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Genetic Improvements in Pea (*Pisum sativum*l.) Through Irradiation by Gama Rays

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ABSTRACT

Mutation breeding using radiation has been shown to be an effective and important tool in incorporating specific desirable agronomic values characteristics in vegetable crops including pea (Pisum sativum L.). In the present study, dry seeds of "Master - B" Pea cultivar were exposed to 0, 10, 20 and 30 Krad doses of gamma rays, and variability in growth characteristics, earliness, and seed yield were investigated in M1, M2 and M3 generations. Analysis of variance showed significant difference among M1-plants as well as M2 and M3-families for the studied traits with higher variability in high doses of gamma ray treatments. In M3–generation, lines with significant increases in yield parameters such as number of pods per plant and number of seeds per plant were obtained. Mutant line number 8 was out yielded in terms of number of pods and seeds per plant. These results indicate that induced mutation breeding by gamma ray is a valid and effective crop breeding method in pea.

Keywords: Gamma rays, genetic variability, Mutation breeding, pea, seed yield

INTRODUCTION

Pea (*Pisum sativum* L.) belongs to the family Fabaceae and is a relatively major vegetable crop in Egypt. It is one of the world's oldest agricultural products, high yield, short-term crop yield and high protein content. In dried seeds, the protein and carbohydrate content was 19.7% and 56.6% respectively.

Mutation breeding using radiation has been shown to be an important tool in incorporating specific desirable agronomic values characteristics. Gamma-ray irradiation of plant materials is commonly used to induce genetic mutation that alters the number of biochemical processes leading to beneficial genotype changes. The primary injury to plant material due to gamma irradiation is physiological damage, which is mainly limited to the M_1 generation. It can be detected and assessed cytologically as well as by the reaction of the whole organism in general by growth retardation and at higher doses of death (Sparrow, 1961). It is possible to create new variations in a short time using the mutation breeding method. The most used mutation breeding agent is physical mutagens such as ultraviolet (UV) light which leads to breaks on DNA double strain and deletions. Gamma rays and neutrons have high energy radiation applications (Koornneet, 2002).To enhance genetic variation for desirable traits, mutation breeding program can beinitiated to develop stabilized mutants with respect to high yield and some other desirable traits following selection in successive advance generations (Dewanjee and Sarkar, 2017).The ease of application irradiation plays an important role on widely spreading of the technique, about 90% of the obtained mutant cultivars were obtained with gamma-rays method, and 22% with X-rays (Jain, 2010). Reduction-induced mutation is one of the most widely used method to improve direct mutant varieties compared to acclimatization, selection, hybridization, which are laborious, time consuming and also with limited genetic variation (Hanafiah *et al.*,2010). Any change in growth pattern will ultimately affect maturity and yield. Gamma irradiation of seeds has been shown to have a significant effect on plant growth (Rubaihayo, 1978). Radiation leads to an increase in the formation of reactive oxygen species (ROS) in plant cells due to damage to cell homeostasis and causes progressive oxidative damage and, finally, cell death (Sharma *et al.*, 2012). In M_2-generation of *P. sativum*, gamma irradiation significantly affected morphological and proximate parameters. The most important dose was 7 Krad. That can be used for agricultural purposes to improve pea yield attributes. (Khan *et al.*, 2018).

Irradiating snap bean seeds with gamma rays strongly induced morphological changes. (Eiiyfa *et al.*, 2007).Seven superior mutants were selected when exposed seeds of cowpea to three doses from gamma rays 10, 20 and 30 Krad (Metwally *et al.*, 1998). A significant variation in the protein patterns of cowpea seeds is due to the new expression of some polypeptide, the silence of others, and the expression of third-class polypeptides (Mohammed *et al*, 2012). Gamma rays are known to affect the development and advancement of plants by affecting cytological, morphogenetic changes in cells and tissues. Several new cultivars in cowpea (Metwally *et al.*, 1998), in tomatoes (Sikder *et al.*, 2013) and mungbean (Sangsiri *et al.*, 2005) have been successfully developed by using gamma rays.

