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# EFFECT OF GAMMA IRRADIATION OF LOCAL BRACHIARIA RUZIZIENSIS (GERMAIN & EVRARD) SEEDS ON AGRONOMIC PERFORMANCE AND YIELD

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**A B S T R A C T**

Appropriate methods of mutation induction are of high importance in pastures for increased genetic variability and improved performance. The objective of this research was to improve the agronomic and nutritive performance of mutant lines (M7) through induced mutagenesis to seeds of a local landrace *Brachiaria ruziziensis.* The seeds were irradiated with 0, 10, 20, 30 and 40Gy doses of gamma radiation from Cobalt 60 ($^{60}Co$). Treatments were; KE 0Gy, KE 10Gy, KE 20Gy, KE 30Gy and KE 40Gy resulting into M1 seeds. The M1 seeds were planted in the greenhouse in germination pots for one month and the seedlings transplanted to the field. Seeds of M6 plants (M7 seeds) were used to establish field experiment in a completely randomized block design, with three replications. Parameters measured included; tillering, leaf-stem ratio, dry matter weight, and seed weight. Data collected was analyzed using the SAS package. Mutant lines exhibited better agronomic performance compared to the wild type. Performance increased with increased gamma-ray exposure with 40Gy treatment outperforming all other treatments whereas the control performed dismally. There was a significant difference ($P<0.05$) in the dry matter with 40Gy treatment having the highest values of dry matter yields, whereas control had the lowest values. Application of nuclear technology to other grasses would lead to increased biomass and improved nutrition for increased animal productivity leading to food and nutrition security.

**Keywords**: Gamma rays, agronomic performance, *Brachiaria* grass, Mutant lines.

## INTRODUCTION

Population growth in sub-Saharan Africa (SSA) is among the fastest in the world, with a population of over one billion in 2017 (World Bank, 2018). However, growth in the production of livestock products is not keeping pace with the growth in the human population and sub-Saharan Africa has the lowest per capita consumption levels of livestock products in the world (Cardoso, 2012). In addition to the requirements of the increasing population, demand for dairy products is also increasing with rising per capita income, urbanization and westernization of diets (Knips, 2006). To meet the market demand for milk, cattle productivity in these areas needs to be increased through improved pasture and forage productivity. However, the main challenge facing cattle productivity in SSA is the lack of enough feed for the livestock emanating from poor crop performance. Therefore, there is a need to improve the local pasture and forage landraces to meet this demand.

Exploitation of natural or induced genetic diversity is an established strategy for the improvement of major food crops, and the use of mutagenesis to create novel variation is particularly valuable in crops. Mutation induction is an effective method for increasing the diversity of plants and their performance (Sutapa and Kasmawan, 2016). Mutations can be induced using physical or chemical mutagens. Physical mutagenesis includes irradiation with non-ionizing gamma rays, alpha and beta rays, fast and slow neutrons (Tadele, 2016; Oladosu *et al.*, 2015). Gamma rays are vital in developing mutant crop varieties and increasing genetic variability (Jan *et al.*, 2012; Ali *et al.*, 2016). Uses of

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gamma rays are more effective and economical compared to other ionizing radiations since they are easily available and greater penetration power (Moussa, 2006). The gamma rays normally influence the growth and development of plants by inducing morphological, genetically, physiological and biochemical changes in cells and tissues (Jan *et al.*, 2012; Haris *et al.*, 2013).

Seed irradiation before sowing is one of the most effective methods that can be used to improve plant performance. The effect of gamma radiation in improving plant performance has been shown to be highly related to the level of doses use (Mudibu *et al.*, 2011; Lima *et al.*, 2011; Respati *et al.*, 2018). However, information is scanty on the effect of gamma radiation dose on the local Brachiaria species in Kenya. The objective of this research was therefore to determine the effect of gamma irradiation of local *Brachiaria ruziziensis* (Germain & Evrard) seeds on the grass performance.

