling emergence increased with increasing gamma ray dosage, with the highest rate at 350 Gy, above which, it reduced significantly to 10 and 5% at 450 and 500 Gy, respectively. Similar findings have been documented by Kim et al. (2000) on Chinese

Table 3. Analysis of variance table for the four varieties in response to seedling emergence (7 DAS) and survival (14 and 21 DAS).

SOV	Df	Experiment 1			
		Mean Sq. (Basmati 217)	Mean Sq. (Basmati 370)	Mean Sq. (ITA310)	Mean Sq. (Komboka)
Dosage	7	0.059476***	0.81820***	0.104400***	0.48482***
Rep	2	0.018533	0.00258	0.039981*	0.00955
DAS	1	0.014008	0.04877**	0.036300	0.00012
Residual	37	0.007807	0.00530	0.008998	0.00905

		Experiment 2		
		Mean Sq. (Basmati 217)	Mean Sq. (Basmati 370)	Mean Sq. (ITA310)
Dosage	10	0.101157***	0.36907***	0.077076***
Rep	2	0.002045	0.00881	0.017955*
DAS	2	0.007576	0.02859.	0.004773
Residual	84	0.004996	0.01021	0.005451

		Mean Sq. (Komboka)
Dosage	10	0.153123***
Rep	2	0.104785***
DAS	2	0.019895
Residual	76	0.012815

Significant codes: '***'0.001, '**' 0.01, '*' 0.05 '.' 0.1 ' ' 1.
Source: Authors

cabbage and radish, where seedling emergence was significantly higher at low dosages compared to the non-irradiated samples. Abdel-Hady et al. (2008) in their review paper indicated that the stimulatory effect of low dosages of gamma ray radiation on germination is attributed to activation of RNA and protein synthesis.

Effect of gamma ray radiation on seedling survival

The rate of seedling survival was recorded on 14 and 21 DAS. In the first experiment (as shown in Figures 6 and 7 in the Appendix), at 21 DAS, most of the entries including the controls had dried up due to some external factors (so the data at 21 DAS was skewed). Therefore, in the analysis of variance and estimation of LD50, data on emergence and survival at 7 and 14 DAS were utilized. In the second experiment, data on emergence at 7 DAS and survival at 14 and 21 DAS were included in the analysis.

In both experiments, gamma radiation caused significant reduction on seedling survival rates as shown in Tables 3 and 4. This is consistent with similar studies in common beans, cowpeas, groundnuts and rice (Harding, 2012; Kang et al., 2020; Mondal et al., 2017; Ulukapi and Ozmen, 2018). DAS group in the ANOVA Table 3 included the rate of emergence at 7 and 11 DAS (for the first and second experiments, respectively), as well as seedling survival rates at 14 and 21 DAS. DAS group was only significantly different for Basmati 370 in the first experiment, indicating that the seedling emergence rate was significantly different from seedling survival rate for this variety. However, it was not significantly different on Basmati217, ITA310 and Komboka varieties.

Upon computing the mean separation of the significant DAS for Basmati 370, the number of seedlings at 14 DAS was significantly reduced compared to what was recorded as emergence at 7 DAS, implying that radiation significantly affected this variety's seedling survival.

Mean separation of the dosage group is presented in Table 4. Of the four varieties, Basmati 370 was the most sensitive, with gamma ray dosages from ≥300 and ≥350 Gy in the first and second experiments, respectively, causing significant reduction in seedling survival. Significant reduction on seedling survival rate for ITA310 mutants was reported at 400 and 500 Gy in the first and second experiments, respectively. For Basmati 217, mutant seedlings emanating from seeds irradiated at 100 Gy had the highest survival rate at 93.89%, this was significantly higher than the other treatments in the group, including the non-irradiated samples. Among the four varieties, Basmati 217 was the least sensitive to gamma

Table 4. The rate of seedling survival per variety and gamma ray dosage treatment (data collected at 21 DAS).