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The decline in the percentage of germination caused by the high doses of radiation applied may have resulted in a decrease in internal growth regulators (Kiong *et al.*, 2008). Gamma rays have induced reduction in plant height may be due to destructions or damage to apical meristem or reduction in the level of amylase activity and to temporary suspension of cell division or delay in mitosis or increase in the production of active radicals that are responsible for lethality or due to the increase in gross structural chromosomal changes induced by radiation (Esnault and Chenal , 2010).

The aim of this study was to induce a large scale of genetic variability by exposing Master-B pea cultivar seeds to different doses of gamma-ray in order to select new lines of pea from the superior mutant that could become a new cultivar and adapted to Egyptian environmental condition.

MATERIALS AND METHODS

The present study was carried out during the three consecutive growing seasons of 2016, 2017 and 2018 in a private farm in the Sidy Salim district, Kafr-Elsheikh Governorate and Sakha Experimental Station at Horticulture Research Institute, Kafr-Elsheikh. This work was completed in two stages, the first stage was started from November 2016 to April 2017 to induce mutation in pea (Master-B cultivar), and the second stage from November 2017 to April 2019 to characterize pea mutants produced and select desired plants in the following generations.

In the first stage, Pea seeds of Master-B cultivar were exposed to three doses of gamma-rays 10, 20 and 30 krad at a dose level of 7.69 rad / sec along with the control "0 Krad" at the Nuclear Research Center, Atomic Energy Establishment, Cairo. The source of gamma-rays was produced from Cobalt 60.

On November 1^{st}in both seasons irradiated and un-irradiated seeds (500 seeds per treatment) were sown on two sides of the ridge (0.8x10 m), 20 cm apart in a randomized complete block design with three replications. In the first season 2016, about 200 plants were chosen from each irradiated treatment which called M_1- plants. The seeds of these plants were separately harvested to generate the M_2-seeds.

M_2- seeds from the individual M-plants from each treatment were sown as a family on November 2017season under field conditions, as well as one row of unirradiated seeds as a control. During the growth period, macro-mutations such as dwarf, vigorous plants, early or late mature plants, and other morphological changes were collected separately to give rise to M_2–families. At harvest stage, seed yield and its components were measured for each plant from each family to determine the differences of M_2 - micro mutations. Data were recorded on plant height, number of branches, pods and seed yield per plant on M_2-plants.

In the second stage, M_3-seeds from 20 selected mutants with the control (unirradiated) were sown in open field on November 2018 for further selections. According to superior in vigorous growth, pod number, juvenility period (Number of days till flowering), high yield and large seeds than the original cultivar (Master B), 11 mutants were selected. The experimental design was a complete randomized block with three replications. For collecting the data, five plants were uprooted from each plot at the full blooming stage and the following data were recorded: plant height, number of branches/plant, number of pods/plant. In addition, number of seeds/pod and number of seeds/plant, seed index were recorded and the percentage of total protein in dry seed was determined using micro-Kjeldated method according to A.O.A.C. (1995). The total protein percent was calculated by the multiplication of nitrogen values by 6.25%

Statistical analysis

The data on M_2-Mutation were analyzed by calculating minimum value, maximum value (Range of variability) the mean treatment (X^-) variance (S^2), standard error (S.E), coefficient of variation (C.V.%), the ratio of coefficient of variation of treatment and control (C.V.% of treatment/ C.V.% of control) to determine the relative variability induced by gamma rays. In addition, the data on M_1 and M_3-Mutatiowere analyzed of variance according to Snedecor and Cochran (1971).

RESULTS AND DISCUSSION

Effect of gamma-rays on seed germination and M_1-plants

The results presented in Table (1) revealed that increasing rate of gamma ray from 0 to 30krad significantly decreased the percentage of seed germination. The maximum germination percent (90.13%) was observed with the unirradiated seeds (control) compared with irradiated seeds. The lowest germination percent (11.8%) was obtained with the seeds treated by gamma ray at rate 30 krad. The effect of mutagens on seeds is expressed through delayed emergence of roots, reduction in vigor, low metabolic and enzymatic activity, losses of membrane integrity and finally loss of germ inability, suggesting that germination is a radio sensitive phenomenon (Sushil and Dubey, 1997).These results are in agreement with those of Katyayani *et al.* (1980) observed the drastic reduction in the percentage of seed germination under higher doses of gamma irradiation in *Phasedusaureus*.