## MATERIALS AND METHODS

**Plant Material and Gamma Irradiation:** Landrace Brachiaria splits *Urochloa ruziziensis* (R. Germ. & C.M. Evrard) were sourced from the wild at Coordinates: 00$^{o}$ 47' 53.0"S, 034$^{o}$ 58' 37.3"W; at an altitude of 1.919 m asl (Oliveira, 2018) at Borabu location, Kisii County. The splits were established at KALRO-Lanet using established agronomic methods. At maturity, the seeds were harvested, dried under shade and cleaned manually. The seeds were packed into sterile Petri dishes and labelled according to required gamma ray exposure.

The seeds were irradiated at the International Atomic Energy Agency (IAEA), Agriculture and Biotechnology Laboratory, A-2444 Seibersdorf, Austria using a CO60 source Gamma cell Model No. 220. The seeds were gamma irradiated at (0, 10, 20, 30, and 40 Gy) as preliminary dose to monitor agronomic performance, The 0 Gy dose served as a comparative control.

**Field Trials:** Field trials were conducted at KALRO - Lanet (0° 27' 09' S and 390 38' 45' E (Oliveira, 2018), at an elevation of 1600 meters above sea level. The site is located in Nakuru County, Kenya. The area has a bimodal rainfall pattern with an annual mean rainfall of 800mm ranging from 534 to 1,049mm and 83% relative humidity. Temperature ranges between 8- 20$^{o}$C (Pratt and Gwynne, 1977). Soils are deep sandy loam with good water holding capacity with pH range of 5.5 to 6.5 (Mwangi *et al.*, 2017).

**Field Experimental Design:** The land for setting M1 Brachiaria seedlings was ploughed and harrowed using a tractor. At the time of planting, Single superphosphate (0:18:0) at a rate of 250 kg/ha was used. In the field, the six treatments; 0, 10, 20, 30 and 40 Gy obtained on basis of gamma-ray exposure to seeds using Cobalt 60. The experiment was laid out as a complete randomized block design (RCBD) with three replications. The seeds were planted into holes at a depth of 3.0cm at a spacing of 60 cm x 30cm. The Brachiaria grass was top dressed using CAN (27% N) at 250 kg/ha when the crop was 60 cm. The agronomic performance was recorded at booting (Rana and Kumar, 2014). The grass was cut at 2.0 cm above ground at booting stage by throwing a $1.0^2$ m quadrant three times at each treatment and sward within the square was harvested using a sickle, homogenized and used to determine leaf to stem ratio and herbage yield (kg/ha).

Other data collected during the growth of the plant included the days to 50% flowering, Stem elongation, internode length, and leaf length. Soil samples were collected before planting and after harvest and there chemical properties analyzed. Total soil N, available P (Mehlich III), exchangeable K, Ca, and Mg were estimated following standard methods as described by Okalebo *et al.* (2002). Cations $Ca^{2+}$, $Mg2^{+}$, and $K^{+}$ were determined by atomic absorption spectrometry and soil P was measured as described by Murphy and Riley (1962).

**Mutant Selection:** The M1 mutant seedlings ($M_1$) were planted in the field at KALRO- Lanet using the standard cultural practices. Seeds of $M_1$ plants were harvested (M2 seeds) dried and bulked according to their respective radiation doses for evaluation. Consequently, the harvested $M_2$ seeds were planted in the field at KALRO-Lanet as $M_2$ population in the form of progeny rows for individual plant selection and to develop the $M_3$ seeds. The $M_3$ plants were evaluated in the field using morphological and agronomical attributes. Evaluation continued up to M4 population. $M_5$ seeds obtained from the selected $M_4$ population were planted as single-plant progenies and selection were made toward the desired trait on a single plant basis. Uniform, non-segregating mutant progenies, were bulked at this stage to hasten the breeding cycle. The $M_6$ populations were evaluated for suitable lines and selected for herbage and seed yield (Figure 1). At this stage, the mutant lines were stable in terms of the plant phenotypic characteristics.

