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Mutagenic Effect of Gamma Radiations on Forage Yield of Egyptian Clover (*Trifolium alexandrinum* L.)

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THE OBJECTIVE of the present study was to evaluate the effectiveness of different gamma-ray doses (0, 15, 30, and 45 Kr) during M_1 and M_2 generations on four multi-cut cultivars of Egyptian clover, or berseem (*Trifolium alexandrinum* L.), namely Hatuor, Narmar, Helaly, and Khadrawy. The results revealed that the four cultivars responded differently to treatment with all gamma-ray doses, including a control, and time of cuts in terms of plant height, tillering capacity and fresh forage yield. The M_1 generation showed significant differences in plant height and tillering capacity at the second cut due to gamma doses, while the M_2 generation showed highly significant differences in plant height at the fourth cut. Moreover, in the M_1 populations, the Helali cultivar produced the tallest plants (87.6 cm) at dose of 15 Kr, followed by the Khadrawy cultivar (87.1 cm) at dose of 45 Kr. In the M_2 generation, the Khadrawy cultivar recorded the highest plant height (85.1) at 30 Kr followed by Hatour cultivar (82.4 cm) at 15 Kr of gamma ray at the first cut. The results indicated also that there are no significant differences between gamma-ray doses across all cultivars on fresh and dry yield at each cut and total cuts in both M_1 and M_2 populations. However, significant interaction effects were recorded between cultivar and gamma radiation dosage on such traits in the M_1 generation. This suggests that the optimum dose of gamma radiation in Berseem mutation breeding is significantly influenced by the genotype. In this concern, Hatour cultivar recorded the highest total dry yield in the M_1 generation (18.41 ton h^{-1}), whereas with no mutagen application, the Hatour recorded the highest forage fresh yield (13.5 ton h^{-1}) at the fourth cut in the M_2 generation, it was statistically comparable to 15 Kr of the same cultivar (13.1 ton h^{-1}). The estimates of high heritability in broad sense coupled with high genetic advance as percent of mean were observed for total fresh yield at 45 Kr and 30 Kr in M_1 and M_2 generations, respectively.

Keywords: Gamma irradiation, Egyptian clover, forage yield, heritability, genetic advance.

Introduction

The Egyptian clover or berseem (*Trifolium alexandrinum* L.) belongs to the Leguminasae (Fabaceae) family and is the main annual winter forage leguminous crop in Egypt. In terms of long- and short-season crops as well as winter seed production, it makes up around 30% of the cultivated area. Berseem is high nutritional quality for animal feed. Additionally, it enhances the physical properties of the soil and increases soil fertility (Graves *et al*., 1996). Breeding of Berseem is highly restricted due to a narrow genetic base. There may be additional factors contributing to the low level of genetic improvement in Berseem, such as self-fertility, small, delicate, and complex flower structure, limited outcrossing, and low seed setting (Jindal *et al*., 2014). Therefore, it seems that creating genetic variability through mutation breeding is crucial for enhancing this significant crop. Furthermore, developing crop cultivars through mutation breeding takes less time than conventional breeding (Bolbhat and Dhumal, 2009; Manjaya, 2009).

According to Pathirana (2011), mutation breeding is a crucial tool for creating new genetic resources and severing undesirable connections. While a variety of mutagens, both chemical and physical, are employed to induce mutations, radiation such as X-rays and gamma rays is a physical mutagen that is used more frequently than chemical mutagens. Gamma-ray mutagenesis has been the predominant technique in plant mutation breeding since the 1960s. Gamma-ray irradiation was used to produce 1,600 of the 3,281 mutant cultivars that are officially registered in the FAO/IAEA mutant

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variety database (http://mvgs.iaea.org). Successful utilization of gamma-rays to generate genetic variability in plant breeding has been reported in different crops like *Oryza sativa* (Abdelnour-Esquivel, *et al*. 2020; Choi, *et al*. 2021), *Vicia faba* (Ali *et al*., 2019), *Cicer arietinum* (Amri-Tiliouine, 2018), *Trigonella foenum-graecum* L (Parchin *et al*., 2019), *Vigna unguiculata* L.Walp. (Kang *et al*., 2021; Raina *et al.*, 2022).

Determining the genetic variability between genotypes and assessing the effects of both genetics and environment on the characteristics under study was made easier by estimating various genetic parameters, such as phenotypic and genotypic coefficient of variability (PCV, GCV), heritability (h^2), and genetic advance (GA) for various quantitative traits. The plant trait's range of genetic variability is measured by the genotypic coefficient of variation (GCV); however, the GCV cannot identify how much variation is heritable on its own (Wani, 2011). Understanding heritability is crucial for selection-based improvement because it shows how much a character can be passed down to subsequent generations (Kozgar, 2014). Heritability estimates must be compared with estimates of genetic advance (GA), or the change in mean value between generations, in order to determine the expected gain in the following generation (Wani, 2011). Thus, the goal of this study was to find out how gamma radiation doses, as a mutagenic agent, affected the forage yield of some Egyptian clover cultivars throughout the M_1 and M_2 generations. It also looked into how the gamma-ray treatments affected the genetic variability of the forage yield.

Materials and Methods:

Four cultivars of Egyptian clover (*Trifolium alexandrinum* L.) were employed in this investigation. They are specifically Hatuor, Narmar (cultivars developed by the Department of Agronomy, Faculty of Agriculture, Cairo University), Helaly, and Khadrawy (cultivars developed by the Department of Forage Research, Field Crop Research, Institute of ARC, Egypt). Three doses of gamma radiation (15, 30, and 45 Kr) were applied to the dry seeds of the four Berseem cultivars under investigation. Radiation was obtained from the ^{60}co source installed at the Nuclear Research Center at Inshas, Atomic Energy Authority (AEA) in Egypt with a dose rate of 20,000 Gy/hour.