Regarding to plant height, results presented in Table (1) showed that increasing irradiation doses caused gradual decrease in plant height; where the control treatment gave the highest value in this respect as compared with the lowest values obtained from the high doses of gamma ray treatments. Similarly, Ramesh and Reddi (2002) reported that irradiation by gamma ray decreased plant height of three cultivars of *Oryza sativa* L.. This may be due to destruction or damage happen to apical meristem when high doses of gamma rays used (Patel and Saha, 1974),hampered respiratory enzyme synthesis and reduction in the level of amylase activity (Reddy and Vidyavathi, 1985), or temporary suspension of cell division or delay in mitosis. In the same line, Machaiah and Vakil (1979) mentioned that ionizing radiation causes inactivation of growth regulators leading to retarded plant growth. In addition, Selim *et al.* (1974) obtained that reductions in plant height may be due to an increase in the production of active radicals due to increase radiation doses.

The effects of gamma doses on number of branches and leaves/plant, fresh weight / plant and number of pods / plant were given in Table 1.The results showed that there were no significant differences among the radiation doses. Furthermore, plant dry weight was decreased with increasing of radiation doses from 0 to 30 krad. The highest dry weight was obtained by the control plants and radiation dose at 10 krad without significant differences. Radiation treatment dose of 10krad significantly increased number of seed per plant followed by control, radiation at 30 and 20krad. The decreasing in plant dry weight could be attributed to reduction plant height, branches and leaf number (Amjad *et al.*, 1993). In addition, radiation lead to increase the formation Reactive oxygen species (ROS) in plant tell due to damage of cellular homeostasis and cause progressive oxidative damage and finally cell death (Sharma *et al.*, 2012). Bajaj (1970) reported that gamma irradiations causes problems with RNA and protein synthesis and then inhibits growth of plants. The reduction in seed yield and its components with increasing the doses may be due to the reduction in fertility which may be attributed to chromosomal aberrations or attributed to physiological damage (Abd El-Rahman, 2000).

Table 1. Effect of gamma irradiation on growth characteristic and yield of Master B pea cultivar and M_1-plants during 2016 season

Radiation doses(KR)	Germination (%)	Plant height (cm)	No .of branches/ Plant	No. of leaves /plant	Plant fresh weight (g)	Plant dry weight (g)	No. of pods/plant	No. of seeds/plant
Control	90.1 a	30.00 a	0.40	9.40	11.70	2.21 a	5.60	29.70 ab
10	76.3 b	25.40 ab	0.30	9.00	10.60	1.80 a	7.10	32.90 a
20	43.7 c	14.60 bc	0.10	7.00	5.40	0.99 ab	4.50	16.00 b
30	11.8 d	3.90 c	0.00	4.40	2.60	0.39 b	5.00	19.90 ab
L.S.Dat5%	11.98	12.19	1.12	5.85	9.36	1.26	3.54	15.57

Means followed by different letters within column are different by Duncan's multiple range test in 0.05 *P*-significance.

2-Effect of gamma irradiation on M_2-generation

Results in Table 2 show that high doses of gamma rays treatments were significantly induced genetic variability higher than control. The measurements of variation (rang of variability and coefficient of variation) in the treated plants were higher than those of the control. Therefore, the increments in variability in M_2- irradiated generation could be due to genetically variations affecting the plant height (Hussein *et al.* 1974), suggesting ware opportunities for the selection of the desirable plant height.

Data presented in Table 3 show that the estimates for number of branches per plant under doses of gamma rays was not statistically variable in spite of the appearance of some differences among the treatments means for this trait. This response for number of branches per plant to radiation might be due to the balance between the stimulating effect of the lower doses of mutagen and inhibiting effect of the higher doses (Badr *et al.*, 2000). Furthermore, the depression effect of the high mutagen doses on vegetative traits maybe attributed to the active disturbances of some enzyme involved in the synthesis of the growth (Abd El-Rahman, 2000). Data presented in Table 3 showed that the number of pods per of pea plant cultivar Master B was affected by the different mutagenic treatments in M_2-generation.The response of seed yield per plant to mutagenic treatments differed in the cultivar Master after irradiated with gamma rays. The highest percentage of mutants in the M_2-generation was obtained and selected 11 mutants with more seeds per pod, larger seeds and plant height than initial form (Khan *et al.* 2018) on pea reported that gamma irradiation significantly affected morphological and proximate and yield parameters in M_2- generation.

