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Induction of Genetic Variability for some Agronomic Traits and Blast Disease Resistance in Egyptian Rice Variety Sakha101

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ABSTRACT

The present study was undertaken to assess the differential sensitivity of Egyptian rice variety Sakha101 to gamma radiation, to study the genetic variability induced and to get desirable phenotypic mutants especially of economic importance traits and blast disease resistance. The study was conducted at the Experimental Farm of Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt, during 2016 and 2017 rice growing seasons. Four gamma ray doses; 100, 200, 300 and 400 Gy were used to treat the seeds of the Egyptian rice variety Sakha 101. The results illustrated that heading date was early in all doses applied in M_1-generation, except in 400 Gy. Plant height was decreased by increasing gamma irradiation in both M_1 and M_2-generations. Number of panicles/plant were decreased by using the dose of gamma rays for 100 and 200Gy, while it increased for 300 and 400 Gy treatments in both of M_1 and M_2 generations. The doses of gamma rays increased the means of sterility % and 100-grain weight in both of M_1 and M_2-generations. For grain yield/plant, the mean values decreased by increasing the dose of gamma rays up to 300 Gy while, increased at 400 Gy, where the grain yield per plant increased by 47.7 grams as compared with the control (44.0 grams) in M_2-generation plants. All studied genetic parameters viz, genotypic variance (GV), genetic coefficient of variation (GCV), heritability in broad sense (h^2bs) and genetic advance of mean (GS%) increased by increasing the dose of gamma rays in M_2-generation indicating individual plant selection for these characters should be effective and satisfactory for successful breeding purposes. A total of 101 M_2 mutant plants were found to be earlier in heading by 10-20 days than the mean value of the original variety Sakha 101; and 105 mutant plants were having grain yield/ plant more than 42 grams comparing with its original plants. About 591 plants from different irradiated treatments were found to be resistant to blast disease from M_2-generation plants. Hence, the induced genetic variability is consider important as it is a basic requisite for successful rice breeding program via direct selection or by crossing with the commercial cultivated varieties.

Keywords: Gamma irradiation, rice (*Oryza sativa* L.), Genetic variability, Mutation, Yield and its contributes.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal food crops in the whole world (Kohnaki *et al*., 2013). The demand for rice is continuously increasing due to unabated growth of population. In order to feed the ever-increasing population, achieving self-sufficiency in production and to maintain price stability, more variability is required to develop new higher yielding varieties with tolerance to biotic and abiotic stresses.

Rice diseases are one of the major limiting factors of rice production worldwide and Egypt. Rice blast disease is caused an economic loss up to 65% yield in susceptible cultivars under favorable conditions (Singh *et al.* 2015). The genetic control of blast resistance is a complex trait, and involves both major and minor genes with complementary or additive effects (Wu *et al.,* 2005 and Li *et al.,* 2007). However, the breakdown of blast resistance frequently occurs due to the rapid change in the blast pathogenic races after a few years of new cultivar release (Song *et al.,* 2014).

Rice has been a common to mutagenesis because it is important as one of the popular food crops. Being diploid species, maximum genetic variability is available for selection in M2-generation. Mutations can create new unique variations when natural variability is not capable of providing the gene for desired traits (Velmurugan *et al.,* 2010). Mutation breeding is a kind of method for affecting genes either by treating seeds and plant parts by using chemical or physical mutagens (Datta and Chakrabarty, 2009).

Induced mutations had important role for rice improvement using developing a huge number of semi-dwarf as well as high yielding genotypes in many countries besides important traits contributing to overall yield (Maluszynski *et al.,* 1986 and Baloch *et al.,* 2003). Significant improvements through the use of induced mutations have been reported for high yield (Singh, 2006 and Shehata *et al.,* 2009), plant height, harvest index, leaf area index, dry matter production and grain yield per plant (Bughio *et al.,* 2007 and Babaei *et al.,* 2010), short stature (Singh and Singh, 2003 and Kumar, 2005), early maturing (Minn *et al.,* 2008 and Shehata *et al.,* 2009), 1000 grain weight and fertile tillers (Babaei *et al.,* 2010). Germination and survival is linearly decreasing with increase of radiation dose in case of γ- rays and fast neutrons (Din *et al.,* 2004). In rice, to allow 60% survival of seeds (percentage of seeds germinated and developed to adult plants) and effective dosage of gamma rays generally ranged from 150 to 300 Gy (Rutger, 1992).

In Egypt, mutation breeding method was started for rice in 1960s by Serry and Masoud when they subjected the old rice varieties Nahda and Agami to gamma rays (Serry and Masoud, 1970). High doses of gamma rays caused higher sterility of M_1 plants, but low doses of gamma rays in some cases stimulated the growth and yield of M_1 plants (El-Keredy, 1990). Irradiating seeds with suitable doses of gamma rays produces physiological and genetical changes that may be affected the yield of plant (Hammad and El- Geddawi, 1988).