In the 2020/2021 season (M_1 generation), treated and untreated check seeds were sown in the field on the second day after irradiation (October 22, 2020) at the Experimental Station, Faculty of Agriculture, Cairo University, Giza, Egypt. Regrowth after the fourth cut was left for seed production. M_2 seeds from each radiation treatment were harvested in bulk.

In the 2021/2022 season, original parental seed and open, randomly harvested M_2 seed representing each cultivar-dose treatment were sown on October 18, 2021 as the M_2 population.

The experimental design for each generation was spilt plot design in Randomized Complete Block Design (RCBD) arrangement using three replications, with the Berseem cultivars placed in the main plots and the gamma-ray doses in the subplots with corresponding controls. Each experimental plot had one ridge bed that was 3 m long and 1.5 cm apart, with five rows spaced 25 cm apart. Each plot had a seed rate of 25 g. The recommended cultural practices of growing Egyptian clover were followed to raise average plant growth. In each season, four cuttings were taken. The first cut was taken after 70 days from sowing, and subsequent cuts were made every 40 days.

For both M_1 and M_2 populations, ten plants from the inner rows of each plot were randomly chosen to measure plant height (in cm). Tillering capacity was measured by counting the number of tillers in 30 cm length from each plot at each cut. Plots as a whole were cut to determine the fresh yield and converted to tons hectar^{-1}. 500g samples were taken, dried to constant weight in a 105°C oven, and the dry matter percentage and dry forage yield were computed. The result of four cuts at each season was added up to determine seasonal fresh and dry yield (ton hectar^{-1}). Data were regularly subjected to the analysis of variance in each generation, following the procedure described by Gomez and Gomez (1984) and utilizing the

MSTATC computer program (MSTATC, Michigan State Univ., 1986). The comparison test between treatment means was conducted using the least significant differences method (LSD) according to Snedecor and Cochran (1989).

Additionally, data from each gamma dose was examined independently in order to determine genetic parameters using the randomized complete blocks design (RCBD) form shown in (Table 1).

Table 1. Analysis of variance of each gamma dose as RCBD form.

S.V.	df	MS	E.M. S
Reps. (r)	r-1		
Cultivars (cv)	(cv-1)	MS_g	$\delta^2_e + r\,\delta^2_g$
Error	(r-1) (cv-1)	MS_e	δ^2_e

Where: r and cv are the number of replications and cultivars, respectively.
σ^2_e = error variance, σ^2_g = genotypic variance.

The variances associated with error (σ^2_e), genotypic (σ^2_g), and phenotypic (σ^2_{ph}) were estimated using expected mean squares (E.M. S.), which were obtained from the RCBD ANOVA table in accordance with Hallauer *et al.* (2010) in the following ways:

$\delta^2_e = MS_e/ r$, $\delta^2_g = (MS_g - MS_e)/r$ and $\delta^2_{ph} = \delta^2_e + \delta^2_g$

Broad-sense heritability (h^2_b) and expected genetic advance (GA %) from direct selection of total fresh and dry forage yield for each gamma dose were calculated according to Singh and Chaudhary (1999**)** as follows:

$$h^2_b\% = \frac{\delta^2 g}{\delta^2 ph} \times 100$$

Where, $\sigma^2 g$ = genotypic variance; and $\sigma^2 ph$ = phenotypic variance

$$GA\% = 100\ K\ h^2_b\ \sigma_{ph} / \bar{x}$$

Where $\bar{x}$ = general mean of the appropriate gamma dose, σ_{ph} = square root of the phenotypic variance estimates and K= Selection differential, the value of which is 2.06 at 5% selection intensity in this study.

Moreover, the following formulas were used to compute the genotypic (GCV%) and phenotypic (PCV%) coefficients of variations:

Genotypic coefficient of variations (GCV %) = $\left(\sqrt{\delta^2 g} / \bar{X}\right) \times 100$

Phenotypic coefficient of variations (PCV %) = $\left(\sqrt{\delta^2 ph} / \bar{X}\right) \times 100$

Results and Discussion:

Analysis of variance for the M_1 and M_2 generations

Table 2 shows the mean square for the studied traits of Berseem cultivars and their interaction with gamma-ray doses in the M_1 and M_2 generations. In the M_1 generation, cultivar mean squares showed significant ($P\leq0.05$) difference in plant height at the fourth cut and dry yield at the third cut, as well as a highly significant ($P\leq 0.01$) increase in total dry yield. These variations demonstrated the diversity of the tested berseem cultivars. Dalal *et al*. (2012) reported previously similar results for plant height in berseem as affected by gamma-ray. The plant height and no. of tillers at the second cut showed significant mean squares resulting from gamma doses. The interaction between Berseem cultivars and gamma-ray doses was significant for plant height at the 4th cut. In the M_2 generation, the results presented in Table 2 indicated that the mean squares for the Berseem cultivars were highly significant ($P\leq 0.01$) for plant height at the first cut and significant at the second cut. On the other hand, differences between gamma doses were highly significant for plant height at the 4th cut and fresh yield at the 1st and 4thcuts.

Also, in the M_2 generation, the interaction between cultivars and doses was significant for fresh yield at the fourth cut and highly significant ($P\leq 0.01$) for plant height at the first cut. However, It was observed that, the mean squares of the interactions between cultivars and doses for plant height at the second, third, and fourth cuts; fresh yield at the first, second, and third cuts; and dry yield at all cuts were not significant. This suggests that the interaction between doses and cultivars had a different effect from cut to cut.