Gamma ray dosage in Gy	The rate of seedling survival per variety in response to irradiation							
	Basmati 217		Basmati 370		ITA310		Komboka	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
0	0.9283^{a}	0.7778^{b}	0.9500^{a}	0.8444^{a}	0.7217^{a}	0.9222^{ab}	0.7850^{a}	0.2222^{cd}
50	-	0.8167^{b}	-	0.8722^{a}	-	0.8833^{ab}	-	0.3444^{abc}
100	0.8283^{a}	0.9389^{a}	0.9733^{a}	0.8556^{a}	0.8000^{a}	0.9222^{ab}	0.8733^{a}	0.4500^{ab}
150	0.9367^{a}	0.8556^{ab}	0.9667^{a}	0.7444^{a}	0.7067^{ab}	0.8833^{ab}	0.8283^{a}	0.5111^{a}
200	0.9633^{a}	0.8111^{b}	0.8883^{ab}	0.8167^{a}	0.7000^{ab}	0.9333^{ab}	0.7967^{a}	0.4500^{ab}
250	0.8650^{a}	0.8111^{b}	0.9400^{a}	0.8056^{a}	0.6900^{ab}	0.8888^{ab}	0.8817^{a}	0.4722^{ab}
300	0.8900^{a}	0.8500^{ab}	0.7667^{b}	0.7444^{a}	0.7117^{a}	0.9389^{a}	0.7617^{a}	0.3833^{abc}
350	-	0.8278^{b}	-	0.5722^{b}	-	0.9222^{ab}	-	0.4111^{abc}
400	0.8500^{a}	0.8000^{b}	0.2517^{c}	0.5278^{b}	0.5150^{bc}	0.8389^{ab}	0.2900^{b}	0.3111^{bc}
450	-	0.7500^{b}	-	0.4722^{b}	-	0.8167^{b}	-	0.0833^{d}
500	0.6450^{b}	0.5111^{c}	0.0283^{d}	0.2389^{c}	0.3950^{c}	0.6167^{c}	0.0980^{c}	0.0500^{d}

Source: Authors

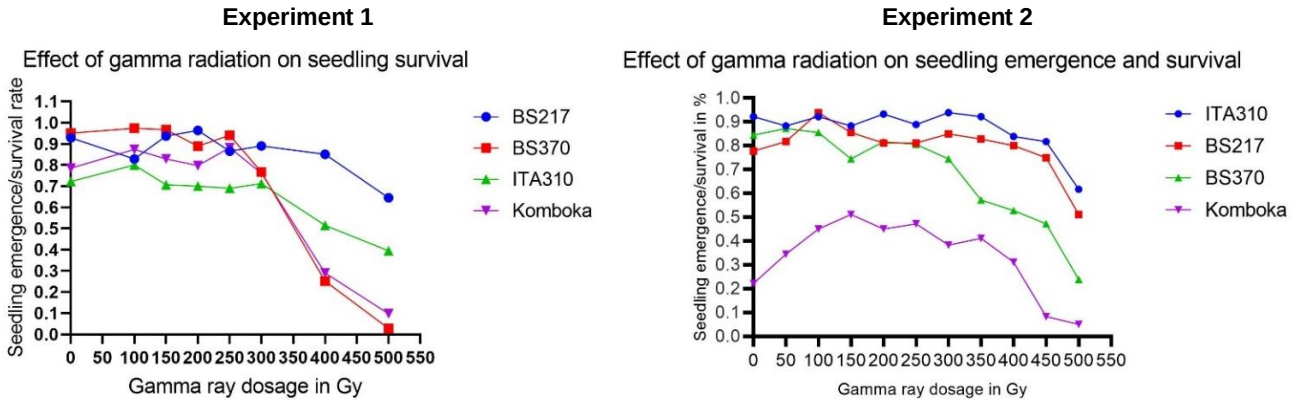

Figure 2. The effect of gamma radiation on seedling survival rate.
Source: Authors

irradiation, with significant reduction on survival rate only at 500 Gy in both experiments.

