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RESEARCH ARTICLE

Inducing potential mutants in rice using different doses of gamma rays for improving agronomic traits

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ABSTRACT

Mutation breeding offers a simple, fast, and efficient way to rectify major defects without altering their original identity. The current research aimed to study interesting traits in a mutant population generated by gamma rays. Five gamma rays doses (100, 200, 300, 400, and 500 Gy) were applied to develop M_1 and M_2 mutant populations from the Egyptian rice (*Oryza sativa* L.) 'Giza177'. Both populations were evaluated in the field during two successive years 2022 (M_1) and 2023 (M_2). Several traits related to growth and yield were recorded such as plant height, number of branches and panicles, panicle weight, grain yield, and harvest index. Significant differences were found among radiation treatments for most traits. Overall, low radiation doses (100, 200, and 300 Gy) promoted the growth and productivity of plants; however, high doses (400 and 500 Gy) decreased these traits and increased the number of sterile plants. The effect of radiation doses was different on some traits between M_1 and M_2. The mutant population M_2 displayed several promising mutant lines with desirable traits; early flowering (4-7 d earlier), short plant height (< 95 cm), panicle length (> 23 cm), tiller number per plant (> 25), and grain yield per plant (> 43 g). Conclusively, low gamma rays doses (inferior to 300 Gy) are of interest in generating desirable traits and promising lines. The current promising mutant lines should be further evaluated in multi-year trials to validate their behavior across various field conditions.

Key words: Agronomic traits, gamma rays, induced mutation, *Oryza sativa*, rice improvement.

INTRODUCTION

Rice (*Oryza sativa* L.) is the primary cereal food staple for 3.5 billion people around the world, providing 20% of the calories in their diets (Fukagawa and Ziska, 2019). Its cultivated area is approximately 165 million hectares and produces annually about 776 million tons (FAOSTAT, 2023). Global consumption of rice is projected to increase to 852 million tons by 2035 (Brar and Khush, 2018). Besides, the genetic diversity of rice, which is indispensable for any crop improvement, was narrowed down because of a high-pressure application in rice breeding (Viana et al., 2019); accordingly, elite rice cultivars show narrow genetic variability. In this context, increasing rice genetic variability and releasing new rice cultivars adapted to climate change, that could satisfy the growing population need, are among the main goals of rice breeders. In the last decades, breeders have given more attention to mutation breeding as a fast method to increase genetic variability (Ma et al., 2021), followed by selecting the superior cultivars to create new genotypes (Robertson et al., 2018).

Mutation breeding could be started by inducing variability through physical, chemical, and biological agent applications (Serrat et al., 2014). Gamma rays are the most prevalent type of physical mutagen as these rays could enhance mutation frequency (Ali et al., 2015). The gamma rays act by altering molecules directly within cells (Barela et al., 2022) or by producing radicals from water in a very short time, then these radicals react with one another or neighboring, unaffected molecules, breaking chemical bonds or oxidizing the molecules. Gamma rays cause firstly DNA breakage in cells (Di Pane et al., 2018); therefore, single or double DNA strands can be affected. These inaccurate repairs result in DNA mutations that affect the structure and metabolism of cellular plants (Chatterjee and Walker, 2017). Mutations induced by gamma rays influence cytological, biochemical, physiological, and morphogenetic changes in cells and tissues, and ultimately on the growth and development of plants (Abdulhafiz et al., 2018). Moreover, Robertson et al. (2018) reported that radiation-induced mutagenesis could be a source of mutants tolerant to drought and salinity.

The effect of gamma rays depends on crop species, irradiation dose (Caplin and Willey, 2018), and germination stage (Majeed et al., 2018). For instance, increasing gamma rays dose resulted in a decline in plant fertility (Kadhimi et al., 2016). According to Ali et al. (2015), the effect of gamma rays on plant height and tiller number differed according to wheat cultivars. In addition, seed irradiation during the pre-sowing is one of the most effective methods to improve plant production, yield components, and chemical composition (Majeed et al., 2018). Several major crops such as wheat, rice, barley, cotton, and chickpea have been improved by induced mutations (Oladosu et al., 2016). New barley cultivars were successfully created, through induced mutation, to have early maturity, high protein levels, and stiff straw (Prasad et al., 2022). Likewise, the induction of useful short-height and early-maturing mutants rice cultivars was successfully performed using gamma rays (Andrew-Peter-Leon et al. 2021).

Rice is among the crops with the highest number of mutants (821 entries) (FAO/IAEA, 2019), with differential characteristics for agronomic traits and consumer-defined quality recognized (Abdelnour-Esquivel et al., 2020). This high number of mutants might be due to that the mutant cultivars are readily accepted better than those created by transgenesis and can be developed in a shorter time than by conventional breeding methods (Masoabi et al., 2018). Mutation breeding, among other breeding methods, aims at inducing genetic variations followed by trait selection to improve some trait defects without altering the genuine characteristics of plants (Oladosu et al., 2016). In this respect, the present study aims to investigate the effect of different doses of gamma rays on several traits of interest in growth and production in a mutant population created from the Egyptian rice ‘Giza177’.