The Egyptian rice variety Sakha 101 is the most popular variety and wide distribution in Egypt virtue of its higher yield, but unfortunately in the last few years it's having some defects such as susceptibility to blast, whereas, its resistance was broken-down from 2005 until now and became susceptible due to appearance of specific virulent race IG-1, EL-Shafey, (2002) and Sehly *et al.,* (2008). In addition, late maturity recently comparing with the new released varieties that have short maturity duration, and considering the problem of the shortage of water irrigation. Keeping in view the drawbacks of this common variety, a study was undertaken to explore the possibility of improving it with respect to these defects through quantitative mutational approaches. So, the present study was undertaken to assess the mutagenic effects of gamma rays and to find out the radiation dose of a given mutagen in order to create variability of cultivated rice variety Sakha101 and also to explore the possibilities of isolating desirable phenotypic mutants for further utilization directly or indirectly cross breeding programs for the quick improvement of the defects of the respected varieties.

MATERIALS AND METHODS

The present study was carried out at the Experimental Farm of Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt, during 2016 and 2017 rice growing seasons. Four different doses of gamma rays were used in the present study. Samples of 250 dry and healthy seeds of uniform size from the Egyptian rice variety Sakha101 (stabilized at 14% moisture level) were irradiated in the Co^{60} source at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. Gamma rays doses were 100, 200, 300 and 400 Gy and dry un-irradiated seeds were used for comparison.

M_1-generation:

In 2016 growing season the different doses of gamma irradiation treated seeds of the rice variety Sakha101, along with its respective control were directly sown after treatments in germination plastic plates in order to raise M_1 plants. Germination percentage was recorded after 15 days of sowing. Shoot length of thirty days old seedling were transplanted (one seedling/hill) in the experimental plots. Plant to plant and row to row distances of 20 and 20 cm, respectively were maintained. To avoid out-crossing, first three panicles in each of the 50 randomly selected plants in each treatment were bagged at the time of panicles emergence. At the time of maturity, the seeds were harvested from the three bagged tillers, as well as un-bagged tillers, separately from each individual plant.

M_2-generation:

In 2017 season, the M2 generation was raised in a randomized complete block design (RCBD) with three replicates. The seeds from the main, Lateral-I and Lateral-II tillers in each of the 50 selected plants were planted in nursery beds. Thirty days old seedlings were transplanted in a well prepared field. Progeny of the selected plants in each treatment was transplanted in a progeny row trial, with three replications, with spacing 20 x 20 cm. Each replication had 50 rows per treatment (900 plants). All recommended agricultural practices were applied during the growing season. Viable mutation in M2 progeny was examined periodically through the entire growth period for visible mutations affecting various growth and morphological attributes. In order to study the magnitude and nature of induced polygenic variability in M2 generation, observations on various quantitative traits were recorded on all the plants in each treatment. The following observations were recorded: germination percentage (%), heading date (days), plant height (cm), number of tillers/plant, chlorophyll content (%), number of panicles/ plant, panicle length (cm), sterility percentage (%), one hundred grain weight(g) and grain yield/ plant (g).

Blast disease scoring:

Blast nursery test:

Progeny of the selected plants from M_1 generation in each treatment was evaluated under field conditions at Sakha location (Experimental Farm of Sakha Agricultural Research Station) for blast resistance at seedling stage for major gene resistance with natural infection at blast nursery test. Seedbeds were prepared during the first week of July and fertilized with nitrogen in the form of urea (46.5% N) at the rate of 60 Kg nitrogen per feddan and manured (8 m^3 farmyard manure /fed.). Width of the seedbed was one meter and 10.5 m long, at the beginning and end of each seedbed. Five rows of Giza 159 (blast spreader) were sown, then five of the considered seeds of M_2 generation, and again one row of the spreader, with 15 cm apart. Another five of the considered seeds of M_2 generation were sown, followed by one row of the resistant check (Giza 181). The susceptible and resistant checks were sown alternatively, surrounding five of the considered seeds of M_2 generation. The seedlings of M_2 generation were left exposed for natural blast infection at seedling stage. About forty-days from sowing, the typical blast lesions were scored, according to the standard evaluation system using 0-9 scale (IRRI, 1996) as follows:

0-2 = resistant (R)
3 = moderately resistant (MR)
4-6 = susceptible (S)
7-9 = highly susceptible (HS)

Statistical analysis:

The obtained data were subjected to the statistical analysis to estimate the mean ($\bar{x}$), rang (R) and standard error ($S_{\bar{x}}$). Means of different treatments in M_1 and M_2 generations were compared by the use of Duncan's Multiple Range Test (DMRT), Duncan, (1955). For comparing the mean values and variance within and between families, the analysis of variance was done for each character separately as suggested by Pause and Sukhatme, (1957). Genotypic and phenotypic variances were computed by the formula suggested by Burton (1952). Heritability in broad sense (h^2b.s), Genetic advance (GS) and Genetic advance of main (GS %) were computed.

Chi-square (x^2), test was performed to assay goodness of fit of the M_2-generation for the phenotypic data to determine the mode of inheritance and number of genes involved in resistance. Chi-square (x^2) test was computed as follows:

$$x^2 = \frac{(O_1 - E_1)^2}{E_1} + \frac{(O_2 - E_2)^2}{E_2}$$

Where: O_1 and O_2 are the observed values, and E_1 and E_2 are the expected values (Gomez and Gomez, 1976).

RESULTS AND DISCUSSION

The present investigation was undertaken to assess the differential sensitivity of the Egyptian rice variety Sakha101 to gamma rays in order to estimate the genetic variability induced and to obtain desirable phenotypic mutants especially of economic importance and blast disease resistance as well.