In the first experiment, Komboka mutants at 400 and 500 Gy had significantly reduced survival rates compared to the non-irradiated samples. But in the second experiment, significantly higher survival rates were exhibited by doses between 100 and 250 Gy compared to the control. In the second experiment, at 350 Gy, seedling survival reduced from 53.33% recorded as emergence rate at 11 DAS to 41.11% at 21 DAS as shown in Tables 2 and 4, respectively.

Data on seedling emergence and survival was used in generating the plots on Figure 2 using GraphPad Prism 9 software. As shown in Figure 2, Basmati 370 in the first and second experiments, and Komboka in the first experiment were most affected by gamma radiation. From 300 Gy, the number of seedlings reduced with the increasing gamma ray dosage. Dosages at 400 and 500 Gy significantly caused stunted growth in most of the seedlings, with 500 Gy causing more than 90% seedling death in Komboka and Basmati 370 varieties in the first experiment. On the contrary, ITA310 and Basmati 217 were less sensitive to irradiation in both experiments, with survival rates above 50% at 500 Gy relative to the 0 Gy dose. The results are consistent with similar studies in varied crops, including rice and cowpeas, where gamma radiation above 300 Gy caused significant reduction on seedling survival (Harding, 2012; Kadhimi et al., 2016; Kang et al., 2020).

Seedling emergence and survival at 0 Gy was used as the reference (converted to 100%), where all the other doses were compared against. From the output in Table

Table 5. The estimated LD50 values and the regression models from seedling emergence and survival rates.

Variety	Experiment	LD50	Regression model
Basmati 217	1	555.6757 Gy	r =0.9061, r^2=0.821, $y = a/(1 + (\frac{x}{b})^c)$; where a=0.1871, b=76.7839, c=-14.7525
	2	521.5158 Gy	r =0.9329, r^2=0.8705, $y = a/(1 + \exp(b - cx))$; where a=1.0702, b=-14.5025, c=-0.0280
Basmati 370	1	354.3508 Gy	r=0.9973, r^2=0.9945, $y = a/(1 + (\frac{x}{b})^c)$; where a=1.0013, b=354.9963, c=8.9019
	2	450.6149 Gy	r=0.9718, r^2=0.9445, $y = a(b - \exp(-cx))$; where a=0.0108, b=94.2896, c=-0.0086
ITA310	1	517.8957 Gy	r=0.9642, r^2=0.9297, $y = a/(1 + (\frac{x}{b})^c)$; where a=1.0274, b=510.5239, c=4.0701
	2	521.2337 Gy	r=0.9715, r^2=0.9439, $y = a/(1 + \exp(b - cx))$; where a=0.9892, b=-13.5057, c=-0.0259
Komboka	1	385.1236 Gy	r=0.9907, r^2=0.9814, $y = a/(1 + (\frac{x}{b})^c)$; where a=1.0656, b=378.2225, c=9.6489
	2	437.2305 Gy	r=0.9867, r^2=0.9736, $y = a + br^x + cx$; where a=5.8871, b=-4.9486, c=-0.0114, r=0.9942

Source: Authors

4, the reduction on the rate of seedling emergence and survival, relative to the control (dose 0Gy) was calculated for each dose and variety, then fitted on regression models using Curve Expert Professional version 2.7.3. where estimation of LD50 was done.

From the curve expert output presented in Table 5, more than 80% of the data fitted the regression models perfectly as depicted by the coefficient of determination (r2). LD50 values across varieties ranged from 354.3508 to 555.6757 Gy. Basmati 217 had the highest LD50 values, thus the least radio-sensitive variety with LD50 ranging from 521.5158 to 555.6757 Gy, followed by ITA310 with LD50 ranging from 517.8957 to 521.2337 Gy. Komboka rice variety had its LD50 ranging from 385.1236 to 437.2305 Gy, while Basmati 370 had the lowest LD50 ranging from 354.3508 to 450.6149 Gy.