MATERIALS AND METHODS

Plant material and irradiation treatment

The current study was carried out during two successive summer seasons (2022 and 2023) at the Experimental Farm of the Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt. Samples of 500 dry, healthy, and uniformed size grains of Egyptian rice (*Oryza sativa* L.) ‘Giza177’ (Giza171/Yomji No.1//Pi No.4) were exposed to gamma rays from ^{60}Co source using five doses: 100, 200, 300, 400, and 500 Gy. A sample of non-irradiated seeds was used as a control. Irradiation was performed at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt.

M_1-generation

In the first year (2022), the irradiated rice seeds by different gamma rays dosages along with the control (non-irradiated seeds) were grown on germination plastic plates, and the percentage of germinated seeds was recorded 15 d after sowing. Then, the 30-d-old rice seedlings were transplanted in rows in the field on a plot using an intra-row spacing of 20 cm with one seedling per hill and a row spacing of 20 cm. Randomized complete block design (RCBD) with three replicates was applied. During the emergence of the panicles, 50 rice plants were randomly chosen from each treatment, and their first three panicles were bagged to prevent outcrossing. At the maturity stage, the bagged and unbagged tillers were harvested separately for every single plant.

M_2-generation

In the second year (2023), the seeds harvested from the three tillers (main, lateral-I, and lateral-II) of the 50 chosen plants were sown in the nursery. Afterward, the 30-d-old seedlings were transplanted into a carefully prepared field area. The chosen 50 plants progenies (M_2 generation) were grown in a randomized complete block design (RCBD) with three replicates in a progeny row trial with a 20 × 20 cm spacing for each treatment. Each treatment included 20 rows per replicate (500 plants). Viable mutant rice plants in M_2 progeny were evaluated for various morphological and growth traits to identify any visible mutations. All farming practices were performed as recommended in the region.

Trait's measurements

The following traits were recorded on M_1 and M_2 generation plants. Germination percentage (G%) was calculated as 100 times of the ratio of number of seeds germinated to the total number of seeds sown for germination. Survival percentage (S%) was calculated as 100 times of the ratio of the number of survival seedlings at transplanting to the total number of seedlings. Days to heading (DTH, d). Plant height (PH, cm) was measured from the base of the plant to the top of the panicle. Number of tillers per plant (TN) and number of panicles per plant (NPPt). Panicle length (PL, cm) was recorded from the base of the rachis to the tip of the panicle. Fertility percentage (F%): 100 times the number of filled grains to the total number of grains. The 100-grains weight (100GW, g) was recorded based on 100 grains randomly selected from each plant and weighed at about 14% moisture content. Number of sterile plants (NSPt). Harvest index (HI): (Grain yield/(Grain yield + Straw yield)). Grain yield per plant (GYPt, g) was the weight of grains harvested from each single plant.

Statistical analyses

Variances in M_1 and M_2 populations were calculated using Burton's proposed formula (Burton, 1952). Duncan's multiple range test (DMRT) was used for comparing means. Genetic advance (GA) expected genetic advance (EGA), were computed following Johnson et al. (1955) and heritability in the broad sense meaning (H) following Falconer (1989). The principal component analysis (PCA) was performed using the FactoMineR R package (Husson et al., 2024).

RESULTS

Traits related to growth

The ANOVA displayed highly significant differences among treatments (radiation treatment) for the percentage of germination (G%) and plant survival (S%), plant height (PH), and the number of tillers per plant (TN) (Table 1). All the above-mentioned traits were significantly different among treatments for M_1 and M_2 generations except for G%, which was only significant for M1. However, the ANOVA revealed nonsignificant difference among treatments regarding days to heading (DTH) and the number of branches per plant (Table 1).

The number of sterile plants (NSPt) was much higher in mutants generated from grains treated with 400, and 500 Gy compared to control, and low radiation doses; however, these radiation doses (400 and 500 Gy) showed, overall, the lowest values for the remaining traits related to growth. The S% was higher in control and 100 Gy radiation treatment. The M_1 plants, treated with 300, 400, and 500 Gy were taller compared to other treatments. Compared to M_1 plants, the PH in M_2 showed the highest values for 200 and 300 Gy, and the lowest values for control (Table 1). The highest number of tillers was observed for the M_1 plants treated with 100 and 200 Gy and for the M_2 plants treated with 100 and 300 Gy compared to other treatments including control. The 100 Gy dose along with the control treatment displayed the highest values for panicle length (PL) compared to other treatments (Table 1).