Table 2. Mean squares for studied traits of berseem cultivars (cv), gamma doses, and the interaction between them at each cut in M_1 and M_2 generations.

M_1 generation

S.V.	df	Plant height				No. of tillers (30 cm in length)			
		Cut 1	Cut 2	Cut 3	Cut 4	Cut 1	Cut 2	Cut 3	Cut 4
cvs	3	90.5 ^{ns}	56.3	24.1 ^{ns}	319.8*	86.2 ^{ns}	35.2 ^{ns}	50.6 ^{ns}	316.6 ^{ns}
Error	6	126.9	31.6	29.8	39.4	172.9	58.9	143.0	379.2
dose	3	23.4 ^{ns}	85.8*	47.9 ^{ns}	66.2 ^{ns}	15.9 ^{ns}	176.6*	59.8 ^{ns}	159.0 ^{ns}
cvs x	9	77.0 ^{ns}	49.2	83.6 ^{ns}	59.3*	109.7 ^{ns}	44.7 ^{ns}	64.0 ^{ns}	160.8 ^{ns}
Error	24	48.9	28.1	44.6	26.1	68.9	47.3	63.0	181.9

S.V	df	Fresh forage yield					Dry forage yield				
		Cut 1	Cut 2	Cut 3	Cut 4	Total	Cut 1	Cut 2	Cut	Cut4	Total
CVs	3	52.2 ^{ns}	32.7	154.3^{ns}	26.2 ^{ns}	456.1^{ns}	0.7 ^{ns}	0.4 ^{ns}	7.4*	1.3 ^{ns}	14.2**
Error	6	21.7	14.2	37.6	50.1	110.5	0.6	0.2	1.1	1.6	0.91
dose	3	7.3 ^{ns}	16.6	17.7 ^{ns}	0.9 ^{ns}	41.2^{ns}	0.2 ^{ns}	0.4 ^{ns}	0.4 ^{ns}	0.4 ^{ns}	2.3^{ns}
cvs x	9	66.3 ^{ns}	17.9	51.0 ^{ns}	13.7 ^{ns}	399.6^{ns}	0.8 ^{ns}	0.3 ^{ns}	1.7 ^{ns}	0.4 ^{ns}	6.8^{ns}
Error	24	43.1	17.1	36.6	47.6	397.3	0.6	0.4	2.0	1.4	9.8

M_2 generation

S.V.	df	Plant height				No. of tillers (30 cm in length)			
		Cut 1	Cut 2	Cut 3	Cut 4	Cut 1	Cut 2	Cut 3	Cut 4
cvs	3	470.05**	76.9*	7.0 ^{ns}	103.5^{ns}	99.9 ^{ns}	27.0 ^{ns}	46.8 ^{ns}	23.7 ^{ns}
Error	6	19.7	17.2	131.5	34.6	111.2	58.4	26.7	130.1
dose	3	23.3 ^{ns}	16.0	49.0 ^{ns}	56.3*	67.5 ^{ns}	28.7 ^{ns}	45.4 ^{ns}	5.2 ^{ns}
cvs x	9	63.6**	11.2	17.4 ^{ns}	20.6 ^{ns}	78.2 ^{ns}	79.7 ^{ns}	85.6 ^{ns}	62.3 ^{ns}
Error	24	12.8	9.6	19.9	18.7	56.0	59.9	71.4	48.6

S.V.	df	Fresh forage yield					Dry forage yield				
		Cut 1	Cut 2	Cut 3	Cut 4	Total	Cut 1	Cut 2	Cut	Cut4	Total
cvs	3	35.5 ^{ns}	1.4 ^{ns}	5.6 ^{ns}	20.0 ^{ns}	20.8^{ns}	0.4 ^{ns}	0.02 ^{ns}	0.3 ^{ns}	0.3 ^{ns}	0.69^{ns}
Error	6	16.5	11.1	24.0	12.4	57.7	0.3	0.08	0.7	0.36	1.05
dose	3	41.2*	7.5 ^{ns}	5.2 ^{ns}	5.9*	57.9^{ns}	0.5 ^{ns}	0.14 ^{ns}	0.2 ^{ns}	0.1 ^{ns}	0.48^{ns}
cvs x	9	2.9 ^{ns}	6.2 ^{ns}	2.1 ^{ns}	3.6 *	29.6^{ns}	0.2 ^{ns}	0.10 ^{ns}	0.1 ^{ns}	0.04	0.70^{ns}
Error	24	9.7	6.8	4.8	1.5	35.4	0.2	0.10	0.2	0.04	0.61

Mean performance of berseem cultivars

Based on the mean performances of berseem cultivars' overall gamma-ray doses, M_1 generation data indicated that the Khadrawy cultivar exhibited the highest values for plant height (87.9cm) at the 4^{th} cut and was significantly different from the other cultivars (Figure 1). While the Hatour cultivar had the highest values (6.82 ton h^{-1}) for dry yield at the 3^{rd} cut (Figure 2) and (18.41 ton h^{-1}) for total dry yield, it was significantly different from other cultivars except for the Narmar cultivar (Figure 3). In the M_2 generation, the data showed that the Hatour cultivar had the highest values for plant height at the first cut (80.0 cm), but without significant difference from that of the Khadrawy cultivar. Conversely, the Khadrawy cultivar had the highest plant height at the second cut (66.2 cm), which was only significantly different from the Narmar cultivar (Figure 4). Berseem cultivars did not affect the number of tillers per plant at any gamma-ray dose in the M_1 or M_2 generations.