A low LD50 consistent with that of Basmati 370 has been reported on MRQ74, MR269 and White Ponni rice varieties on similar studies, which had LD50 at 365.1061, 351.3429 and 354.8000 Gy, respectively (Kadhimi et al., 2016; Ramchander et al., 2015).

Varieties with low sensitivity, Basmati 217 and ITA310 had higher LD50 values than the highest value of the thirteen cultivated varieties in Sierra Leone studied by Harding (2012), whose LD50 ranged from 345 to 423 Gy. These were also slightly lower than Galon rice genotype in Bangladesh which was reported at 575 Gy by Gupta et al. (2021). Higher LD50 values in rice varieties have also been reported by Solim and Rahayu (2021) for Mira-1 and Bastari in Indonesia whose LD50 ranged from 521.40 and 663.68 Gy. Suliartini et al. (2020) in a similar study on four lowland rice varieties, reported an LD50 value of 518 Gy for one of the varieties, Inpago Unram-1.

The outcomes are consistent with the varieties with the lowest sensitivity in our study, Basmati 217 and ITA310 whose LD50 ranged from 517.8957 to 555.6757 Gy.

Effect of radiation on seedling height at 14 and 21 DAS

Seedlings height was measured and recorded at 14 and 21 DAS. Analysis of variance was computed in R software, from which the output in Table 6 was

Table 6. Analysis of variance table in response to seedling height at 14 and 21 DAS.

Variety	SOV	Df	Mean Sq	SOV	Df	Mean Sq
Basmati 217	Dosage	7	2079.1***	Dosage	10	452.2***
	Rep	2	7661.2***	Rep	2	10.6 .
	DAS	1	30716.5***	DAS	1	4391.2***
	Residual	951	45.9	Residual	1017	5.5
Basmati 370	Dosage	7	550.1***	Dosage	10	452.79***
	Rep	2	244.0***	Rep	2	14.78
	DAS	1	11565.8***	DAS	1	2589.84***
	Residual	576	29.1	Residual	890	5.62
ITA310	Dosage	7	344.6***	Dosage	10	640.32***
	Rep	2	654.8***	Rep	2	15.91**
	DAS	1	6958.7***	DAS	1	2161.92***
	Residual	574	16.7	Residual	1127	3.44
Komboka	Dosage	7	3525***	Dosage	10	184.96***
	Rep	2	6460***	Rep	2	53.74***
	DAS	1	44384***	DAS	1	1037.54***
	Residual	732	42	Residual	415	5.22

Source: Authors

Table 7. The output for the mean separation of seedling height (in cm) at varied gamma ray dosage.

Gamma ray dosage (Gy)	Seedling height in cm of seedlings grown from irradiated seeds per variety and experiment							
	Basmati 217		Basmati 370		ITA310		Komboka	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
0	13.9932^{b}	13.0064^{ab}	9.3282^{b}	12.5667^{a}	9.4891^{a}	13.1164^{ab}	21.0008^{ab}	8.4643^{ab}
50	-	13.0208^{ab}	-	11.1000^{ab}	-	13.9275^{a}	-	10.5429^{a}
100	15.4037^{ab}	13.1518^{a}	12.9718^{a}	11.3077^{ab}	8.8283^{ab}	14.2855^{a}	22.8576^{a}	9.8815^{ab}
150	18.4587^{a}	13.0519^{ab}	10.4330^{ab}	10.5644^{b}	6.7771^{bc}	12.5396^{bc}	20.2934^{ab}	8.8969^{ab}
200	16.4667^{ab}	13.8896^{a}	8.6931^{b}	10.7000^{b}	8.7691^{ab}	12.3411^{bc}	17.7800^{bc}	8.3393^{ab}
250	13.1479^{b}	12.4314^{abc}	10.0436^{ab}	10.5708^{b}	8.1281^{ab}	11.6692^{cd}	15.9599^{c}	7.9034^{b}
300	15.6434^{ab}	11.4269^{bc}	4.7186^{c}	8.8159^{c}	4.5809^{cd}	11.3148^{cd}	6.5695^{d}	5.3522^{c}
350	-	10.7898^{cd}	-	6.4189^{d}	-	10.4722^{d}	-	4.3381^{cd}
400	8.6231^{c}	9.0149^{de}	2.8238^{c}	6.3688^{d}	4.8059^{cd}	8.5592^{e}	3.0500^{d}	3.1462^{cd}
450	-	8.7318^{e}	-	5.8481^{d}	-	7.6714^{e}	-	0.7000^{d}
500	4.5815^{c}	7.1259^{e}	0.7500^{c}	5.0500^{d}	2.7697^{d}	5.1382^{f}	4.6400^{d}	0.0000^{d}