Table 1. Mean performance of M_1 and M_2 'Giza177' rice generations for growth-related traits in rice. Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test. ^{ns}Nonsignificant; $^{*}P < 0.05$; $^{**}P < 0.01$.

	Germination percentage		Survival percentage		Days to heading		Plant height		Number of tillers per plant		Number of branches per panicle		Panicle length	
Mutants	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M^2
	—— % ——		—— % ——		—— d ——		—— cm ——		Nr plant^{-1}		Nr panicle^{-1}		—— cm ——	
Control	95.33±1.53^{a}	95.66±2.27^{a}	95.26±1.42^{a}	95.26±1.71^{a}	95.33±0.85^{a}	95.33±0.47^{a}	100.00±2.57^{ab}	99.00±2.84^{c}	19.33±0.49^{b}	19.00±1.11^{c}	12.00±0.94^{a}	11.33±1.15^{a}	21.03±1.12^{b}	21.03±1.30^{a}
100GY	91.00±2.65^{a}	92.00±3.46^{ab}	87.66±2.08^{b}	93.40±2.00^{b}	95.33±1.24^{a}	95.33±0.53^{a}	95.00±1.73^{b}	103.00±3.11^{ab}	21.00±0.23^{a}	22.66±1.18^{a}	11.66±0.78^{a}	11.33±1.21^{a}	23.90±0.85^{a}	19.90±0.67^{ab}
200GY	85.00±2.00^{b}	92.66±1.69^{ab}	83.33±2.89^{c}	91.16±1.93^{c}	95.33±0.58^{a}	94.66±1.36^{a}	99.00±1.25^{ab}	104.66±1.53^{a}	21.00±0.35^{a}	21.66±0.91^{b}	11.66±0.65^{a}	11.66±0.82^{a}	18.80±0.72^{c}	19.00±1.41^{b}
300GY	83.66±1.53^{b}	93.66±1.53^{ab}	80.33±1.26^{d}	90.96±1.11^{c}	95.33±1.33^{a}	94.66±0.81^{a}	103.00±1.73^{a}	104.66±2.08^{a}	19.66±0.71^{b}	23.66±1.07^{a}	10.66±0.88^{a}	12.00±1.00^{a}	18.33±0.91^{c}	19.33±0.94^{b}
400GY	80.00±2.65^{b}	92.00±1.73^{ab}	74.66±1.58^{d}	90.20±1.53^{c}	95.66±0.98^{a}	94.66±0.98^{a}	102.66±2.01^{a}	102.66±1.92^{ab}	18.33±0.95^{c}	18.33±0.72^{c}	10.66±1.13^{a}	11.33±0.79^{a}	19.33±0.64^{c}	18.40±0.53^{b}
500GY	69.66±2.03^{c}	89.66±1.74^{b}	54.33±2.66^{e}	88.16±2.41^{d}	96.00±1.27^{a}	94.66±0.65^{a}	104.33±1.53^{a}	101.33±2.36^{b}	14.66±0.64^{d}	17.33±0.66^{d}	10.66±0.67^{a}	11.66±1.53^{a}	19.66±1.23^{c}	18.50±0.79^{b}
ANOVA	Mean square													
Replicate	9.55^{ns}	1.55^{ns}	6.84^{ns}	6.04^{ns}	1.16^{ns}	0.05^{ns}	1.5^{ns}	5.05^{ns}	1.16^{ns}	0.12^{ns}	0.06^{ns}	0.38^{ns}	2.12^{ns}	1.01^{ns}
Mutant	239.95**	11.92^{ns}	592.24**	92.07**	0.23^{ns}	0.35^{ns}	42.23*	13.95**	16.66**	17.46**	1.16^{ns}	0.22^{ns}	12.51**	2.92**
Error	7.68	3.95	10.56	3.86	0.0007	0.38	9.83	0.85	0.0023	0.29	0.92	1.05	0.67	0.42

Traits related to fertility and grain yield

The results indicated that the traits related to productivity were highly affected by radiation treatment (Table 2) for both generations M_1 and M_2. The number of panicles per plant (NPPt) was improved in the exposed ones to 100, 200, and 300 Gy doses; however, the lowest NPPt was found in the highest gamma rays doses (400 and 500 Gy). The control plants produced the heaviest panicle followed by 100 Gy, while the lowest values of panicle weight were observed for 400 and 500 Gy. For the M_1 generation, the weight of 100 grains (100GW) was found to be the highest in 100 and 300 Gy and the lowest in 400 and 500 Gy; however, for the M_2 generation, the 300 Gy treatment allowed to have the highest 100GW values, whereas the lowest values were observed for control and 200 Gy treatments. The fertility percentage (F%) was higher in control and decreased progressively with increasing gamma rays doses. The grain yield (GY) showed different patterns between M_1 and M_2 in response to different radiation treatments. In M_1, the control yielded higher grains, whereas the 400 and 500 Gy showed the lowest values. Regarding M_2, the control along with 400 and 500 Gy treatment displayed the lowest GY values whereas the 300 Gy showed the highest GY (Table 2). For the harvest index (HI), the highest values were observed for 300 Gy, and the lowest ones were found for 500 Gy for M_1 and control, 400 and 500 Gy for M_2 (Table 2).