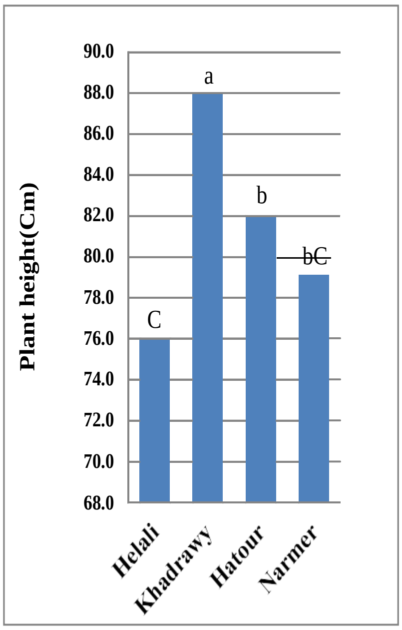

Figure 1: Mean performance of berseem cultivars overall gamma-ray doses for plant height (cm) at the 4th cut in the M_1 generation

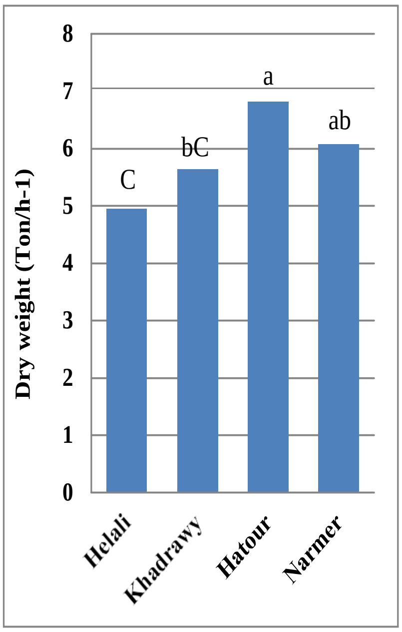

Figure 2: Mean performance of berseem cultivars overall gamma-ray doses for dry yield (ton h^{-1}) at the 3rd cut in the M_1 generation

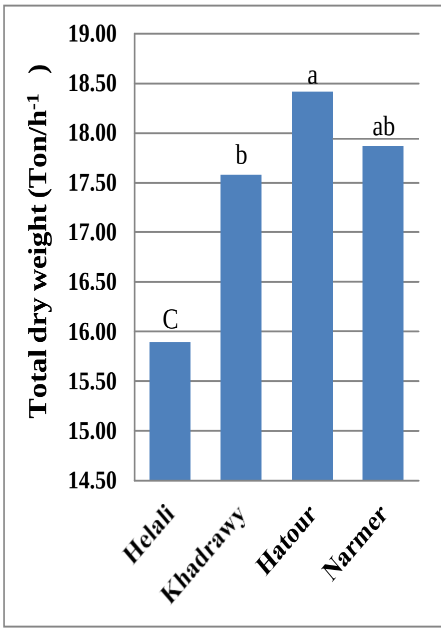

Figure 3: Mean performance of berseem cultivars overall gamma-ray doses for **total dry yield (ton h^{-1}) in the M_1 generation**.

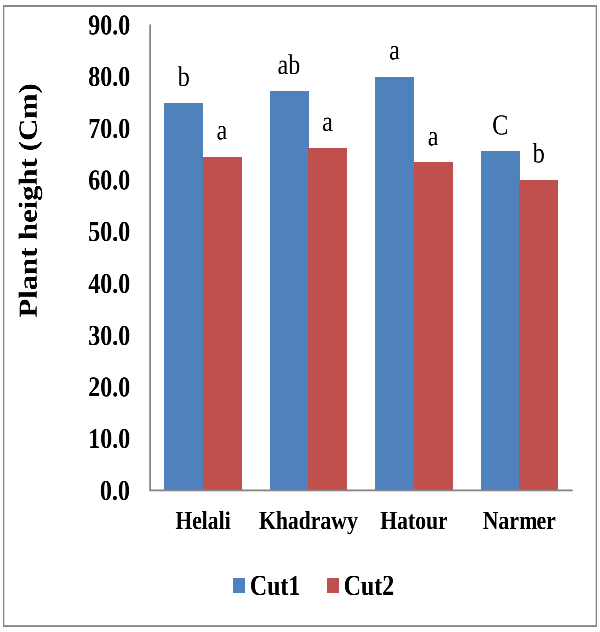

Figure 4: Mean performance of berseem cultivars overall gamma-ray doses for plant height (cm) at 1st and 2nd cuts in the M_2 generation

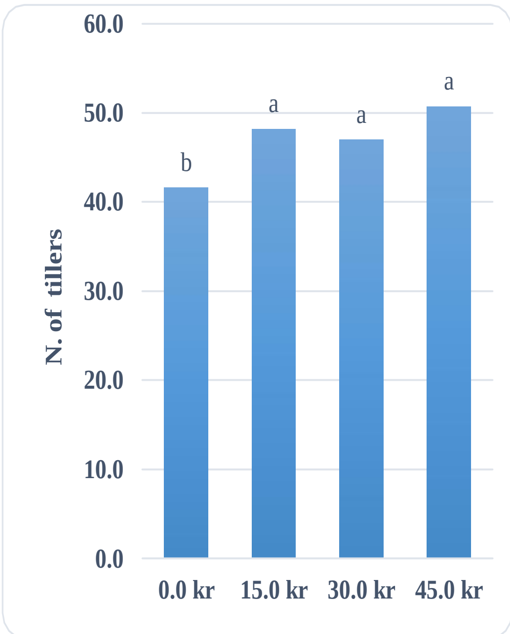

Figure 5: Means of gamma-ray doses over all cultivars for number of tillers (30 cm in length) at 2nd cut in the M_1 generation**.**