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**SELECTION METHOD**

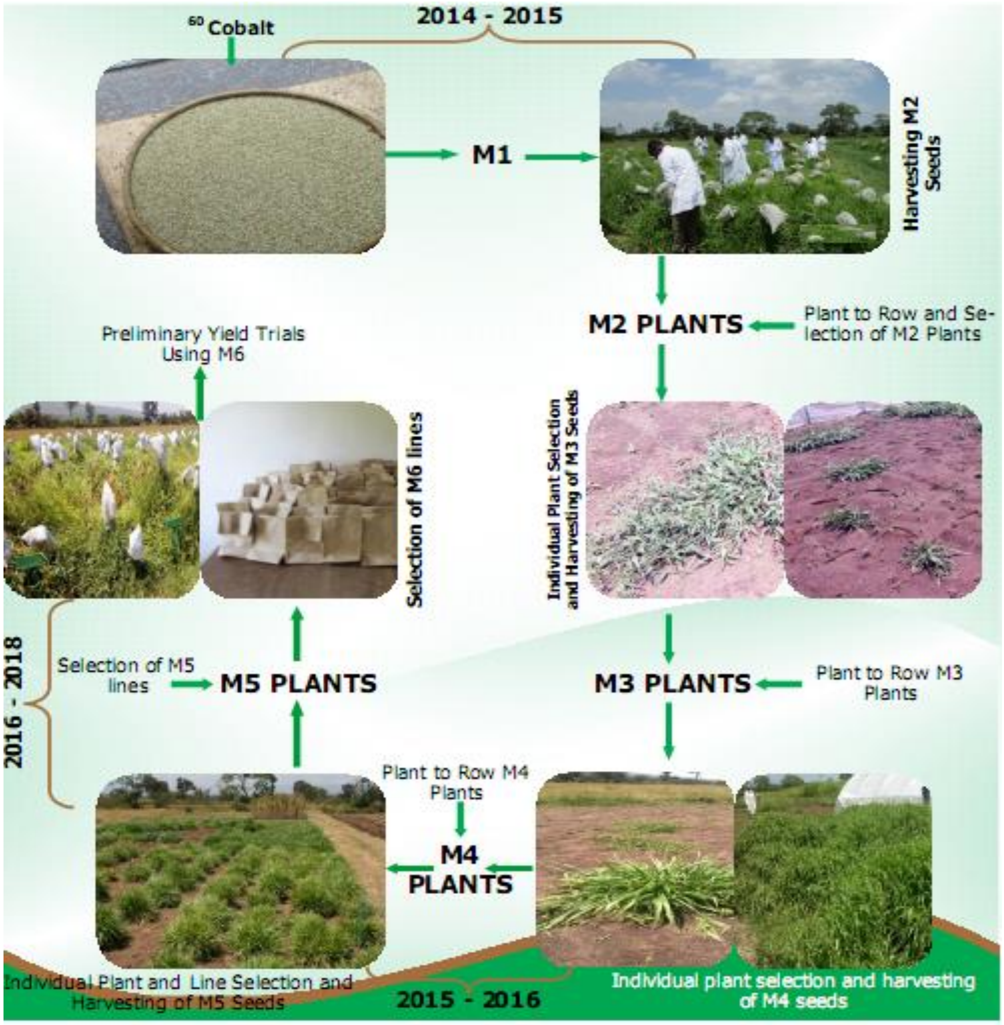


Figure 1: Diagrammatic presentation of selection methods used.

**Data analysis:** Analysis of variance was carried out for the data collected. Mean separation was done using Tukey's whenever there was a significant treatment effect. Statistics analysis software (SAS) Version 9.3 was used for the analysis (SAS Institute, 2010).