Mean performance of M_1-generation plants:

Significant differences were observed among the treatments of gamma rays and the control on one side, and among the treatments itself on the other side for most of the studied traits (Table 1), only panicle length trait showed in-significant differences. Germination percentage in general was decreased by increasing the dose of gamma radiation and the highest germination percentage was detected at 100 Gy (90.20%), while the lowest percentage was observed at 400 Gy (52.60%). Chemma *et al.,* (2003), found a decrease in germination percentage with increasing the dose in M_1 generation.

For days to heading trait, the results illustrated that heading was early in all doses applied, except in 400 Gy. Maturity was earlier in 100 Gy treatment (15 days) than the control one and in the same trend nine days in 200 Gy treatment. Using a low dose of radiation (100 Gy) significantly exhibited higher negative mean value (94.45 day) over all treatments but, the differences between 200 and 300 Gy were not significant. This may be ensuring that low doses of radiation in some cases stimulated the growth of M_1 plants.

There was a gradual decrease for plant height by the increasing the dose of gamma rays and significant differences were found between control and all treatments. Similar results were obtained by Mohamed *et al*., (2006) and El-Degwy (2013) they found a gradual decrease of plant height and days to heading with increase of radiation dose.

The results in Table 1 showed that both of number of tillers/plant and number of panicles/plant traits in general increased significantly with gamma rays treatments. The maximum increases in no. of tillers/plant were observed for 400Gy (23.5 tillers), while in no. of panicles/plant (21.95 panicles). It could be concluded that the most effective dose of gamma rays was 400 Gy for these characters. These results were in close agreement with the results obtained by Shehata *et al.,* (2009).

The data also revealed that there were significant differences in 100-grain weight trait and the most effective dose of gamma rays was 200 Gy. Sterility percentage was significantly increased by increasing the dose of gamma rays and the highest sterility percentage was recorded at 400 Gy treatment (54.25%). The pervious results reported that the decrease in seed fertility or the increase in sterility percentage with gamma rays doses from 10 to 20, 30, 40, and/or 50 Kr (Shadakshari *et al*., 2001). Also, Sasikala and Kalaiyarasi (2010) reported that seed fertility percentage was decreased with the increase of gamma radiation doses from 100 up to 350 Gy in linear fashion.

Decrease in grain yield per plant was recorded with the increasing dose of treatments as shown in Table 1. The lowest values of grain yield per plant were recorded at 400 Gy (22.25 g). The decreasing trend in grain yield per plant may be attributed to the increasing in sterility percentage. Similar results were reported by El-Degwy (2013).

Table 1. Effect of gamma irradiation doses on the growth and yield characters of rice variety Sakha101 in the M_1 generation during 2016 growing season.

Doses (Gy)	Germination Percentage (%)	Heading date (day)	Plant height (cm)	No. of tillers /plant	No. of panicles /plant	Panicle length (cm)	100-grain weight (gm)	Sterility percentage (%)	Grain yield/plant (g)
Cont.	99.22a	109.25a	95.90a	22.60b	20.90b	22.50	2.72b	8.12c	43.00a
100	90.20b	103.57b	89.25b	19.25c	18.50c	22.58	2.85b	13.35bc	31.40b
200	85.25b	94.45c	85.50b	20.25b	18.85c	22.99	3.51a	25.60b	35.52b
300	57.75c	105.54b	83.50c	22.50b	21.05a	21.90	2.87b	50.50a	32.25b
400	52.60d	110.50a	82.15c	23.50a	21.95a	21.75	2.70b	54.25a	22.25c
F-Test	**	**	**	*	*	ns	*	**	*

***, ** and ns: significant at the 0.05, 0.01 level of probability and not significant, respectively.**
Means followed by a common letter(s) aren't significantly differed at 0.05 level by DMRT.

It could be concluded that the gamma rays treatments widened the range of various characters in most of the cases, indicating thereby that the treatments were effective in creating additional variability. However, this increased variability is not of permanent nature wholly, as the plants in M_1 generation are chimeric in nature and their phenotype is the expression of changed genetic composition as well as disturbed physiological processes. The effect of physiological disturbances is eliminated almost completely in the subsequent generations, while the gene mutations and chromosomal aberrations are transmitted to the M_2 and later generations. Thus, the extent of change in the genetic composition can be estimated efficiently in the M_2 and subsequent generations.

M_2- generation:

Behavior of mean and range:

The effect of gamma irradiation doses on the mean values of growth and yield and its attributes characters in M_2-generation are shown in Tables 2 and 3. The data obtained with respect to germination percentage, revealed that, as compared to the control gamma rays treatments in general had drastic effect on the germination percentage. Chemma *et al.,* (2003) found a decrease in germination percentage with increasing dose in M_2-generation as found in the present study. The most effective dose of gamma rays was found to be 400 Gy. Regarding days to heading, the results illustrated that accelerate in heading occurred gradually by increasing the doses of gamma rays and maximized with 400 Gy. The obtained results were in a good agreement with those obtained by Gomma *et al.,* (1995) that they found gamma-ray treatments widened the range of variability of heading date which could allow to select early mutants in advanced generations.