Effect of gamma-ray doses

Plant height

The mean performances of each dose of gamma radiation across all cultivars at each cut for studied traits in M_1 and M_2 generations are presented in Table 3. In the M_1 generation, data from Table 3 indicated that the 45 Kr irradiated seeds had the highest plant height (82.2cm), but without significant difference as compared with the control at the first cut. In the second cut, a significant decrease in plant height was observed in the 30 Kr (71.2 cm) as compared to the control (77.7 cm). In the third cut, plant height showed an increasing trend as gamma- ray doses increased from 15 to 30 and 45 Kr, however there was no significant difference in plant height between the control and all irradiation treatments. In the fourth cut, seeds irradiated with 15 Kr recorded the highest plant height (84.2 cm), but without significant difference from the plant height attained by the control (81.8) or other gamma-ray treatments (30 and 45 Kr), which recorded 79.4 and 79.2 cm, respectively. These results are in harmony with those found by HobAllah (1985), who reported that the low and moderate gamma-ray doses (10-50 Kr) had a stimulating, though insignificant effect on plant height of Berseem clover. Also, Arora *et al*. (1994) observed the doses 25 kR followed by 10 Kr of gamma-rays to be the most effective in creating a positive shift for plant height in Berseem. Moreover, another study in Berseem (Anonymous, 2006) indicated that plant height increased with increasing doses of gamma rays. Further, Zayed *et al*. (2013) on alfalfa, found that the dose treatment 300 Gray increased plant height over all cuts.

In the M_2 generation, data in Table 3 revealed that there were no significant differences between gamma-ray treatments including the check control in the first, second and third cuts. However, the fourth cut showed significant differences between gamma-ray treatments, in which the 30 Kr treatment recorded the shortest plant height (74.6 cm), and there were no significant differences between the control and both 15 and 45 Kr doses. In their study of mutation induction on Berseem using three doses (50, 70 and 100 Kr) of gamma-ray, Dalal *et al*. (2012) revealed a wide range of variability for the plant height in M_2 generation. A wide range (35.0-67.2 cm) for plant height was observed in the case of seeds treated with 70 Kr gamma rays. The population raised from 70 Kr also showed a significant positive shift in mean values of plant height, whereas for the remaining treatments (50 and 100 Kr) shift was either negative or non-significant.

The results also agree with previous findings in alfalfa (Natsir *et al***.,** 2018) and grasspea (Waghmare and Mehra, 2000). Furthermore, Dalal *et al.* (2012) found that the best way to induce a positive shift in plant height in Berseem was to administer gamma-ray doses of 25 Kr and 10 kr consecutively. In this study, there was a stimulatory effect on plant height, particularly in M_2 generation at lower doses of gamma irradiation during first, second, and third cuts (Table 3). This is consistent with the detection made by Wi *et al*. (2007) that plants may be stimulated to grow when exposed to low levels of gamma radiation. These authors explained that stimulation of plant growth by low doses of gamma rays occurs through changes in the hormonal signaling network or by increasing the anti-oxidative capacity of the cells to overcome stress factors such as fluctuations in light intensity and temperature during the period of plant growth and development. Further, Zaka *et al*. (2004) stated that variations in the rates of cell division and the activation of growth hormones like auxin in the treated populations are responsible for the stimulatory effect on plant growth.

Tillers/plant

The mean number of tillers of 30 cm length at different cuts in the M_1 and M_2 generations of the various radiation treatments is presented in Table 3. In the M_1 generation, data from Table 3 showed that all gamma-ray doses had significantly more tillers/plant in the second cut, in which the doses of 15, 30 and 45 Kr recorded 48.2, 47.0 and 50.7 tillers/plant compared to the control (41.6) (Fig. 5). Tillers/30cm length did not show significant differences between gamma-ray doses in the 1^{st}, 3^{rd} and 4^{th} cuts. However, gamma-ray doses of 15 and 30 Kr in the 1^{st} cut, and dose of 15 Kr in the 3^{rd} cut as well as all doses in the 4^{th} cut showed numerically more tillers/plant than the corresponding control.

Table 3. Means of different doses of gamma rays for the studied characters at each cut across all cultivars of berseem in the M_1 and M_2 generations.

Cuts	Doses	M_1 generation				M_2 generation			
		Plant height (cm)	No, of tillers (30 cm)	Fresh forage yield	Dry forage yield	Plant height (cm)	No .of tillers (30 cm)	Fresh forage yield	Dry forage yield
1st	Control	79.4	48.8	32.517	4.232	73.0	45.5	11.274	1.454
	15	79.7	50.4	31.611	3.963	74.2	42.7	7.974	1.022
	30	79.1	49.1	31.418	4.005	76.4	39.9	7.031	1.063
	45	82.2	47.6	33.074	3.95	74.2	41.5	8.045	1.036
	$LSD_{0.05}$	ns	ns	ns	ns	ns	ns	2.6	ns
2nd	Control	77.7	41.6	28.496	3.742	64.6	43.7	11.309	1.205
	15	74.8	48.2	29.437	3.967	64.6	44.2	12.067	1.333
	30	71.2	47.0	27.837	3.664	62.5	40.7	11.15	1.153
	45	75.0	50.7	26.635	3.538	62.6	42.8	10.143	1.073
	$LSD_{0.05}$	4.5	4.8	ns	ns	ns	ns	ns	ns
3rd	Control	91.3	43.4	38.204	5.898	73.1	38.6	13.374	1.691
	15	86.8	46.1	36.356	5.986	72.7	41.3	14.364	1.732
	30	87.6	41.0	35.498	5.995	71.2	36.6	14.976	1.892
	45	88.0	42.0	37.574	5.593	68.6	38.1	14.268	1.975
	$LSD_{0.05}$	ns	ns	ns	ns	ns	ns	ns	ns
4th	Control	81.8	54.3	21.609	3.889	79.8	35.8	11.733	1.513
	15	84.2	60.0	22.124	3.941	78.2	35.0	11.239	1.426
	30	79.4	61.3	21.693	3.52	74.6	35.6	10.184	1.334
	45	79.2	62.5	22.12	3.863	77.7	34.3	10.513	1.356
	$LSD_{0.05}$	ns	ns	ns	ns	3.6	ns	1.0	ns
Total	Control	-	-	120.83	17.76	-	-	47.69	5.86
	15	-	-	119.53	17.86	-	-	45.65	5.52
	30	-	-	116.45	17.19	-	-	43.34	5.44
	45	-	-	119.41	16.95	-	-	42.97	5.44
	$LSD_{0.05}$	-	-	ns	ns	-	-	ns	ns