**RESULTS**

**Soil characteristics of the study site:** Analysis of the soil before planting showed that the soil had a medium acidic pH and adequate amounts of nitrogen (N), phosphorous (P), copper (Cu), potassium (K), calcium (Ca), magnesium (Mg) and manganese (Mn) both in the top and soil. The total organic carbon was moderate (Table 1). Soil analysis aster harvest of the *Brachiaria* grass showed that the organic carbon was moderate while the N, Cu, K, Ca, Mg and Mn were adequate. There was a change in the concentration of available P after harvest with the soil having an increased amount of P in both the topsoil and subsoil (Table 1).

**Field agronomic performance of *Brachiaria ruziziensis*:** There was significant ($P<0.05$) difference in the establishment, number of tillers, stem elongation, internode length, leaf elongation rate, leaf to stem ratio, plant height, dry matter and seed weight (Table 2). Tillering increased with an increase in gamma-ray exposure. The 40 Gy dose recorded the highest number of tillers (34) compared to the other doses and the control (Table 3). For the days to 50% flowering, the control took significantly the longest to flower (179 days). The 40Gy treatment took the least number of days though not significantly different from the 10, 20 and 30 Gy doses respectively (Table 3).

Table 1. Soil characteristics of the study site.

| Stage | Depth (cm) | pH | Total N | Organic Carbon | P | Cu | K | Ca | Mg | Mn |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | (%) | | (ppm) | | (%me) | | | |
| Pre-planting | 0-15 | 5.89 | 0.25 | 2.48 | 60 | 2 | 1.3 | 0.7 | 3.21 | 0.38 |
| | 15-30 | 5.89 | 0.23 | 2.3 | 50 | 1.93 | 1.22 | 4.9 | 2.96 | 0.44 |
| Post-harvest | 0-15 | 5.89 | 0.23 | 2.27 | 110 | 1.85 | 0.88 | 3.9 | 2.29 | 0.39 |
| | 15-30 | 5.85 | 0.22 | 2.13 | 115 | 1.99 | 0.9 | 4.3 | 2.35 | 0.43 |

Table 2. ANOVA mean square values for the gamma irradiation effect agronomic performance of *Brachiaria ruziziensis.*

| SOV | *df* | Establishment | Tillering | Days_50 | SER | IL | LER | LL | LSR | PH | DM | SW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replicates | 2 | 0.72 | 1.72 | 40.2 | 0.003 | 0.3 | 0.01 | 24.5 | 0.001 | 93.4 | 0.1 | 166.7 |
| Treatment | 5 | $3.56^{ns}$ | $192.19^{***}$ | $1014.1^{***}$ | $0.02^{**}$ | $087^{ns}$ | $0.06^{**}$ | $83.2^{***}$ | $0.007^{**}$ | $2103.7^{**}$ | $879.6^{***}$ | $9485.7^{***}$ |
| Error | 10 | 0.72 | 0.59 | 19.9 | 0.001 | 0.45 | 0.01 | 5.28 | 0.001 | 237.3 | 0.23 | 316.3 |
| CV (%) | | 0.85 | 2.9 | 2.7 | 10.9 | 11.2 | 20.8 | 8.5 | 10.8 | 14.5 | 1.0 | 8.4 |
| $R^2$ | | 0.73 | 0.99 | 0.96 | 0.88 | 0.52 | 0.8 | 0.89 | 0.76 | 0.82 | 0.99 | 0.94 |

SOV- Source of variation; df- degrees of freedom; *, **, ***- significant at p<0.05, p< 0.01 and p<0.001 respectively; ns- not significant; DAY_50- Days to 50% flowering, SER-Stem elongation rate, IL-Internode length, LER-Leaf elongation rate, LL-Leaf length, LSR-Leaf to stem-ratio, PH-Plant height, DM-Dry matter, SW-seed weight.