Genetic parameters

The induced variation was appreciable for all the recorded traits (Table 3). In M_1 plants, the harvest index had the lowest genotypic variance (GV) and phenotypic variance (PV) values, while the survival percentage had the highest values (Table 3). Grain yield (GY) showed the greatest genetic advance (GA) and expected genetic advance (EGS) compared to other traits, as well as it (GY) was among the traits having the highest heritability (H) values like panicle weight, number of sterile plants and 100-grains weight. Phenotypic and genotypic coefficients showed similar values, where the highest ones were found for the number of sterile plants (Table 3).

For M_2 plants, grain yield displayed the highest values in genotypic variance (GV), phenotypic variance (PV), and genetic advance (GA), while harvest index, days to heading, panicle weight, and number of branches per panicle showed the lowest values. The heritability was appreciable for all traits except for days to heading. The genetic and phenotypic coefficients of variation were highest in number of sterile plants and 100-grains weight and lowest for most traits (Table 3).

Table 2. Mean performance of M_1 and M_2 'Giza177' rice generations for traits related to panicle and grain yield in rice. Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test. ^{ns}Nonsignificant; $^{**}P < 0.01$.

Mutants	Number of sterile plants		Number of panicles per plant		Panicle weight		100 grains weight		Fertility percentage		Grain yield		Harvest index	
	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
	—— Nr ——		— Nr $plant^{-1}$ —		—— g ——		—— g ——		—— % ——		— g $plant^{-1}$ —		—— % ——	
Control	$0.001±0.00^c$	$0.001±0.00^c$	$19.33±1.58^{ab}$	$19.00±0.86^c$	$3.23±0.36^a$	$3.90±0.11^a$	$2.77±0.11^b$	$2.76±0.26^c$	$95.2±2.16^a$	$95.23±1.51^a$	$42.33±1.47^a$	$40.50±1.87^d$	$0.44±0.007^c$	$0.43±0.013^c$
100GY	$0.05±0.00^c$	$0.001±0.00^c$	$20.20±1.35^a$	$22.56±1.28^a$	$2.80±0.23^b$	$3.53±0.28^b$	$2.83±0.17^a$	$2.77±0.26^b$	$89.93±2.64^b$	$94.53±1.79^a$	$37.20±1.68^b$	$51.00±1.35^b$	$0.46±0.019^b$	$0.47±0.012^{ab}$
200GY	$0.08±0.00^c$	$0.005±0.00^c$	$20.10±1.17^a$	$21.33±1.14^b$	$2.53±0.31^c$	$3.46±0.14^b$	$2.77±0.24^b$	$2.73±0.18^d$	$86.76±2.14^b$	$93.73±1.64^a$	$33.30±1.73^c$	$45.00±1.29^c$	$0.45±0.010^{bc}$	$0.46±0.014^{abc}$
300GY	$0.01±0.00^c$	$0.006±0.00^c$	$18.50±1.50^b$	$23.33±1.29^a$	$2.80±0.49^b$	$3.40±0.20^b$	$2.82±0.16^a$	$2.82±0.14^a$	$82.46±2.58^c$	$91.16±1.80^b$	$34.00±1.51^c$	$55.33±1.53^a$	$0.52±0.005^a$	$0.49±0.020^a$
400GY	$34.66±3.06^b$	$3.33±0.90^b$	$16.33±1.58^c$	$17.63±1.35^d$	$2.33±0.82^d$	$3.36±0.58^b$	$2.74±0.11^c$	$2.78±0.10^b$	$82.06±2.83^c$	$83.83±1.59^c$	$29.10±1.36^d$	$38.33±1.56^e$	$0.44±0.009^c$	$0.43±0.010^{bc}$
500GY	$96.33±4.77^a$	$6.33±1.08^a$	$12.00±1.23^d$	$16.00±1.00^e$	$1.96±0.78^e$	$2.90±0.10^c$	$2.70±0.21^d$	$2.78±0.13^b$	$71.80±2.75^d$	$83.16±1.61^c$	$21.13±1.81^e$	$33.00±1.70^f$	$0.38±0.016^d$	$0.43±0.011^{bc}$
ANOVA	Mean square													
Replicate	2.16^{ns}	0.22^{ns}	0.82^{ns}	0.85^{ns}	0.0005^{ns}	0.002^{ns}	0.001^{ns}	0.002^{ns}	9.22^{ns}	0.48^{ns}	1.39^{ns}	1.26^{ns}	0.001^{ns}	0.007^{ns}
Mutant	4573.03^{**}	21.38^{**}	29.79^{**}	25.24^{**}	0.57^{**}	0.31^{**}	0.007^{**}	0.002^{**}	192.14^{**}	88.49^{**}	157.03^{**}	206.21^{**}	0.005^{**}	0.002^{**}
Error	16.7	0.08	0.249	0.22	0.002	0.002	1.76	7.22	3.82	0.64	0.64	1.03	0.006	0.001