ns:non-sinificant

In the M_2 generation, data from Table 3 indicated no significant differences between gamma-ray doses on the no. of tillers at all cuts. Moreover, all gamma-ray doses showed insignificant reduction for no. of tillers at all cuts except for doses of 15 Kr in the 2nd and 3rd cuts as compared to the control. These results are in harmony with those obtained in Berseem by HobAllah (1985) who stated that the lower gamma-ray doses (20-50 Kr) showed numerically more tillers/plant than the control at the second and third cuts in the M_1 generation. Also, all differences in tillers/plant between such radiation treatments and untreated control did not reach the level of significance in the M_2 generation. On the other hand, Arora *et al*. (1994) observed that the dose of 25 Kr followed by 10 Kr of gamma-rays to be the most effective to create a positive shift for tillers/plant in Berseem.

Fresh forage yield

Results in Table 3 showed that**, in the M_1 generation**, the irradiation treatment at a dose of 45 Kr increased fresh yield at the 1st, 3rd, and 4th cuts but was not significantly different from the control or other doses at the same cuts. In the second cut, an insignificant increase of fresh yield was recorded for 15 Kr gamma-ray dose (29.43 ton h^{-1}) as compared to the control (28.49 ton h^{-1}). Although there are no significant differences in total fresh yield between the gamma-ray treatments including the untreated control, the highest value of total fresh yield (120.83 tons h^{-1}) was recorded for the control treatment, which is comparable to those of plants treated with 15 Kr (119.53 tons h^{-1}) and 45 Kr (119.41 tons h^{-1}). Compared to the control or other gamma-ray doses, plants exposed to a 30 Kr radiation dose produced the lowest total fresh yield (116.45 ton h^{-1}) (Table 3). These results are in accordance with HobAllah (1985) who found that treating Egyptian clover seeds with low and moderate gamma radiation doses (10-50 Kr) insignificantly increased fresh yield over the untreated control in the first cut of M_1 generation. While, in the second cut the 20 Kr dose significantly increased fresh yield by 17% over the control, and other doses *viz*., 10, 30, 40 and 50 Kr insignificantly increased fresh yield. At the last third cut, the differences between radiation treatments and untreated control were not significant. Moreover, total fresh yield of the three cuts (seasonal yield) for the M_1 generation was increased by 13 and 10% over the control at 10 and 20 Kr radiation doses, respectively; and the same trend was generally noted in the M_2 generation. Also in this concern, Shala (2019) reported that the total fresh yield of basil (*Ocimum basilicum* L.) plants gradually decreased as the dose of gamma rays increased from 15 to 30 Kr. Khaled *et al*. (2022) found that irradiation with all gamma-ray doses (0, 50, 150, and 250 Gy) in M_1 generation significantly ($P \leq 0.05$) increased the fresh forage yield of guar plants.

The results in Table 3 revealed that, in the M_2 generation, there was a significant reduction at higher doses compared to the control for fresh yield at the 1st and 4th cuts. In contrast for the 2nd cut, the total fresh yield reduction was insignificant. The highest decrease in fresh yield was recorded in the 30 Kr gamma rays treatment at the 1st cut (7.031 ton h^{-1}) and (10.184 ton h^{-1}) at the 4th cut as compared to the control (11.274 and 11.773 ton h^{-1}) at the 1st and 4th cuts, respectively. The irradiation treatment at a dose of 45 Kr recorded a higher insignificant reduction in fresh yield at the 2nd cut (10.143 ton h^{-1}) compared to the control (11.309 ton h^{-1}). Also, gamma rays at a dose of 45 Kr recorded the lowest total fresh yield (42.97 ton h^{-1}) compared with control (47.69 ton h^{-1}) or other doses (Table 3). In comparison to the control, there was a negligible increase in total fresh yield in the third cut across all gamma-ray doses, in which the 30 Kr yielded the maximum total fresh yield (14.976 ton h^{-1}) compared to the control (13.374 ton h^{-1}).