Table 3: Effect of gamma irradiation on agronomic performance of *Brachiaria ruziziensis*.

| Treatment | Tillers | Day_50 | SER | IL | LER | LL | LSR | Chlorophyll | PH |
|---|---|---|---|---|---|---|---|---|---|
| Control | $15.7^{d}$ | $179^{a}$ | $0.25^{bc}$ | 6.5 | $0.15^{b}$ | $17.8^{c}$ | $0.25^{c}$ | $46.4^{a}$ | $127.3^{ab}$ |
| 10Gy | $21.0^{d}$ | $160^{b}$ | $0.32^{ab}$ | 6.6 | $0.18^{b}$ | $28.3^{ab}$ | $0.27^{bc}$ | $41.8^{ab}$ | $138.7^{a}$ |
| 20Gy | $24.0^{c}$ | $154^{b}$ | $0.37^{a}$ | 5.7 | $0.17^{b}$ | $31.3^{a}$ | $0.30^{abc}$ | $40.0^{bc}$ | $121.7^{abc}$ |
| 30Gy | $26.7^{b}$ | $154^{b}$ | $0.40^{a}$ | 5.6 | $0.5^{a}$ | $30.3^{ab}$ | $0.31^{abc}$ | $38.0^{bc}$ | $94.3^{bcd}$ |
| 40Gy | $34.0^{a}$ | $150^{b}$ | $0.35^{a}$ | 6.2 | $0.2^{b}$ | $30.7^{a}$ | $0.36^{ab}$ | $35.9^{c}$ | $80.7^{cd}$ |
| Tukey MSD (α=0.05) | 2.2 | 12.7 | 0.09 | NS | 0.3 | 6.5 | 0.1 | 5.3 | 43.7 |

Means followed by different letters within a column are significantly different from each other at α=0.05. MSD- Tukey's mean significant difference; NS- not significant; DAY_50- Days to 50% flowering, SER-Stem elongation rate, IL-Internode length, LER-Leaf elongation rate, LL-Leaf length, LSR-Leaf to stem-ratio, PH-Plant height.

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For the shoot elongation rate, the 30Gy dose recorded the highest shoot elongation rate though not significantly different from the 10, 20 and 40 Gy doses respectively. The control had the least stem elongation rate. The 30Gy dose had the highest leaf elongation rate than the other gamma radiation doses and the control (Table 3). The 20Gy recorded the highest leaf length (31.7 cm). Gamma radiation at the low doses had the leaf length significantly different from the control (Table 3).
The leaf shoot ratio increased with increase in the gamma radiation dose. Conversely, the chlorophyll content decreased with an increase in gamma-ray dosage. The plant height was significantly highest when the seeds were treated with 10Gy radiation dose, while the 40 Gy dose had the shortest plants (Table 3).

**Effect of gamma radiation dose on the dry matter and seed yield of *Brachiaria ruziziensis*:** There was a general increase in the dry matter yield of Brachiaria with the increase in gamma radiation dose. The control had the least dry matter yield while the 40 Gy had the highest dry matter yield (Figure 2). As opposed to the plant height an increase in the gamma irradiation dose led to shorter plants with much lateral growth and more tillers hence the high total dry matter. Similarly, the 40Gy dose led to the highest seed weight yield compared to the low gamma radiation doses of 10 Gy and the control. There was an increase in seed weight as the radiation dose increased from 10 Gy to 40 Gy (Figure 3).