Source: Authors

generated. As shown in Table 6, dosages, replication and DAS groups were significantly different for all the varieties in the first experiment. In the second experiment, dosage and DAS groups were significantly different in all varieties, while the replication group was significantly different in ITA310 and Komboka varieties. Mean separation using Tukey HSD at $P<0.05$ was computed for the significant groups in R software. Seedling height at 21 DAS was significantly greater than the height at 14 DAS.

As shown in Table 7, in the first experiment, Basmati 217 and Komboka were significantly taller at lower dosages than other entries in the first and second experiments. In addition, at 100 and 150Gy doses of

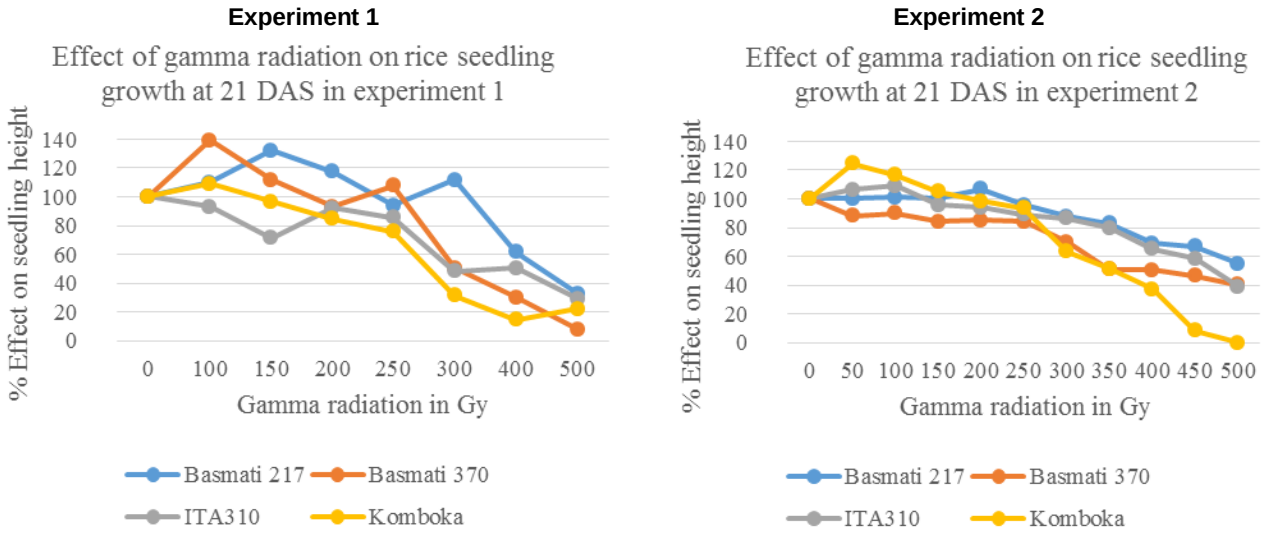

Figure 3. The effect of gamma ray radiation on seedling height grown from irradiated seeds.
Source: Authors