Table 3. Genetic parameters of the yield and its attributes characters in M_1 and M_2-irradiated 'Giza177' rice populations. PV: Phenotypic variance; GV: genotypic variance; EV: environmental variance; PCV: phenotypic coefficient of variation; GCV: genotypic coefficient of variation; GA: Genetic advance; EGA: expected genetic advance; H: heritability.

Characters		PV	GV	EV	PCV	GCV	GA	EGA	H
Germination, %	M1	79.98	77.42	2.56	10.63	10.46	17.28	20.55	0.94
	M2	3.97	2.66	1.32	2.15	1.76	3.06	3.31	0.50
Survival, %	M1	197.41	193.89	3.52	17.73	17.57	55.05	69.45	0.96
	M2	30.69	29.40	1.29	6.05	5.92	25.78	28.16	0.92
Day to heading, d	M1	0.08	0.07	0.00	0.29	0.29	1.58	1.65	0.94
	M2	0.13	0.01	0.13	0.38	0.06	0.03	0.03	0.01
Plant height, cm	M1	14.08	10.80	3.28	3.73	3.27	18.82	18.73	0.62
	M2	4.65	4.37	0.28	2.10	2.04	17.29	16.86	0.89
Panicle length, cm	M1	4.17	3.95	0.22	10.12	9.85	18.45	91.47	0.90
	M2	0.97	0.83	0.14	5.10	4.72	8.17	42.19	0.75
Tillers per plant, nr $plant^{-1}$	M1	5.55	5.55	0.00	12.41	12.40	28.41	149.56	1.00
	M2	5.82	5.72	0.10	11.87	11.77	30.48	149.91	0.97
Panicle weight, g	M1	0.19	0.19	0.00	16.71	16.68	6.09	233.32	0.99
	M2	0.10	0.10	0.00	9.39	9.38	4.83	141.16	1.00
Branches per panicle, nr $panicle^{-1}$	M1	0.39	0.08	0.31	5.54	2.52	0.15	1.32	0.12
	M2	0.07	0.06	0.02	2.34	2.06	0.52	4.52	0.63
Sterile plants, nr	M1	1524.30	1523.79	0.56	178.84	178.80	158.40	725.54	1.00
	M2	7.13	7.10	0.03	165.81	165.50	13.41	832.76	0.99
Panicles per plant, nr $plant^{-1}$	M1	9.93	9.85	0.08	17.76	17.69	18.78	105.84	0.98
	M2	8.41	8.34	0.07	14.52	14.46	20.12	100.75	0.98
100 grain weight, g	M1	0.59	0.58	0.00	27.63	27.58	6.12	220.97	0.99
	M2	2.41	2.41	0.00	55.94	55.93	14.05	506.66	1.00
Fertility, %	M1	64.05	62.77	1.27	9.45	9.35	77.37	91.34	0.96
	M2	29.50	29.28	0.21	6.02	5.99	59.21	65.58	0.99
Grain yield, g	M1	52.34	52.13	0.21	22.03	21.98	86.54	263.51	0.99
	M2	68.74	68.39	0.34	18.90	18.86	107.20	244.42	0.99
Harvest index	M1	0.02	0.02	0.01	9.14	8.59	0.45	101.79	0.79
	M2	0.01	0.01	0.01	5.57	5.41	0.34	74.73	0.89

Trait's relationships

In M_1 plants, GY presented high and positive correlations (0.97-0.99) especially with G%, S%, PW, and F%, whereas negative correlations were found with days to heading (DTH, -0.90), number of sterile plants (NSPt, -0.89) and plant height (PH, -0.62) (Table 4). Days to heading and PH were positively correlated (0.63) and they presented negative correlations with most of the traits. Remarkably, NSPt was negatively correlated with all measured traits but with DTH and PH. Other interesting correlations of DTH with panicle weight (PW) (-0.86) and number of panicles per plant (NPPt, -0.98) indicate early M_1 lines would have a high number and heavier panicles (Table 4).

Regarding the correlations among M_2 plants and in comparison, with those among M_1 plants, GY presented a high correlation with NPPt (0.98), tiller number (0.97) and HI (0.95), while only a negative correlation was found with NSPt (-0.79) (Table 4). Both, PH and DTH were negatively associated (-0.56) unlike M_1 (0.63). In addition, DTH presented a negative correlation with NSPt (-0.47) and PH was negatively correlated with PL (-0.52) (Table 4).