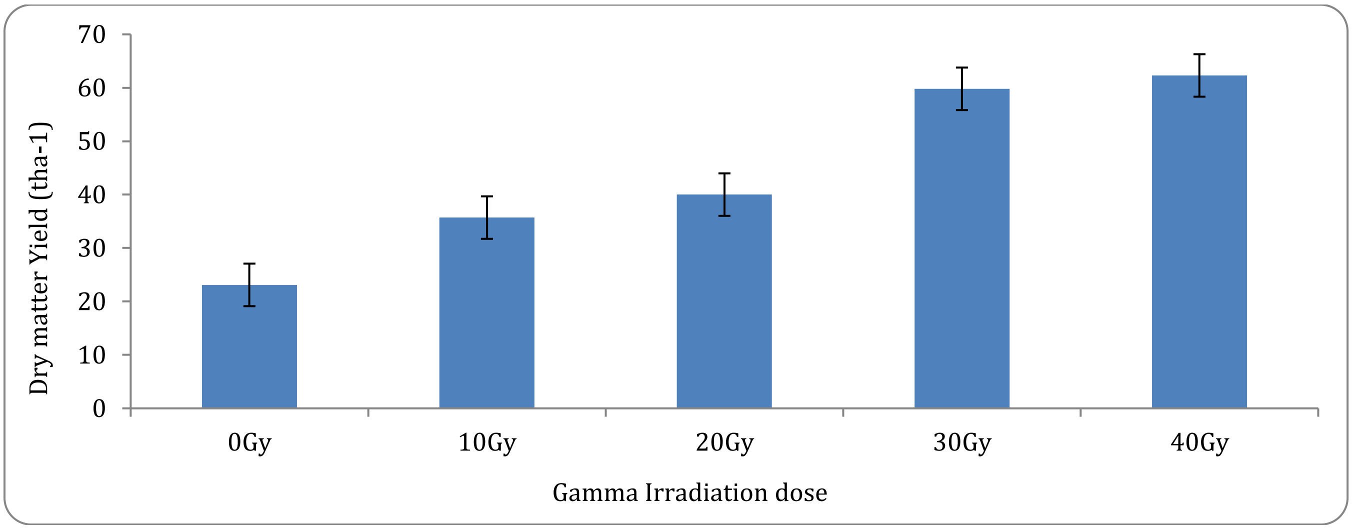


Figure 2. Effect of gamma irradiation dose on the dry matter yield of Brachiaria. Error bars represent the standard error of the means.

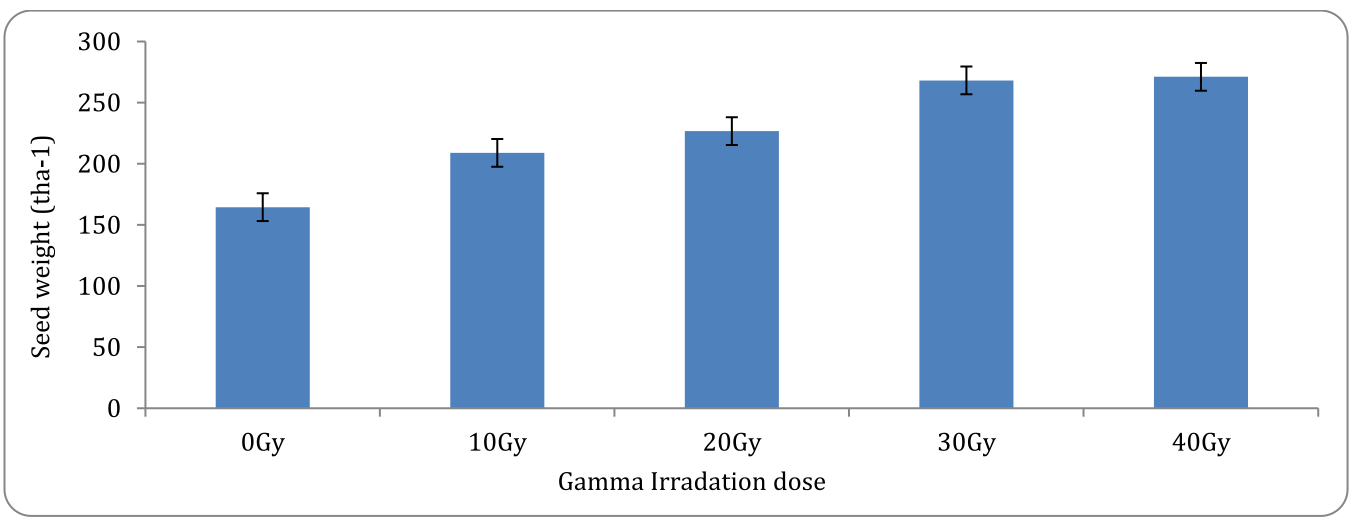


Figure 3: Effect of gamma irradiation dose on the seed yield of Brachiaria. Error bars represent the standard error of the means.