Plant height was decreased by increasing gamma rays doses (Table 2). The most effective treatment was 400 Gy. It caused a decrease in plant height reached 7.0 cm. Insignificant differences were detected between 200 Gy, 300 Gy and 400 Gy treatments. These obtained results were in a good agreement with those reported by Gomma *et al*., (1995) and Abdallah, (2000). From the previous results, it is clear that gamma-rays was able to create and extent an additional variations in maturity and plant height of the M_2-generation.

The number of tillers per plant was affected significantly by increasing gamma rays treatments and the highest number of tillers was obtained for 400 Gy treatment. Insignificant differences were detected between 300 Gy and 400 Gy treatments. These results indicated that gamma rays treatments proved effective, as was evident from the tendency of the treated M_2-generation plants showing increase in mean values of number of tillers per plant particularly for 300Gy and 400Gy treatments, respectively. These results were in agreement with Bentota (2006).

Regarding chlorophyll content, the results revealed a consistent trend in mean values by increasing the dose of gamma rays as well as significant differences were detected between means of both control and the different doses of gamma rays. These findings were in agreement with those reported by (Datta and Chakrabarty, 2009).

Table 2. Effect of gamma irradiation doses on the growth characters of rice variety Sakha101 in the M_2-generation during 2017 growing season.

Doses (Gy)		Germination Percentage (%)	Days to heading (day)	Plant height (cm)	No. of tillers/plant	Chlorophyll content %
	Rang	95-100	109-113	95-100	22-25	43-50.5
Cont.	X¯	99.00a	110.00a	96a	23.00ab	45.05b
	Sx¯	0.80	0.28	1.75	0.9	1.99
	Rang	90-93	106-115	98-101	18-23	40-52
100	X¯	91.00b	110.00a	97.18a	19.62b	47.33b
	Sx¯	0.32	0.61	1.11	0.289	1.09
	Rang	79-90	105-110	89-100	17-25	41-51
200	X¯	87.00b	108.50a	91.10b	22.25ab	48.74b
	Sx¯	1.44	0.53	0.11	1.60	0.22
	Rang	75-83	101-110	87-101	20-26	37-65
300	X¯	80.00c	104.00b	90.56b	25.25a	50.13a
	Sx¯	1.09	1.10	1.14	1.01	2.64
	Rang	70-80	96-110	82-100	19-29	35-62
400	X¯	73.00d	101.3b	89.56b	27.94a	51.13a
	Sx¯	1.09	1.2	1.57	1.08	2.64

Means followed by a common letter are not significantly different at the 5% level by DMRT.

It is clear from Table 3 that the gamma rays treatments had different effects depending on the dose for number of panicles/plant. Significant differences were observed between the control and most of the treatments on one hand and among the treatments itself on the other hand. The highest significant increase in number of panicles per plant was recorded for 400Gy treatment. These results indicated that the effectiveness of gamma rays was found with the increase of the doses with either plus or minus effect. These results were in close dis agreement with those obtained by El-Degwy (2013). Meanwhile, (Babaei *et al.,* 2010) found that no definite trend was evident with regard to such character.

The results illustrated that panicle length at maturity was increased by increasing the dose of gamma rays. The data indicated significant differences between the control and the treatments l00 and 200 Gy while, insignificant differences between these two treatments were observed. The most desirable dose of gamma rays of panicle length was 200 Gy treatment. In general, there was an inconsistent trend in mean values of different treatments for panicle length. These results were in agreement with reports of Abdallah, (2000).

Table 3. Effect of gamma irradiation doses on yield and its attributes characters of rice variety Sakha101 in the M_2-generation during 2017 growing season.

Doses (Gy)		No. of panicles /plant	Panicle length (cm)	100-grain weight (gm)	Sterility percentage (%)	Grain yield/plant (g)
	Rang	20-24	20-23	2.7-3.0	5.0-10.0	43.0-50.0
Cont.	X¯	22.00b	22.50ab	2.78a	7.35c	44.00b
	Sx¯	0.82	0.32	0.01	0.42	1.60
	Rang	17-22	21-25	2.5-3.0	14.0-19.0	37.0-48.0
100	X¯	18.34c	23.34a	2.60a	13.80b	40.80cb
	Sx¯	0.18	0.06	0.19	1.39	1.82
	Rang	16-24	21-25	2.0-3.0	20.0-27.0	32.0-50.0
200	X¯	21.91b	24.01a	2.70a	16.00b	37.16c
	Sx¯	0.01	0.09	0.07	2.29	0.25
	Rang	19-24	19-24	1.9-2.9	25.0-40.0	40.0-60.0
300	X¯	23.00a	22.21ab	2.80a	22.85a	42.00b
	Sx¯	1.50	0.55	0.05	2.40	0.87
	Rang	18-28	17-25	1.9-2.8	25.0-50.0	45.0-58.0
400	X¯	26.00a	22.50ab	3.00a	25.53a	47.70a
	Sx¯	2.42	0.62	0.06	2.10	1.25

Means followed by a common letter are not significantly different at the 5% level by DMRT.

Regarding 100-grain weight, the results showed that, no significant differences were found between the control and the gamma rays treatments. However, the range extremes indicated the possibility of selecting promising heavy grain mutants from the different dose of gamma rays of the rice variety Sakha101. These results were in agreement with those reported by Mohamed *et al.,* (2006).