The number of branches per plant was positively correlated with 100 grains weight in M_2 plants but not in M_1 plants. The percentage of fertility (F%) was significantly associated with all traits in M_1 generation except for HI, with negative correlations with DTH, PH, NSPt. For M_2 generation, F% displayed correlations with traits except PH, NBP, and 100 GW, with a negative correlation with NSPt (Table 4).

Table 4. Pearson correlation coefficients for the measured traits among the M_1 (below diagonal) and M_2 'Giza177' rice plants (above diagonal). *P < 0.05; **P < 0.01. G%: Percentage of germination; S%: survival percentage; DTH: days to heading; PH: plant height; PL: panicle length; TN: tiller number; PW: panicle weight; NBP: number of branches per panicle; NSPt: number of sterile plants; NPPt: number of panicles per plant; 100GW: weight of 100 grains; Fertility %: percentage of fertility; GYPt: grain yield per plant; HI: harvest index.

		M_2 generation													
		G%	S %	DTH	PH	PL	TN	PW	NBP	NSPt	NPPt	100GW	F%	GYPt	HI
M_1 generation	G%		0.82**	0.48*	-0.24	0.80**	0.31	0.92**	-0.09	-0.78**	0.42	-0.05	0.71**	0.40	0.16
	S%	0.99**		0.87**	-0.39	0.94**	0.34	0.95**	-0.47*	-0.77**	0.42	-0.27	0.85**	0.37	0.1
	DTH	-0.88**	-0.93**		-0.56*	0.87**	0.16	0.70**	-0.63**	-0.47*	0.22	-0.22	0.66**	0.18	-0.05
	PH	-0.70**	-0.65**	0.63**		-0.52*	0.68**	-0.28	0.58*	-0.26	0.61**	0.14	0.02	0.61**	0.75**
	PL	0.50*	0.38	-0.22	-0.81**		0.25	0.85**	-0.31	-0.66**	0.34	-0.15	0.81**	0.30	0.07
	NT	0.80**	0.86**	-0.95**	-0.74**	0.27		0.33	0.38	-0.80**	0.99**	0.12	0.68**	0.97**	0.95**
	PW	0.95**	0.94**	-0.86**	-0.49*	0.35	0.69**		-0.41	-0.82**	0.43	-0.28	0.81**	0.37	0.09
	NBP	0.82**	0.78**	-0.64**	-0.74**	0.55*	0.61**	0.69**		-0.01	0.35	0.48*	-0.08	0.40	0.61**
	NSPt	-0.88**	-0.93**	0.99**	0.61**	-0.2	-0.96**	-0.84**	-0.60**		-0.85**	0.16	-0.92**	-0.79**	-0.61**
	NPPt	0.89**	0.93**	-0.98**	-0.74**	0.31	0.98**	0.80**	0.71**	-0.98**		0.14	0.73**	0.98**	0.93**
	100GW	0.70**	0.71**	-0.86**	-0.65**	0.39	0.83**	0.70**	0.36	-0.84**	0.82**		-0.35	0.33	0.36
	F%	0.99**	0.98**	-0.85**	-0.69**	0.47*	0.79**	0.91**	0.86**	-0.85**	0.88**	0.61**		0.62**	0.47*
	GYPt	0.99**	0.99**	-0.90**	-0.62**	0.42	0.78**	0.98**	0.78**	-0.89**	0.88**	0.71**	0.97**		0.95**
	HI	0.53*	0.61**	-0.80**	-0.25	-0.09	0.74**	0.60**	0.06	-0.82**	0.71**	0.87**	0.44	0.58*	

Treatment's classification

Relationships among treatments and traits were further explored using the principal component analyses (PCA). The PCA results for M_1 and M_2 generation were overall similar (Figure 1), therefore the mean of M_1 and M_2 was used for the final PCA. The first principal components (PC1 and PC2) explained 64.79% and 22.87% of the total variability, respectively (Figure 1). The most of traits were positively correlated and located on the positive side of the PC1. Only the number of sterile plants and plant height, with less degree, were located on the negative side of PC1, indicating that they were negatively correlated with most of the traits. For PC2, it was mostly formed by days to heading (DTH) and, with less degree, by plant height (PH) and panicle length (PL). The five radiations doses were obviously distributed on the PC1, where the control and 100 Gy treatments were similar and they favored the establishment of plants (germination and survival), growth (number of branches and tillers, panicle length), and productivity (grain yield, weight, and number of panicles). The radiation doses 200 and 300 Gy were more advantageous for higher harvest index and grain weight whereas the 400 and 500 Gy radiation doses increased the number of sterile plants and reduced all the traits related to growth and productivity (Figure 1).