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***Correlation of agronomic variables***: Dry matter weight was significantly (P<0.05) positively correlated to a number of tillers (r = 093) and leaf to stem ratio (r = 0.78); but negatively correlated to the days to 50% flowering, internode length and plant height (Table 4). The seed weight was positively correlated to shoot elongation rate, leaf elongation rate and leaf length; but negatively correlated to the days to 50% flowering (Table 4).

Table 4. Correlation of Agronomic Variables and yield attributes of *Brachiaria ruziziensis*.

| | Tillers | DAY_50 | SER | IL | LER | LL | LSR | PH | DM | SW |
|---|---|---|---|---|---|---|---|---|---|---|
| Tillers | 1 | | | | | | | | | |
| DAY_50 | $0.17^{ns}$ | 1 | | | | | | | | |
| SER | $-0.09^{ns}$ | $-0.88^{***}$ | 1 | | | | | | | |
| IL | $-0.46^{ns}$ | $-0.33^{ns}$ | $0.16^{ns}$ | 1 | | | | | | |
| LER | $-0.03^{ns}$ | $-0.40^{ns}$ | $0.57^{*}$ | $-0.18^{ns}$ | 1 | | | | | |
| LL | $0.34^{ns}$ | $-0.62^{**}$ | $0.64^{**}$ | $-0.18^{ns}$ | $0.33^{ns}$ | 1 | | | | |
| LSR | $0.86^{***}$ | $0.12^{ns}$ | $-0.09^{ns}$ | $-0.38^{ns}$ | $0.03^{ns}$ | $0.32^{ns}$ | 1 | | | |
| PH | $-0.82^{***}$ | $-0.16^{ns}$ | $0.08^{ns}$ | $0.34^{ns}$ | $-0.15^{ns}$ | $-0.15^{ns}$ | $-0.90^{***}$ | 1 | | |
| DM | $0.93^{***}$ | $-0.00^{***}$ | $0.14^{ns}$ | $-0.47^{*}$ | $0.27^{ns}$ | $0.43^{ns}$ | $0.78^{***}$ | $-0.82^{***}$ | 1 | |
| SW | $0.04^{ns}$ | $-0.88^{***}$ | $0.83^{***}$ | $0.03^{ns}$ | $0.59^{**}$ | $0.68^{**}$ | $0.09^{ns}$ | $-0.04^{ns}$ | $0.24^{ns}$ | 1 |

*, **, ***- significant at p<0.05, p< 0.01 and p<0.001 respectively; ns- not significant; DAY_50- Days to 50% flowering, SER-Stem elongation rate, IL-Internode length, LER-Leaf elongation rate, LL-Leaf length, LSR-Leaf to stem-ratio, PH-Plant height DM-Dry matter, SW- seed weight.

**DISCUSSION**

The present results show that seed treatment with $^{60}Co$ gamma radiation decreased plant germination and the establishment in the field. The restriction in germination may have been caused by the high amount of irradiation that resulted in the cell cycle arrest at the G2/M phase during somatic cell division or genome damages, changes in protein synthesis and reduced amount of endogenous growth regulators (Preuss and Britt, 2003; Abdel-Hady *et al.*, 2008; Kiong *et al.*, 2008). Additionally, Shah *et al.* (2008) stated that processes such as destruction of auxin, ascorbic acid content changes, biochemical and physiological disturbances could induce the inhibition of plant germination. These results are in agreement with the findings of previous researchers who reported decreased germination potential and survival rates of different crops due to increased irradiation dose (Irfaq and Nawab, 2001; Chaudhuri, 2002; Marcu *et al.*, 2013; Songul *et al.*, 2015; Warid *et al.*, 2017).