For Sterility percentage (%) as shown in Table 3, there were significant differences among the gamma rays treatments. It is worthy to note that sterility percentage was increased by increasing the doses of gamma ray. The most effective dose was 400Gy and this treatment recorded the highest sterility percentage. Similar results were obtained by El-Degwy (2013).

It is clear from Table 3 that grain yield per plant was significantly affected by mutagenic treatments. The results indicated that the mean values of grain yield per plant decreased by increasing the dose of gamma rays up to

300 Gy then increased at 400 Gy. So, the most effective treatment was 400 Gy, where the grain yield per plant increased by 47.7 gram as compared with the control and the other treatments as well. On the other hand, range extremes indicated the possibility of selecting high yielding mutants from the irradiated populations in subsequent generations. Similar results were obtained by Abdallah, (2000), Shehata *et al.,* (2009) and El-Degwy (2013).

In the present study, increase in mean values of various traits may be due to the selection of normal-looking plants in M_2 which led to the elimination of aberrant plants and also due to changes induced at the genetic level. Gaul (1964) suggested that the selection process should be delayed until the M_3 or later generations following mutagenic treatment. However, here the selection of progenies on the basis of desirable mean and greater variance in the early generation was found to be highly useful, leading to the desirable improvement of yield and its components in the M_3 generation. The same was concluded by Siddiqui and Singh (2010) who reported improvement in mean values from M_2 to M_3 generation. The increased range in the positive direction indicated the scope of selection for the improvement of quantitative characters including yield.

Estimates of genetic variability parameters in M_2-generation:

Considerable improvement and success breeding programs for any crop largely depends on the amount of genetic variability among genotypes, which selected for further manipulation to achieve the breeding target. A survey of genetic variability with the help of suitable parameters such as genotypic (GV) and phenotypic (PV) variance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2bs) in broad sense and genetic advance (GS%) are necessary to start an efficient breeding program.

Heritability estimates along with genetic advance as per cent of mean are normally more helpful in predicting the gain under selection than heritability estimates alone (Johnson *et al*., 1955). Genetic advance is indicative of the expected genetic progress for a particular trait under selection procedure (Kaul and Garg, 1982) and consequently carries much significance in self-pollinated crops. Genetic variability, which is partitioned from environmental effects, is appreciated. The genetic variability in terms of GCV alone is not sufficient for the determination of the amount of heritable variability. In addition, estimation of heritability and genetic advance as per cent of mean is also needed to assess the heritable portion of total variation and extent of genetic gain expected for effective selection (Alt *et al*., 2002). As heritability in broad sense includes both additive and epistatic gene effects, it will be reliable only if accompanied by high genetic advance (Burton, 1952). This indicated that such characters are governed largely by additive gene effects, which may favourably be exploited in M2 generation through selection.

A wide range of induced variations in ten studied traits were observed among M_2-generation plants for the rice variety Sakha101 with respect to growth characters (Table 4) and yield and its attributes traits (Table 5). Table 4 presents estimates of genotypic (GV) and phenotypic (PV) variance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2bs) in broad sense and genetic advance (GS%) for the growth characters in M_2-generation. Expectedly, phenotypic variance was generally higher than the genotypic variance in all the studied growth traits.

For germination percentage, the results showed that highly significant increase in genotypic variance (GV) and coefficients of genotypic variability (GCV) values were obtained by irradiation. Heritability in broad sense estimates (h^2bs) were higher in irradiated populations than the control. The expected gain from selection as percent of the mean (GS%) were increased in irradiated populations and amounted 2.12 %.

Regarding days to heading trait, Extent of induced variation in terms of genotypic variances (GV) and coefficient of genotypic variability (GCV) indicated that the gamma rays induced variation was much significance in the rice variety Sakha101. Gamma rays treatments are known to induce genetic variation as well as chromosomal mutations, along with non-heritable physiological changes, all of which contribute to the total induced variance. In M_2-generation the heritability in broad sense estimates (h^2bs) were found to be moderate to high for irradiation treatments (52.0%). This is indicative of the fact that irradiation not only induces higher proportion of chromosomal and physiological changes but also brings about a high frequency of gene mutations. This finding can, therefore, also be of considerable values in planning mutations experiment with respect to the choice of reliable stage of selection for the improvement of earlier plants. Genetic advance (GS), which depends on the amount of variability induced and the heritability (h^2bs), provides a clue to the degree of response of a segregating population to selection. In the present study, genetic advance (GS) for selection was predicted on the basis of the performance of lines in the M_2-generation for all the treatments. It was noted that expected genetic advance, when calculated gamma rays treated populations than in the control.

Table 4. Genetic parameters of the growth characters in M_2-irradiated populations of rice variety Sakha101.

Genetic parameters	Germination Percentage (%)	Days to heading (day)	Plant height (cm)	No. of tillers /plant	Chlorophyll content %
GV	1.23**	1.12*	9.98**	1.90**	0.45*
PV	2.02	2.14	15.55	2.78	1.45
GCV	1.28	0.94	3.41	6.45	1.42
PCV	1.69	1.34	4.25	7.72	2.58
h^2 bs	0.63	0.52	0.48	0.71	0.32
GS	1.80	2.50	3.75	2.40	0.76
GS%	2.12	3.39	4.10	10.78	1.68

GV: genotypic variance, PV: phenotypic variance,
GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, h^2(bs%): heritability in broad sense,
GS: genetic advance, GS%:genetic advance of means.
NS, * and ** denotes insignificant and highly significant increase in genotypic variance of the irradiated population as compared with that of the control, respectively.