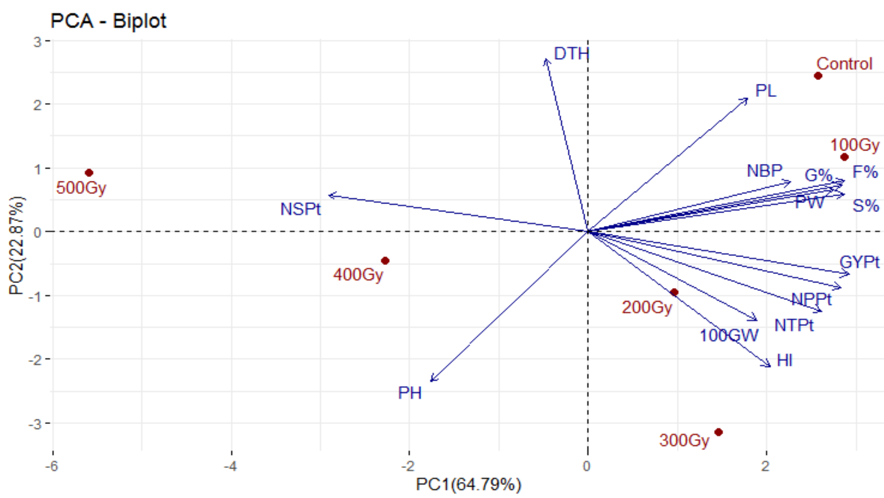

Figure 1. Biplot of the first two principal components based on traits measured in 'Giza177' rice mutant lines treated with different radiation doses based on the mean of the two mutant populations (M_1 and M_2). G%: Percentage of germination; S%: survival percentage; DTH: days to heading; PH: plant height; PL: panicle length; TN: tiller number; PW: panicle weight; NBP: number of branches per panicle; NSPt: number of sterile plants; NPPt: number of panicles per plant; 100GW: weight of 100 grains; Fertility %: percentage of fertility; GYPt: grain yield per plant; HI: harvest index.

Favorable traits in M_2 rice mutants population

Among the M_2-generation (more than 2000 plants), the promising mutant plants, which had important traits (compared to 'Giza177' the original rice) were counted. It was found that 33 mutant plants were 4-7 d earlier than the mean value of 'Giza177' and 168 mutant plants with plant height shorter than 'Giza177'. The desired PH and DTH were found more in the mutants induced by 100 and 200 Gy (Table 5). In addition, 30 mutant plants displayed panicle length less than 23 cm, 213 mutant plants had tiller number greater than 25 tillers, and 306 mutant plants yielded more than 43 g per plant. The irradiated plants with 300 Gy had the greatest number of plants with the desired PL, TN, and GYPt (Table 5).

Table 5. Number of the desirable gamma rays-induced ‘Giza177’ rice mutants based on most important traits in M_2 generation. PH: Plant height; GY: grain yield.

Gy	Early flowering (4-7 d)	PH < 95 cm	Panicle length > 23 cm	Nr of tillers per plant > 25 tillers	GY per plant > 43.0 g
100	9	53	15	51	72
200	8	32	18	44	64
300	7	27	30	55	85
400	4	28	12	35	50
500	5	28	9	28	35
Total	33	168	84	213	306

DISCUSSION

Crop genetic improvement aims to deliver for farmers new cultivars that are tolerant to the new conditions of climate change. In this respect, radiation-induced mutagenesis is an interesting source for induced genetic variability in rice, and it is of great value in the search for genotypes that can tolerate extreme drought and salinity (Robertson et al., 2018). The obtaining of new cultivars based on mutation breeding largely depends on the use of appropriate mutagens, their optimum dose for the induction of desirable mutations, and the selection and handling of mutagenic populations. In the current research, the treatment of the Egyptian rice ‘Giza177’, with various doses of gamma rays, resulted in mutant rice lines that expressed substantial variation for most traits. This indicates that these new lines (mutants) could be used to develop genetically diverse rice cultivars. The presence of high variability among the mutant lines provides room for selection for improved traits of interest. Several reports indicated similar results for yield-contributing traits (Andrew-Peter-Leon et al., 2021). In the present study, the percentage of germination and survival were reduced when the radiation dose was increased. Similar results were reported for crops like barley (Rozman, 2014) and basmati rice (Biomy, 2021). However, a low radiation dose (100 Gy), overall did not have a significant reduction when compared to control in agreement with the findings of Choi et al. (2021). For days to heading (DTH), M_1 and M_2 plants showed nonsignificant difference regardless of the radiation doses used, even though some plants within each treatment had early blooming and others had late flowering. Andrew-Peter-Leon et al. (2021) reported similar findings; the gamma rays induced early flowering and maturing rice mutants in rice. Regarding plant height (PH) of M_1 plants did not display any variation compared to the control treatment, however, in M_2 plants, PH showed a substantial increase for all doses compared to the control. Usually, rice breeders prefer to select short plants because thin and tall plants tend to have low yield potential and are more susceptible to lodging (Da Luz et al., 2016). The number of tillers was highest in M_1 plants of 100 and 200 Gy treatments and in M_2 plants of 100 and 300 Gy; however, increasing gamma rays doses, 400 and 500 Gy, decreased the tiller number, which was in agreement with the findings of Islam et al. (2014).