Dry forage yield

Data on dry forage yield of different cuts of various gamma-ray doses showed no significant difference between treatments in the M_1 and M_2 generations (Table 3). This result was relatively similar to that reported by Hutasoit *et al*. (2022), who found that there was no significant difference between several doses of gamma-ray radiation for dry matter production of Indigofera (*Indigofera zollingeriana*) plants. Although not significantly different in each treatment, numerically, the data obtained in the M_1 generation showed an increase in dry forage yield, but only up to a level of 30 Kr (4.005 and 5.995 ton h^{-1}) at the first and third cuts, respectively, and decreased at a dose of 45 Kr (3.95 and 5.59 ton h^{-1}) in the same cuts. The high values of dry yield at the second and fourth cuts were found at a dose of 15 Kr (3.967 and 3.941 ton h^{-1}, respectively), which are not significantly different ($P > 0.05$) from the untreated control in the same cuts. Generally, for total dry yield in the M_1 generation, there is an insignificant decreasing trend in dry yield with the increasing dose of gamma rays. Table 3 indicates that the 45 Kr gamma-ray dose had the greatest reduction in total dry yield (16.95 ton h^{-1}) when compared to the control (17.76 ton h^{-1}), while the 15 Kr dose yielded the highest total dry yield (17.86 ton h^{-1}). These results are on par with those obtained by HobAllah (1985). In the M_2 generation, data from Table 3 showed no significant differences between gamma-ray treatments compared to control for dry yield at all cuts and total dry yield. However, numerically, the non-irradiation treatment (control) had the highest dry yield at 1st (1.45 ton h^{-1}) and 4th (1.51 ton h^{-1}) cuts and total dry yield (5.86 ton h^{-1}), without a significant difference ($P > 0.05$) as compared to other doses at the same cuts. The highest dry yield, at the second cut, was found in the 15 Kr dose (1.33 ton h^{-1}), which is insignificantly different ($P > 0.05$) from the control (1.20 ton h^{-1}). An insignificant increase in dry yield (0.284 ton h^{-1}) was also noted at 3rd cut for the 45 Kr treatment compared to the non-irradiation treatment (Table 3). These results are not on par with those obtained by HobAllah (1985) who found that the irradiated seeds of berseem with 20, 30 and 50 Kr of gamma-ray doses increased total dry forage yield (ton/fed.) by 10-17% more than the control in the M_2 generation.

Effect of interaction between berseem cultivars and gamma-ray doses

According to Table 2, there was a significant ($P \leq 0.05$) interaction between Berseem cultivars and gamma-ray doses for plant height at the fourth cut in the M_1 generation, as well as a highly significant ($P \leq 0.01$) interaction for plant height at the first cut, and a significant interaction for forage fresh yield at the fourth cut in the M_2 generation. Each of the four cultivars responded differently to gamma ray treatment at all dosages, including control, in terms of plant height and fresh forage yield. In the M_1

generation, plant height varied from 67.6 to 87.6 cm at the fourth cut (Table 4). The tallest plants were recorded in the Helaly cultivar (87.6cm) when irradiated with 15 Kr, followed by the Khadrawy (87.1cm) cultivar when treated with 45 Kr. On the other hand, the shortest plant recorded for Hatour (67.6 cm) with a dose of 15 Kr. Results in Table 4 for the M_2 generation indicated that the cultivar Khadrawy had the tallest plants at the first cut (85.1 cm), whereas the cultivar Narmer had the shortest plants (61.6 cm) after receiving a 45 Kr dose. The highest forage fresh yield (13.5 ton h^{-1}) at the 4^{th} cut was recorded by Hatour receiving no mutagen application (control), and it was statistically similar to 15 Kr of the same cultivar (13.1 ton h^{-1}). These results suggest that the effective dose of gamma radiation, either in a positive or negative direction, in berseem mutation breeding is influenced by the genotype. Yaqoob and Rashid (2001) stated that the desirable mutation/variability in mungbean crop can be possibly created through the gamma-ray, and the effect of gamma-rays was genotype specific for various plant parameters. They suggested that various traits can be improved in various genotypes through variable gamma-ray doses.

Table 4. Effect of interaction between cultivars and gamma rays on plant height at 4^{th} cut in the M_1 generation, plant height at 1^{st} cut and forage fresh yield at 4^{th} cut in the M_2 generation.

Berseem cultivar	Plant height at 4^{th} cut in M_1 generation(cm)				Plant height at 1^{st} cut in M_2 generation(cm)				Fresh yield at 4^{th} cut in M_2 generation (ton h^{-1})			
	Gamma dose (Kr)				Gamma dose (Kr)				Gamma dose (Kr)			
	0	15	30	45	0	15	30	45	0	15	30	45
Helaly	79.1	87.6	82.3	79.0	73.3	70.5	76.4	79.5	12.1	11.7	11.1	11.9
Khadrawy	77.2	82.3	81.1	87.1	70.9	74.7	85.1	78.3	11.7	10.7	9.7	12.4
Hatour	77.3	67.6	78.5	81.1	79.2	82.4	81.0	77.4	13.5	13.1	10.4	10.3
Narmer	84.1	81.3	74.5	81.4	68.6	69.2	62.9	61.6	9.6	9.5	9.6	7.5
$LSD_{0.05}$	7.1				5.0				1.7			

Genetic parameters

Assessing the genetic diversity between genotypes and the influences of both genetic and environmental factors on the traits under investigation was made easier by estimating several genetic parameters. Table 5 presents estimates of the phenotypic (δ^2ph) and genotypic (δ^2g) variances, the phenotypic (PCV) and genotypic (GCV) coefficients of variation, the broad-sense heritability (h^2b), genetic advance (GA), and genetic advance as a percentage of mean (GA%) of the total fresh and dry forage yield traits at each gamma-ray dose in the M_1 and M_2 generations of Berseem. In both M_1 and M_2 generations, the estimations of PCV were greater than those of GCV for the total fresh and dry yield at all gamma-ray treatment dosages, indicating that the environment had an impact on these characteristics. The highest values of PCV and GCV were recorded by 45 Kr of gamma rays in M_1 generation for total fresh yield (15.79; 12.82), followed by total dry yield (15.74; 12.33), respectively.

Moreover, in the M_2 generation, the maximum values of the PCV for total fresh yield (13.66%) and total dry yield (15.81%) were found after 45 Kr of gamma rays. According to Subramanian and Menon (1973), the GCV and PCV values were categorized as low (0%–10%), moderate (10%–20%), and high (>20%). For the fresh and dry forage yield features, the GCV and PCV values found in our study were, respectively, moderate and low. Consequently, in both generations, modest PCV and GCV were predicted for these features (Table 5). This suggested that the phenotype may represent the genotype and that selection may be effective based on these features' phenotypic performance in early mutant generations. Dalal *et al*. (2012) previously published similar results in Berseem.