For plant height trait, it is clear from the obtained results that the variability increased in the mutagen treated populations. Genotypic variance (GV) value was significantly higher by irradiation than the control, where it calculated 9.98. Genetic coefficients of variation (GCV) increased also in irradiated population (3.41%). The heritability (h^2bs) and genetic advance of means (GS%) were comparatively moderately high in the gamma ray-treated populations than those in the control and amounted 48.0% and 4.5, respectively. These results are in a good agreement with those reported by Shehata *et al.,* (2009).

Regarding number of tillers/plant, Extent of induced variation in terms of genetic variance and coefficients of genetic variability in M_2-generation indicated that the mutagen induced variability for this character was more than that of the respective controls (Table 4). Pronounced increases in the genetic coefficient of variation (GCV) were observed by irradiation and amounted 6.45%. The value of heritability in broad sense (h^2bs) was high in irradiated populations and calculated 71.0%. Value of the expected gain from selection as percentages of the mean (GS%) was increased by irradiation as compared with the control and amounted 10.78%. It could be concluded that the heritability and genetic advance estimates were high, there by indicating the easy fixability of this trait by selection, practiced on the basis of phenotypic expression. Similar results were obtained by Abdallah, (2000) and Mohamed *et al*., (2006).

Extent of induced variation for chlorophyll content in terms of genotypic variances (GV) and coefficients of genotypic variability (GCV) indicated that the mutagen induced variation was not of much significance in M_2-generation for rice variety Sakha101. The differential effect of various doses of gamma rays on the induction of variation for such character has been reported by many workers. The heritability estimates were found to be low for treatments with different doses of gamma rays, when it calculated 32.0%.The lower values of heritability for this trait was mainly attributed to the lower values of genotypic variance. Genetic advance (GS), which depends on the amount of variability induced and the heritability, provides a clue to the degree of response of a segregating population to selection. The genetic advance (GS %) as percent of mean by irradiation was low and calculated 1.68%.

Table 5 presents estimates of genotypic (GV) and phenotypic (PV) variance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2bs) in broad sense and genetic advance (GS%) for yield and its attributes characters in M_2-generation of rice variety Sakha101.

Expectedly, phenotypic variance was generally higher than the genotypic variance in the entire studied yield and its attributes characters. For Extent of induced variation for number of panicles per plant in terms of genotypic variances (GV) and coefficients of genotypic variability (GCV) Table 5 indicated an increase in the genetic variability of M_2 generation of the mutagen treated populations and significant increase in the genotypic variance were detected by irradiation and calculated 1.95%. The mean values of the genetic coefficient of variation (GCV%) were found to be higher in the irradiated populations compared with the controls and amounted 6.72%. These results are in agreement with the previous reports in rice that the gamma rays induced considerable genetic variation for number of panicles per plant. Estimates both of heritability (h^2bs) and genetic advance (GS%) were increased by irradiation and calculated 67% and 11.10%, respectively.

Regarding panicle length trait, extent of induced variation in terms of genetic variance and coefficients of variability in M_2 indicated that the different doses of gamma rays induced variability for this characters was more than that of the respective controls in and were amounted 0.32% and 2.37%, respectively. Similar conclusion has been also drawn by Elayaraja *et al.,* (2004). Heritability value was moderate in irradiated populations when it amounted 55%. Considerable increase in expected genetic advance as percent of mean (GS%) was found to be higher in irradiated populations and calculated 3.51%. It could be concluded that the heritability (h^2bs) and genetic advance (GS) estimates were high there by indicating the easy fixability of this trait by selection, practiced on the basis of phenotypic expression. These results are in a good agreement with those reported by Elayaraja *et al.,* (2004).

Table 5. Genetic parameters of the yield and its attributes characters in M_2-irradiated populations of rice variety Sakha101.

Genetic parameters	No. of panicles /plant	Panicle length (cm)	100-grain weight (gm)	Sterility percentage (%)	Grain yield /plant(g)
GV	1.95**	0.32*	0.15*	7.95**	13.75**
PV	2.82	0.58	0.15	13.82	16.88
GCV	6.72	2.37	13.25	10.02	7.36
PCV	8.13	3.21	14.28	35.13	8.45
h^2 bs	0.67	0.55	0.95	0.57	0.64
GS	2.35	1.85	0.72	4.35	6.55
GS%	11.10	3.51	16.91	4.75	13.75

GV: genotypic variance, PV: phenotypic variance, GCV: genotypic coefficient of variation, PCV: phenotypic coefficient of variation, h^2(bs%): heritability in broad sense, GS: genetic advance, GS%:genetic advance of means.
NS, * and ** denotes insignificant and highly significant increase in genotypic variance of the irradiated population as compared with that of the control, respectively.

Genetic parameters of 100-grain weight are given in Table 5. Genotypic variance value was slightly increased by irradiation as compared with their respective control and calculated 0.15. Pronounced increases in the genetic coefficient of variation GCV%) was observed by irradiation and amounted 13.25%. Heritability value in broad sense was high in irradiated populations and found 95%. Considerable increase in expected genetic advance upon selection (GS%) was obtained for this trait by irradiation as compared with the control and found 16.91%. It is very interesting to note that 100-grain weight having high heritability estimates in M_2 generation of rice variety Sakha 101. These results indicated that individual plant selection for this trait should be effective for successful breeding purpose. Similar results were obtained by Mohamed *et al.,* (2006) and (Babaei *et al.,* 2010).