Basmati 370 and Basmati 217, respectively, mutants were significantly taller than the non-irradiated (0Gy) samples as presented in Table 7 and Figure 3. The same was reported in the second experiment on ITA310 mutants at 50 and 100 Gy. Our results were consistent with similar studies in rice, chickpeas and Chinese cabbage (Gupta et al., 2021; Kadhimi et al., 2016; Kim et al., 2000; Toker et al., 2005). In rice, increased seedling height relative to non-irradiated samples at 100 and 200 Gy was reported by Gupta et al. (2021) and Kadhimi et al. (2016). Kim et al. (2000) showed increased seedling height in Chinese cabbage at low dosages of 4 and 10 Gy. Toker et al. (2005) reported increased seedling growth on chickpeas at 100 and 200 Gy, while Ulukapi and Ozmen (2018) reported enhanced root growth in common beans F16 cultivar at 100 Gy compared to the non-irradiated samples.

As shown in Table 7 and Figure 3, gamma radiation caused significant reduction in seedling height from as low as 150 Gy in experiment 2 for Basmati 370 mutants. The height significantly reduced with increasing gamma ray dosages with variations across varieties as shown in Figure 3, and Figures 4 to 12 in the Appendix. This is consistent with similar studies in various crops (Kadhimi et al., 2016; Kang et al., 2020; Sasikala and Kalaiyarasi, 2010).

In the first experiment, significant growth reduction on Basmati 370 was observed from 300 Gy. Radiation caused significant growth reduction on Komboka mutants from ≥250 Gy and ≥300 Gy in the first and second experiments, respectively. For ITA310, mutants were significantly shorter from ≥250 Gy and ≥300 Gy for the second and first experiments, respectively compared to the non-irradiated samples. In the first experiment, ITA310 mutants at 150 Gy were also significantly shorter than the non-irradiated (0Gy) samples, but the mutants at 200 and 250 Gy were not. This is possible because of the random effect of radiation giving different responses (Suliartini et al., 2020). For Basmati 217, significant reduction on seedling height was observed from ≥350 Gy and ≥400 Gy on the second and first experiments, respectively. This placed Basmati 217 as the least sensitive variety and Basmati 370 as the most sensitive variety in our study. The results across varieties on the effect of radiation on seedling height were consistent with reports on similar studies by Harding (2012) and Solim and Rahayu (2021) where significant growth reduction was reported at radiation dosages >300 Gy and >400 Gy, respectively.

The reduction effects on varied growth parameters by gamma radiation have been associated with disruption of cell cycle at G2/M phase, disruption of DNA, RNA, protein, growth hormone synthesis (Kang et al., 2020; Solim and Rahayu, 2021; Ulukapi and Ozmen, 2018), disturbance in hormonal balance and enzyme activity (Kant et al., 2020).

The output in Table 7 was converted as a percentage of the non-irradiated samples and used in generation of growth curves presented in Figure 3. As shown in Figure 3, similar pattern on the effect of radiation on individual variety is notable in both experiments.

The data on seedling height at 21 DAS was fitted on regression models and utilized in estimation of RD30 and RD50 values. Data on seedling height fitted well on

Table 8. Regression models and the estimated RD30 and RD50 using seedling height data at 21 DAS.

Variety	Experiment	RD50	RD30	Regression model
Basmati 217	1	496.2296 Gy	425.9882 Gy	r=0.9825, r2=0.9652 ; $y = a + br^x + cx$, where a=43.5626, b=-22.7030, c=-0.0656, r=99.2821
	2	531.1608 Gy	417.0106 Gy	r=0.9860, r^2=0.97224; $y = (a + bx)/(1 + cx + dx^2)$, where a=12.7855, b=0.0106, c=0.001596, d=3.3595×10^{-6}
Basmati 370	1	368.3795 Gy	311.0258 Gy	r=0.9840, r2=0.9683; $y = a + br^x + cx$, where a=33.3609, b=-17.3821, c=-0.0659, r=0.9926
	2	421.8008 Gy	286.9128 Gy	r=0.9685, r^2=0.9379; $y = a + br^x + cx$, where a=-5.0162, c=-0.02353, r=0.99596
ITA310	1	418.3331 Gy	330.2820 Gy	r=0.7355, r2=0.5410; $y = a(b - \exp(-cx))$, where a=2.2609, b=7.9619, c=-0.0037
	2	470.0900 Gy	392.4310 Gy	r=0.9858, r^2=0.9719; $y = a(b - \exp(-cx))$, where a=69.3933, b=20.8654, c=-0.0518
Komboka	1	335.3659 Gy	266.5063 Gy	r=0.9464, r2=0.8956; $y = 1/(a + bx + cx^2)$, where a=0.03386, b=-1.2602×10^{-4}, c=6.7307×10^{-7}
	2	351.2681 Gy	292.5661 Gy	r=0.9928 r^2=0.9857; $y = a + br^x + cx$, where a=14.9272, b=-6.3269, c=0.0302, r=0.9874