The percentage of fertility decreased when the radiation doses were increased (Gowthami et al., 2017). The negative effect of high radiation doses (especially 400 and 500 Gy), on the most recorded traits, resulted in a decrease of grain yield (GY). For M_1 plants, the GY was decreased according to the increase in radiation doses compared to control, similar to the results reported by El-Degwy and Hathout (2014). Notwithstanding, for M_2 plants, the 300 Gy dose followed by 100 and 200 Gy promoted higher GY compared to control. According to El-Degwy and Hathout (2014), low gamma rays doses could improve grain yield in wheat. Similar to GY; the harvest index (HI) also was higher for the radiation dose of 300 Gy, which indicates the advantage of this treatment for GY in detriment to biomass.

The variability of the recorded traits is a result of alterations in the DNA sequences of ‘Giza177’, resulting in a variability in gene expression and ultimately the phenotype for each mutant line. Depending on the level of exposure, gamma rays have been shown to have various effects on a plant's morphology, anatomy, biochemistry, and physiology (Yasmin and Arulbalachandran, 2022; Barela et al., 2022). The variability found in the current mutant line populations would be useful in breeding activities. The heritability assessed in our work is a broad sense heritability, which includes both additive and epistatic gene effects and it can be reliable only if accompanied by high genetic advance (Limbani et al., 2017). High heritability was found for most traits. In addition, by increasing the dose of gamma rays in the M_2 generation, all studied genetic parameters (genotypic variance (GV), genetic coefficient of variation (GCV), heritability in the broad sense (H), and genetic advance (GA%)) increased for most traits like tillers number, panicle weight, panicle number, 100 grains weight, fertility %, grain yield and harvest index, as a comparison to the original plant 'Giza177'. This indicates that individual plant selection for these characteristics should be successful and satisfactory for breeding purposes in the M_2 generation.

Grain yield, the ultimate target of the plant breeder, was highly significant and positively correlated with several traits like panicle number, tiller number, 100-grains weight, harvest index, and panicle length while it was negatively correlated with others like heading dates and plant height. Accordingly, a group of mutant lines was selected based on agronomic traits of interest in adaptation. The current mutant populations included 33 lines with flowering days earlier by 3 to 7 d than its original, which is consistent with other reports (Hwang et al., 2016; Gautam et al., 2021). Likewise, lines with plant height shorter than their original were isolated in the M_2 generation, similar to the findings of Purwanto et al. (2019), who also isolated semi-dwarf or short-height mutants using physical and chemical mutagens. Regarding grain yield per plant, 306 mutants had grain yield per plant greater than the original plants in agreement with the findings of Katô et al. (2020). The isolated mutants in this generation had changes in many of the traits that had been studied, and the variation between them was greater than that between plants of the original cultivar, indicating that the isolated forms had distinct genetic makeups.

CONCLUSIONS

Using gamma rays generated appreciable variability for most traits, where the low gamma rays doses (100-300 Gy) promoted the growth and fertility of plants in contrary to the higher doses (400 and 500 Gy). Moreover, interesting mutant lines were isolated based on interesting agronomic traits like flowering date, plant height, and grain yield. Therefore, the use of gamma irradiation is a useful tool to generate new lines superior to its originals. Nevertheless, multi-replicated and multi-year yield trials across different locations should be carried out to confirm and select the intrinsic higher-yield mutants.

Author contribution

Conceptualization: H.A.E., M.M.G., H.Sh.H., S.M.S., O.A.E., M.T.W., E.M.B. Methodology: H.A.E., M.M.G., H.Sh.H., S.M.S., O.A.E., M.T.W., E.M.B. Software: K.M.A., R.B., E.M. Validation: H.A.E., M.M.G., H.Sh.H., S.M.S., O.A.E., M.T.W., K.M.A., R.B., E.M., E.M.B. Formal analysis: K.M.A., R.B., E.M., E.M.B. Investigation: H.A.E., M.M.G., H.Sh.H., S.M.S., O.A.E., M.T.W., K.M.A., R.B., E.M., E.M.B. Resources: K.M.A., R.B., E.M. Data curation: K.M.A., R.B., E.M., E.M.B. Writing-original draft: K.M.A., R.B., E.M., E.M.B. Writing-review & editing: K.M.A., R.B., E.M., E.M.B. Visualization: H.A.E., M.M.G., H.Sh.H., S.M.S., O.A.E., M.T.W., K.M.A., R.B., E.M. Funding acquisition: K.M.A., R.B., E.M., E.M.B. All co-authors reviewed the final version and approved the manuscript before submission.

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