Table 5 illustrated the genetic parameters of the sterility %. Significant increase in genotypic variance was found by irradiation and calculated 7.95%. Pronounced increase in the genetic coefficient of variation (GCV%) was observed by irradiation and the value was 10.02 %. Heritability estimate was found to be highly moderate in the M_2 generation effected by irradiation. It is interesting for this trait to note that high genetic gain represented as percentage of the mean (GS%) was associated with relatively high heritability value in sterility %.

Regarding grain yield per plant trait, extent of induced variation in terms of genetic variance and coefficients of variability in M_2 indicated that the different doses of gamma rays induced variability for this characters was more than that of the respective controls in and were amounted 13.75% and 7.36%, respectively. Similar conclusion has been also drawn by Elayaraja *et al.,* (2004) and (Babaei *et al.,* 2010). Heritability value was highly moderate in irradiated populations when it amounted 64%. Considerable increase in expected genetic advance as percent of mean (GS%) was found to be higher in irradiated populations and calculated 13.75%. High genetic gain represented as percentage of the mean (GS%) was associated with relatively high heritability values in grain yield/plant indicating, individual plant selection for this characters should be effective and satisfactory for successful breeding purpose. The obtained results are in a good agreement with those reported by Elayaraja *et al.,* (2004), Shehata *et al.,* (2009) and El-Degwy (2013).

From the foregoing discussion, it could be concluded that estimates of the genetic parameters for studied traits were, in general higher in irradiated populations than in their respective controls. This is logic, since gamma radiation induced more genetic variability and hence other genetic parameters increased. Thus effective selection might be achieved from M2 irradiated populations of rice variety Sakha101 in next generation.

The desirable observed mutant plants in M_2-generations in Sakha 101:

Numbers of desirable observed mutant plants in M2-generations in rice variety Sakha 101 were illustrated in Table 6. About 3600 plants from different irradiated populations of the rice variety Sakha101 were examined during the growth period in M2-generation. A total of 101 M2 mutant plants were found to be earlier in heading date by 10-20 days than the mean value of the original rice variety Sakha 101, (23 from 100 Gy, 14 from 200 Gy, 19 from 300 Gy and 45 from 400 Gy irradiated dosages). Early maturing mutants were isolated in the present study which is in conformity with the reports of Bentota (2006), Domingo *et al.* (2007) and Hallajian *et al.* (2013).

For plant height, 41 dwarf plants were isolated in this generation from irradiated populations of rice variety Sakha101. The forty one mutants were (5 from 100 Gy, 10 from 200 Gy, 12 from 300 Gy and14 from 400 Gy treatments) shorter than the original plants of the variety Sakha 101 (< 90 cm). Abdallah, (2000) and Bentota (2006) also isolated semi-dwarf or short stature mutants through the use of physical and chemical mutagens in their experiments on rice.

With respect to panicle length 44, M2 plants are having panicle length taller than the original variety were identified in the M2-generation (Table 6).

The forty four changed plants were found from the irradiated populations of the variety Sakha 101 (13, 11, 12 and 8 from 100, 200, 300 and 400 Gy, respectively).

Table 6. Number of the desirable observed mutant plants in M_2-generations in Sakha 101.

Treatments	Early flowering (10-20)day	Semi dwarf < 90 cm	Panicle length >24 cm	No. of tillers/plant >25 tiller	Grain yield/plant >42.0 g	Blast resistance (0-3)
100 Gy	23	5	13	12	35	125
200 Gy	14	10	11	10	15	144
300 Gy	19	12	12	15	23	157
400 Gy	45	14	8	25	32	165
Total	101	41	44	62	105	591

For number of tillers/ plant, Sixty two mutants (12, 10, 15 and 25 from the treatments 100, 200, 300 and 400 Gy, respectively) were found to have tillers number/ plant more than the original plants of the rice variety Sakha101. Concerning the changes in grain yield/ plant, 105 mutants (35, 15, 25 and 32 from the treatments 100, 200, 300 and 400 Gy, respectively) were having grain yield/ plant more than 42 grams comparing with its original plants. About 591 plants from different irradiated populations (125 from 100 Gy, 144 from 200 Gy, 157 from 300 Gy and 165 from 400 Gy treatments) were found to be resistant to blast disease from M_2-generation plants of the rice variety Sakha 101.

In general, results in Table 6, evident that the mutated forms isolated in this generation possessed changes in many studied traits and the variation among them is larger than the variation between plants of the original variety, indicating the presence of genetic differences between the isolated forms.

Chi-square test and inheritance analysis for Blast resistance in M_2-generation:

Knowledge on the inheritance of blast disease resistance would facilitate the adoption of appropriate breeding strategies and improve the efficiency of selection procedures. From the data analysis of the chi-square test for the single gene model, on segregation ratios (R: S) were observed for blast lesion traits for the M2-generation for the rice variety Sakha101. The segregation ratio in the M2-generation of susceptible plants to resistant plants was expected to be 3:1 if a single gene provided resistance to blast disease causes fungus. The segregation pattern or reaction type for blast resistance in the M2-generation is shown in Table 7.