Source: Authors

regression models as shown in Table 8, with coefficient of determination ≥0.9 for all the varieties, except for the data of ITA310 in the first experiment, where the r2 was 0.5410, indicating that only 54.10% of the ITA310 data set in the first experiment fitted the model. This can be explained by the fact that only a few plants across dosages for this variety survived per entry to 21 DAS data point (as shown in Figure 7 in the Appendix), so the data on seedling height for this variety was skewed.

From RD30 and RD50 values as presented in Table 8, the least radio-sensitive variety was Basmati 217 followed by ITA310, while the most sensitive variety was Komboka followed by Basmati 370. For Basmati 217, RD30 values ranged from 417.0106 to 425.9882 Gy, while RD50 values ranged from 496.2296 to 531.1608 Gy. For ITA310, RD30 values ranged from 330.3820 to 392.4310 Gy, while RD50 values ranged from 418.3331 to 470.0900 Gy. Basmati 370 had its RD30 ranging from 286.9128 to 311.0258 Gy, while RD50 ranged from 368.3795 to 421.8008 Gy. Komboka's RD30 ranged from 266.5063 to 292.5661 Gy, while RD50 ranged from 335.3659 to 351.3659 Gy.

RD50 values in this study are consistent with the growth reduction 50 values on rice reported by FAO/IAEA (2018), which ranged between 350 and 500 Gy. LD50 and RD30/RD50 values differed slightly across environments consistent with similar studies on rice by Kant et al. (2020) where the researchers studied the effect of gamma radiation *in vivo* and *in vitro*.

Conclusion

In this study, gamma radiation on rice seeds did not have significant effect on seedling germination, but significantly affected seedling emergence, survival and growth. The effect of irradiation on these growth parameters varied across varieties and slightly across experiments/ environments. Basmati 370 and Komboka were the most sensitive to irradiation, ITA310 was moderate, while Basmati 217 was the least sensitive. At low doses of 50, 100, and 150 Gy, irradiation caused increased seedling vigor on Basmati 217, Basmati 370, and ITA310. Across varieties, seedling survival and height reduced with increasing doses from 300 and 350 Gy, respectively. The effective doses were determined for each variety as their

individual LD50, RD30 and RD50 values. LD50 values ranged from 354 to 556 Gy. Basmati 370 had the lowest LD50 values at 354 and 451 Gy, followed by Komboka at 385 and 437 Gy, then ITA310 at 518 and 521 Gy, while Basmati 217 had the highest LD50 values at 522 and 556 Gy in the first and second experiments, respectively. RD30 values ranged from 267 to 426 Gy. Komboka had the lowest RD30 values at 267 and 293 Gy followed by Basmati 370 at 290 and 311 Gy, then, ITA310 at 330 and 392 Gy, while Basmati 217 had the highest values at 417 and 426 Gy in the first and second experiments, respectively. RD50 values ranged from 335 to 531 Gy. Komboka had the lowest RD50 values at 335 and 351 Gy, followed by Basmati 370 at 368 and 422 Gy, then ITA310 at 418 and 470 Gy, while Basmati 217 had the highest values at 496 and 531 Gy in the first and second experiments, respectively. Radio-sensitivity study was successful in determining the effective doses for selected varieties. In the rice mutation breeding program, it is recommended to adopt gamma ray doses between RD30 and LD50 in this study as the optimal doses for each variety to cause the desired mutations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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APPENDIX

Figure 4. The effect radiation on rice growth at 21DAS in experiment 1; Basmati 217.