The estimated heritability helps the plant breeder to select elite genotypes from diverse genetic populations. As stated by Singh *et al*. (2011), thus, prior knowledge regarding the heritability of traits is a requirement for the selection procedure. The heritability percentage categorized as low (0%-30%), moderate (30%–60%), and high (>60%) was, according to Robinson *et al.* (1949). Genetic advance refers to the extent of stability and genetic progress for a particular trait under a suitable selection system. Johnson *et al*. (1955) classified the genetic advance as low (0%–10%), moderate

(10%–20%), and high (>20%). Heritability values coupled with genetic advances would be more reliable in predicting the effect of selection than heritability alone. This is because, according to Khursheed *et al*. (2018), there are estimation errors and genotype-environment interactions that affect the heritability estimates. Results in Table 5 showed that, in general, no trend of increase or decrease in the estimates of heritability and genetic advance was observed with the increasing doses of mutagens for the fresh and dry forage yield traits in the M_1 and M_2 generations. This was in agreement with the report of Dalal *et al*. (2012). However, in the M_1 generation, 45 Kr of gamma rays revealed the highest values of heritability, with genetic advance as a percent of the mean for total fresh yield (65.91; 17.41) and total dry yield (61.33; 15.57). But, in M_2 generation, the estimates of high heritability in broad sense coupled with high genetic advance as percent of mean were observed for total fresh yield (74.86; 14.37) at 30 Kr and for total dry yield (90.05; 19.73) at 15 Kr, followed by (74.09; 12.06) at 30 Kr gamma rays (Table 5). It has been suggested that character with high heritability coupled with high genetic advance as percent of mean would respond to selection better than those with high heritability and low genetic advance. These results are in conformity with the findings of Sangwan *et al.* (2012), who observed in fodder oat (*Avena sativa* L.) that high heritability with high genetic advance for green fodder yield. Additionally, Singh and Singh (2010) reported that the heritability and genetic advance were high for the green fodder yield and dry matter yield of oat.

Table 5. Estimates of genetic parameters of the total fresh and dry forage yield traits at each gamma dose in the M_1 and M_2 generations of berseem.

	Total forage yield (ton h^{-1})							
	Fresh				Dry			
Dose (Kr)	0	15	30	45	0	15	30	45
Parametric	M_1 generation							
δ^2_{ph}	83.00	255.87	211.54	355.61	3.98	4.23	4.53	7.11
δ^2_g	0.00	134.92	11.47	234.39	0.21	1.82	1.12	4.36
PCV%	7.54	13.38	12.49	15.79	11.24	11.52	12.39	15.74
GCV%	0.00	9.72	2.91	12.82	2.57	7.55	6.15	12.33
$h^2_{b\,\%}$	0.00	52.73	5.42	65.91	5.24	43.03	24.65	61.33
GA	0.00	12.62	0.38	20.79	0.05	1.20	0.54	2.64
GA%	0.00	10.56	0.32	17.41	0.28	6.70	3.12	15.57
M_2 generation								
δ^2_{ph}	16.79	8.85	21.78	34.44	0.27	0.38	0.27	0.74
δ^2_g	0.00	1.11	16.31	1.95	0.09	0.34	0.20	0.00
PCV%	8.59	6.52	10.77	13.66	8.87	11.17	9.55	15.81
GCV%	0	2.31	9.32	3.25	4.83	10.56	8.22	0
$h^2_{b\,\%}$	0.00	12.56	74.86	5.65	32.25	90.05	74.09	0.00
GA	0.00	0.27	6.23	0.16	0.20	1.09	0.69	0.00
GA%	0.00	0.60	14.37	0.38	3.37	19.73	12.61	0.00

δ^2_{ph}= phenotypic variances, δ^2_g = genotypic variances, PCV= phenotypic coefficients of variation, GCV= genotypic coefficients of variation, h^2_b = heritability in broad-sense, GA=genetic advance, GA%=genetic advance as percent of mean.

Conclusion

Studying the effects of various gamma-ray dosages on plant height, tillering capacity, fresh and dry yields, and overall yield of fresh and dry forage yields of four Egyptian clover cultivars in the M_1 and M_2 generations revealed that the Helali cultivar, which was irradiated with 15 Kr, produced the tallest plants (87.6 cm), followed by the Khadrawy cultivar (87.1 cm), which was treated with 45 Kr at 4^{th} cut of the M_1 generation. The highest forage fresh yield (13.5 ton h^{-1}) at the 4^{th} cut of the M_2 generation was recorded by Hatour receiving no mutagen application (i.e., control), and it was statistically similar to 15 Kr of the same cultivar (13.1 ton h^{-1}). The estimates of high heritability in a broad sense coupled with high genetic advance as a percent of the mean were observed for total fresh yield at 45 Kr and 30 Kr in M_1 and M_2 generations, respectively.

It is worth noting that by reviewing research related to the effect of different doses of gamma rays to inducing beneficial mutations of Berseem for either the forage yield or its related traits, it became clear that a higher dose rate could be employed than that used in the current study, indicating the tolerance of Egyptian clover seeds to higher doses, possibly up to 100 kr. Accordingly, it is suggested that when Berseem breeding by induced mutations using gamma-rays, higher doses may be used than in this study.

Consent for publication:

All authors declare their consent for publication.

Author contribution:

The manuscript was edited and revised by all authors.

Conflicts of Interest:

The author declares no conflict of interest.

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