Table 2. Effect of gamma irradiation in M_2- generation on plant height and number of branches of pea plants cv."Master B" during 2017 season

Radiation doses(KR)	Number of plants	Plant height (cm)					No. of branches				
		Min.	Max.	$\bar{x}$±S.E	C.V. %	$\frac{C.V.treat}{C.V.Cont}$	Min	Max	$\bar{x}$±S.E	C. V.%	$\frac{C.V.treat}{C.V.Cont}$
Control	450	45	63	56.10 ± 5.57	17.21	1.00	1.20	2.10	1.80 ± 0.33	17.21	1.00
10	381	52	62	57.00 ± 3.00	9.11	0.53	1.00	2.00	1.60 ± 0.30	33.23	1.93
20	217	57	84	67.70 ± 8.33	21.31	1.23	2.00	2.50	2.30 ± 0.14	11.16	0.65
30	59	51	85	65.80 ± 10.13	26.77	1.55	0.80	2.50	1.60 ± 0.49	55.17	3.20

Table 3. Effect of gamma irradiation in M_2- generation on number of pods and seeds of pea plants cv."Master B" during 2017 season

Radiation Doses (Krad)	Number of plants	Number of pods/plant					Number of seeds/plant				
		Min	Max	$\bar{x}$±S.E	C.V.%	$\frac{C.V.treat}{C.V.Cont}$	Min	Max	$\bar{x}$±S.E	C.V.%	$\frac{C.V.treat}{C.V.Cont}$
Control	450	9.00	19.00	14.10 ± 2.98	36.84	1.00	42.00	52.00	48.30 ± 3.40	12.19	1.00
10	381	9.00	14.00	12.10 ± 1.48	21.31	0.58	34.00	68.00	57.00 ± 11.44	34.75	2.85
20	217	12.00	24.00	16.70 ± 0.99	41.36	1.12	51.00	108.00	73.40 ± 17.83	42.07	3.45
30	59	6.00	33.00	17.20 ± 8.23	82.85	2.24	25.00	91.00	57.20 ± 18.94	57.42	4.71

3- Effect of gamma rays on M_3–generation

In M3-generation,11 lines were selected based on superiority in vegetative growth and flowering traits, and high yield with large seeds than the original cultivar (Master B).The results presented in Table 4 showed that there were significant differences between the mutant lines for plant height and number of branches. The tallest line was obtained by Mutant line 10, while the shortest one obtained with line 4 compared with original cultivar (control). In addition, mutant line 8 had the higher number of branches per plant than the original cultivar. On the other hand, mutant line 6 had the lowest number of branches per plant.

It is obvious from Table 4 that there were significant differences in number of days till flowering among M_3-lines selected and the original cultivar (Master B). Mutant line 3 had the shortest days to flowering compared to original cultivar with 9 days differences. Significant differences among 11 mutant lines and original cultivar were also observed for seed yield and its components (number of pods per plants, number of seeds per pod and seed index) as indicated in Table 4. In general, most of mutant lines were produced more pods and heavy seeds than the original cultivar, while the behavior of number of seeds per pod differed among the mutants and the original cultivar. Mutant line number 8 was produced the highest number of pods per plant. Furthermore, mutants lines 5, 8, 9, and 11 were produced the highest number of seeds per plant. In the same line, Metwall *et al.* (1992) found that gamma rays was increased significantly seed yield of cowpea plants which were expressed by number of pods per plant, number of seeds per pod as well as the value of seed index.

Regarding to seed protein percentage, data in Table 4 indicate that one mutant line No. 3 contain higher values than the original cultivar. On the other hand, mutant line No. 7 was decreased significantly in their protein content. Khan *et al*. (2018) found that irradiated pea in M_2 generation reported was varied in seed protein.