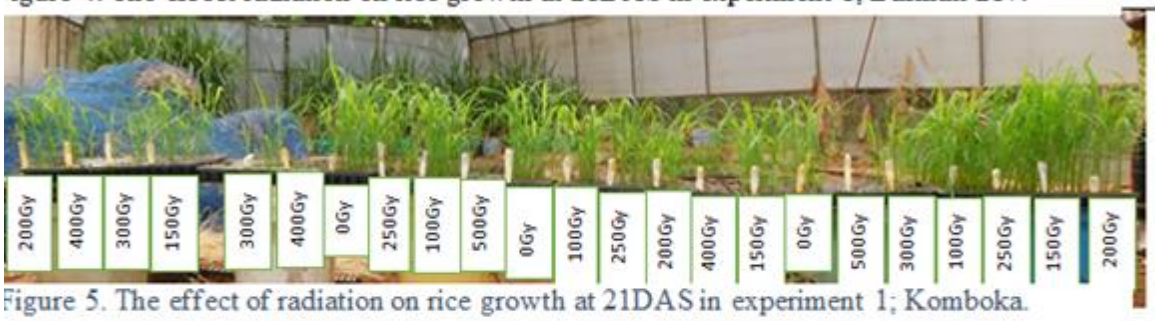

Figure 5. The effect of radiation on rice growth at 21DAS in experiment 1; Komboka.

Figure 6. The effect of radiation on rice growth at 21DAS in experiment 1; Basmati 370.

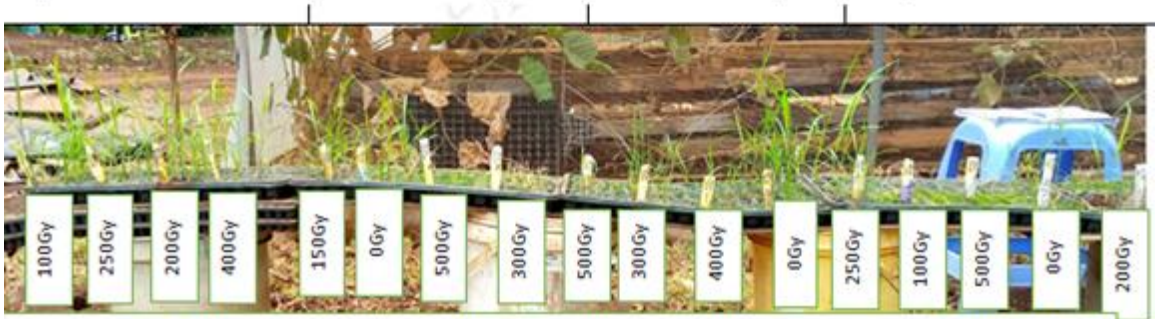

Figure 7. The effect of radiation on ITA310 mutant seedlings in experiment 1 at 21DAS.

Figure 8. Data collection in progress in experiment 2 at 21DAS. Note that the orientation of the dosages is from left to right (0-500Gy).

Figure 9. Basmati 217 mutant seedlings at 21 DAS. The arrangement of the dosages is as shown by the arrow from 0-500Gy.

Figure 10. Basmati 370 mutant seedlings at 21 DAS. The arrangement of the dosage groups is as shown by the arrow from 0-500Gy.

Figure 11. ITA310 mutant seedlings at 21 DAS. The arrangement of the dosages is as shown by the arrow from 0-500Gy.

Figure 12. Komboka mutant seedlings at 21DAS. The arrangement of the dosages is as shown by the arrow from 0-500Gy.

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