Results from this study showed that gamma radiation increased the days to flowering of the Brachiaria grass. These results are in agreement with Khan *et al.* (2000) who reported that gamma irradiation progressively increased the number of days to 50% flowering at various irradiation doses as compared to control. Similar findings were reported by other authors who showed that gamma irradiated plants especially at high doses took longer to flower compared to the control and low dose treatments (Emrani *et al.*, 2012; Khan, 2015).

This study showed a general decrease in the plant height with an increase in the gamma radiation dose with the high dose (40 Gy) having the least plant height. This phenomenon can be attributed to a decrease in the mitotic activity of meristematic tissue in seeds (Khalil *et al.*, 1986). Similar results were reported by Shereen *et al.* (2009) who showed a significant reduction in the plant height of rice parent varieties as a result of gamma irradiation under saline conditions. However, contrary results have been reported whereby the plant height of *Brachiaria brizantha* was increased as a result of increased gamma radiation dose (Respati *et al.*, 2018).

The other plant growth parameters that were negatively affected by the higher radiation dose included the shoot elongation rate, leaf elongation rate, leaf length and seed weight. However, the low radiation doses performed better than the control. This may be as a result of the modification or damage of important component of plant cells caused by the free radicals. These radicals usually affect the anatomy, morphology, physiology and biochemistry of the plants depending on the dose of the radiation (Ashraf *et al.*, 2004). Toker *et al.* (2005)

showed that the growth of chickpea seeds had a significant increase in plant growth parameters but at 400Gy, a depression in the plant shoot length was observed. Low doses of gamma irradiation stimulate the cell proliferation, cell growth and enzyme activity (Moussa, 2006; El-Beltagi *et al.*, 2011) but the high doses of gamma rays disturb the protein synthesis, enzyme activity and water exchange (Aly and El-Beltagi, 2010). This will also explain the observation on the amount of sucrose, iron and zinc content results from this study.

Tiller numbers, leaf to shoot ratio, the leaf length and the dry matter content progressively increased with the increase in gamma radiation dose. This is in agreement with the finding of Songul *et al.* (2015) who reported that gamma-ray irradiation induced useful morphological variation in Bermuda grass. Similarly, Haris *et al.* (2013) found out that the highest number of tillers was found in plants germinated from paddy seeds radiated with gamma radiation at the 300 Gy in a variety of rice.

Finding from this study showed that the irradiated plants had low chlorophyll content than the control. This observation can be attributed to the effect of gamma irradiation that has been shown to damage pigments in the plants leading to loss of photosynthetic ability Strid *et al.* (1990). Saha *et al.* (2010) stated that irradiation caused a decrease in the chlorophyll content of plants which can result from the release of chlorophyll from its protein complex with subsequent dephytolization and possibly pheophytinization. Similar results were reported by Kiong *et al.* (2008) who showed that there is greater destruction of chlorophyll owing to the disturbance of its biosynthesis or degradation of its precursors.

Correlation analysis from this study showed some positive correlation between the dry matter and seed yield and the morphological characteristics of the plant. These findings are in agreement with the finding of Tudsri and Kaewkunya (2002) who reported morphological characteristics were correlated with DM yield and nutritional quality. This implies that manipulation of these morphological traits through mutagenesis could be used to improve the overall performance of the grasses in terms of the herbage yield for enhanced cattle productivity.

**CONCLUSION**

Results from agronomic performance**,** mineral and sugar levels, nutrient profile**s** indicated that the mutant lines differed from their parents and therefore, these mutant lines could be used as donor parents in forage breeding program and some of them can be recommended as new Brachiaria varieties suitable for Kenya. Future studies should focus on total mixed ration formulation using the mutant lines, designed feeding trials, fractionation of amino and fatty acids from the lines for incorporation in poor forages and national performance trials of mutant lines with an aim of forage variety release.

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