Table 7. Reaction type (chi-square X^2) and Expected ratio in M_2-generation plants of rice variety Sakha101 for Blast disease.

Population	Reaction Type	No. of Expected plants	No. of Observed plants	Percentage (%)	Expected Ratio	X^2	P-value
M_2-generation	R	900	591	16.41		106.09	
plants of	S	2700	3009	83.58	1R:3S	35.36	0.007
Sakha101	Total	3600	3600			141.45	

(S): Susceptible; (R): Resistant

From the chi-square analysis of the observed blast lesion traits M_2-generation plants of rice variety Sakha101 did not properly segregate in a 3 : 1 ratio of susceptible to resistant plants (3S:1R). Out of 3600 M_2-generation plants, 591 plants were resistant and 3009 were susceptible to the blast disease causes fungus (X^2 = 141.45, P = 0.003). These results suggested that a single recessive gene may be controlling resistance to blast resistance disease in the M_2-generation plants under this study. Similar studies have been conducted to determine the resistance of F_2 populations with a specific blast isolate. Prasad *et al.,* (2009) and Kumbhar *et al.,* (2013) have also suggested a monogenic control of resistance. Studies conducted in IRRI revealed that most of the traditional varieties have one or two dominant gene/s (Mackill *et al.,* 1985).

Over all, it can be conducted that the success achieved with induced mutation, especially for the improvement of the major cereals of the world like rice, indicates that it is still an important tool to complement the conventional breeding technology in development of new crop varieties. In this study, used irradiation by gamma-ray exhibited different genetic variability such as semi-dwarf, early heading, blast disease resistance and high yield in M_2-generation plants for rice variety Sakha101. Hence, the induced genetic variability was more important as it is a basic requisite for successful rice breeding program through direct selection or by hybridization with the commercial cultivars.

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استحداث تباينات وراثية لبعض الصفات المحصولية ومقاومة مرض اللفحة فى صنف الارز المصرى سخا101

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اجريت هذه الدراسة لتقييم مدى اختلاف حساسية صنف الارز المصرى سخا101 لاشعة جاما وذلك لدراسة التباينات الوراثية المستحدثة للحصول على طفرات مظهرية مرغوبة خاصة للصفات الاقتصادية الهامة ومقاومة مرض اللفحة. اجريت هذه الدراسة فى المزرعة البحثية لمحطة البحوث الزراعية بسخا-كفرالشيخ-مصر خلال موسمين زراعيين للارز2016-2017م. استخدمت اربع جرعات من اشعة جاما هى 300,200,100و 400 جراى لمعاملة بذور صنف الارز المصرى سخا101 فى موسم 2016. وتوضح النتائج ان التزهير كان مبكرا لجميع الجرعات الاشعاعية المستخدمة فى الجيل الاشعاعى الاول ما عدا الجرعة 400 جراى. لقد نقص ارتفاع النبات بزيادة الجرعات الاشعاعية فى كلا من الجيل الاول والثانى الاشعاعيين. قد نقص عدد الفروع المثمرة لكل نبات بزيادة الجرعات باستخدام الجرعات الاشعاعية 100 , 200 جراى بينما زادت بزيادة الجرعات الاشعاعية الى 300 ,400 جراى فى كلا من الجيل الاول والثانى الاشعاعيين. لقد ادت زيادة الجرعات الاشعاعية الى زيادة متوسطات كلا من النسبة المئوية للعقم ووزن ال100حبة فى كلا من الجيل الاول والثانى الاشعاعيين. بالنسبة لصفة محصول الحبوب لكل نبات لقد نقصت متوسطات القيم بزيادة الجرعات الاشعاعية حتى 300 جراى وبعد ذلك زادت عند الجرعة الاشعاعية 400 جراى, وذلك عندما زاد محصول الحبوب لكل نبات وسجل 47,7 جرام مقارنة بالصنف القياسى (44,0جرام) فى الجيل الاشعاعى الثانى. لقد ازدادت كل المدلولات الوراثية التى تم دراستها مثل التباين الوراثى, معامل الارتباط للتباين الوراثى, درجة التوريث بالمعنى الواسع و النسبة المئوية لمعدل التحسين الوراثى لمتوسط الاداء وذلك بزيادة الجرعات الاشعاعية المستخدمة فى الجيل الاشعاعى الثانى ممايشير الى ان انتخاب النبات الفردى لهذة الصفات يكون فعال ومرضى لاغراض برامج التربية الناجحة. لقد اظهر 101 نبات جيل تانى مطفر تزهيرا مبكرا ب10-20 يوم تبكيرا مقارنة بمتوسط قيم صنف الارز القياسى سخا 101ولقد انتج 105 نبات مطفر محصولا اكثر من 42 جرام للنبات الفردى مقارنة بنباتات الصنف القياسى. لقد اظهر حوالى 591 نبات مطفر من الجيل الثانى الاشعاعى من كل المعاملات الاشعاعية المستخدمة مقاومة لمرض اللفحة. ومن ثم, فان التباينات الوراثية المستحدثة تعتبر اكثر اهمية كشرط اساسى لبرنامج تربية ارز ناجح من خلال الانتخاب المباشر او عن طريق التهجين مع الاصناف التجارية المنزرعة.