Table 4. Growth characteristic, flowering date, seeds yield and components of 11mutants of pea comparing with cv. "Master B" during 2018 season.

Number of line	Plant height (cm)	No. of branches/ plant	No. of days till flowering	No. of pods /plant	No. of seeds /pod	No. of seeds /plant	Seed index	Protein % in seeds
Control	63.80 g	1.50 cde	38 a	13.80 c	9.50 a	78.00 bc	14.90 g	19.89 ab
1	92.80 e	1.00 de	38 a	14.30 c	7.00 cd	79.30 bc	17.20 e	15.07 d
2	78.80 f	2.80 ab	38 a	20.80 bc	8.30 b	96.80 abc	18.80 c	18.61 bc
3	97.50 e	2.30 abc	47 d	20.30 bc	6.00 d	71.00 c	20.40 a	21.40 a
4	38.00 h	1.50 cde	38 a	6.00 d	7.80 bc	30.00 d	14.10 h	19.20 bc
5	103.00 de	2.00 bcd	44 b	19.00 bc	7.00 cd	114.50 a	18.70 c	19.64 abc
6	118.00 bc	0.80 e	43 c	18.30 bc	8.00 bc	93.80 abc	18.90 c	18.47 bc
7	129.30 ab	1.00 de	43 c	22.80 b	7.50 bc	107.80 ab	18.00 d	17.61 c
8	102.30 de	3.30 a	43 c	29.30 a	7.30 bc	128.30 a	19.60 b	18.80 bc
9	112.00 cd	1.00 de	43 c	17.00 bc	7.80 bc	118.30 a	17.10 e	18.19 bc
10	136.00 a	0.80 e	43 c	18.30 bc	7.50 bc	104.30 abc	16.00 f	19.75 ab
11	60.30 g	2.50 abc	43 c	17.00 bc	9.50 a	122.50 a	16.80 e	19.74 ab
L.S.Dat5%	12.07	1.002	0.004	6.13	0.97	30.79	0.57	1.81

Means followed by different letters within column are different by Duncan's multiple range test in 0.05 *P*-significance.

CONCLUSION

Induced mutation is a valuable tool for inducing new genetic variability in vegetable crops including pea. In the present study, genetic variability was observed for all morphological and yield measured characters in M_1-plants and M_2 generation, and new mutant families of pea with novel morphological traits and higher seed yield were selected in M_3-generation. These elite mutant families could be used in the future breeding program in pea for development of new cultivars adapted for Egyptian condition.

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دراسة الاختلافات الوراثية فى البسلة باستخدام اشعة جاما

على ابراهيم على مصرى ، عاطف محمد السيد فياض و داليا ابراهيم طاهر
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يعتبر استخدام الطفرات عن طريق الاشعاع من اهم الطرق الفعالة والهامة فى التحسين الوراثى فى محاصيل الخضر وخاصة المحاصيل ذاتية التلقيح مثل البسلة. لذلك إجريت الدراسة خلال ثلاثة مواسم متتالية ابتداء من 2017 حتى 2019على نباتات البسلة صنف ماستر بى المنزرعة بمزرعة خاصة بمحافظة كفرالشيخ – مصر. وذلك بهدف استحداث اختلافات وراثية يمكن استخدامها فى تحسين محصول البسلة .لذا تم استحدام جرعات مختلفة من اشعة جاما (0 , 30,20,10) كيلو راد على خصائص النمو المختلفة والتبكير والمحصول ومكوناتة بالاضافة الى الاختلافات الوراثية على الجيل الاول والثانى والثالث(M_1,M_2,M_3) اظهرت النتائج وجود فرووق معنوية بين نباتات الجيل الاول من الناحية المورفولوجية بالاضافة الى وجود اختلافات وراثية بين نباتات الجيل الثانى والثالث وخاصة مع زيادة جرعة الاشعاع (30كيلو راد) .كما اوضحت النتائج وجود زيادة معنوية فى سلالات الجيل الثالث المنتخبة وخاصة السلالة رقم 8 فى خصائص المحصول مثل (عدد القروون / نبات ,عدد البذور / نبات). لذلك توصى الدراسة الى ان استخدام اشعة جاما من افضل الطرق الفعالة فى تحسين البسلة.