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IMPROVING SEED YIELD AND PROTEIN CONTENT OF COMMON BEANS (*PHASEOLUS VULGARIS* L.) THROUGH MUTATION BREEDING

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ABSTRACT: The common bean is a staple food and source of protein in many African countries. To date, genetic improvement of common bean in Egypt lags behind compared with other legume crops which mainly due to limited genetic resources. This study was carried out in three consecutive growing seasons (2016, 2017 and 2018) at Sakha Experimental Station, Horticulture Research Institute (HRI) at Kafr El-Sheikh governorate, Egypt. The study aimed to increase genetic variations in Nebraska bean cultivar through mutation breeding for selection of novel bean lines with higher seed yield and protein content. The experiment was arranged in a randomized complete blocks design (RCBD) with three replications. Seeds of Nebraska bean cultivar which widely grown in Egypt were treated with gamma rays three different doses (10, 20, and 30 kr), and selection was made at M_2 and M_3 generations for desired traits related to vegetative growth parameters, pod characteristics and seed yield components. These traits were significantly affected by irradiation treatments in M_1 generation, and changes in plant height, number of branches per plant, number of pods and seeds per plant were generally favorable in M_2 generation than control (un-irradiated plants). Analysis of variance in M_3 generation showed a highly significant difference among M_3 lines and control for vegetative and agronomical traits except for the traits of number of days to first flower and number of seeds per pod. Among 25 M_3 lines, ten mutant lines were produced higher seed yield (4.3 to 108.6%) and protein content (0.08 to 2.79%) along with heavier seeds (3.5 to 35%) than original parent Nebraska cultivar. Hence, these lines should be evaluated for the desired traits and wide adaptability to biotic and abiotic stress to release new improved varieties in Egypt.

Key words: Beans, gamma rays, legumes, mutation breeding, protein content, seed yield, selection.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L. 2n = 2x = 22) is a member of the *Leguminosae* (*Fabaceae*) family and represent one of the most widely grown grain legumes worldwide for its edible green pods and dry seeds (Broughton et al., 2003; Porch et al., 2013 and Hayat et al., 2014). Despite being cultivated for its green immature pods and grains, beans are produced and consumed mainly as dry grain. Common bean ranks as the third food legume crop worldwide, after soybean (*Glycine max* L.) and peanut (Arachis hypogea L.), in global production with 26 million tonnes produced in 2017 (FAO. 2017). The common bean is a highly self-pollinating crop and adaptable to different cropping systems which makes it attractive to many farmers in different regions of the world (Raatz et al., 2019). Common bean is propagated primarily using seeds, although it is possible to propagate bean vegetatively, using stem cuttings (Wortmann, 2006 and Brink & Belay, 2006). Based on morphology, Common bean includes numerous cultivars with a considerable variation for several traits including growth habit, and seed color and size (Purseglove, 1968; Singh et al., 1991 and De Ron et al. 2004).

Regarding nutritional value, it is an especially important and cheap source of proteins, starch, dietary fiber, vitamins (folic acid and other B vitamins) and minerals such as iron, zinc, potassium, and beneficial phytochemicals, antioxidants and flavonoids (Brigide et al., 2014; Vaz Patto et al., 2015 and Mukankusi et al., 2019). Additionally, they are used increasingly as substitute for meats and valuable source of complementary protein in the standard diets of low-income consumers (Gonzalez et al., 2011 and Rivera et al., 2018). Previous studies have demonstrated that common bean have specific properties of health benefits when included in the daily diet such as reducing risk of cardiovascular diseases, obesity, diabetes, and colon, prostate and breast cancer (Correa 1981; Hangen and Bennink 2003 and Thompson et al. 2009). Besides their great nutritional value, it contributes to environmental sustainability due to their biological nitrogen fixation, which makes them attractive crops for soil improvement and the control of weeds in farming systems (Rubiales and Mikic, 2015 and Bitocchi et al., 2016).

The common bean is native to the new World and was first domesticated around 8000 years ago in Mesoamerica and the Andes (Bitocchi et al., 2012 and Rendón-Anaya et al., 2017). Around the sixteenth century, Common bean have been introduced into Africa by Spanish traders and Portuguese travelers (Gentry 1969 and Asfaw *et al*., 2009). Nowadays, common bean is a staple food in many African countries, and represented the third most important source of calories after cassava and maize for lower income populations in Africa (Asfaw et al., 2009 and Mukankusi et al., 2019), occupying more than 7 million ha annually and a total production of about 6 million tons (FAO., 2017). In eastern and southern Africa, young bean leaves and green immature pods are occasionally consumed as a vegetable, while straw from the plants is used as forage and bedding for livestock, fuel and roofing material (Broughton et al., 2003; Wortmann, 2006 and Dagnew *et al*., 2014). In the last 10 years, bean production has increased in most African countries as a result of an increase in the demands of consumers, but there are some constraints in their production, such as poor adaptation, pests and diseases, and unstable yield. In this respect, the development of higher-yielding varieties, useful traits such as the size of the seeds, and quality of the crops is the main need of the time Bean farmers (Kelly and Cichy, 2012 and Vandemark et al. 2014).

Common bean has a narrow genetic base due to the bottlenecks associated with its evolution and domestication. Therefore, plant breeders need to broad genetic base of common beans to breed new cultivars. Mutation breeding is an important and effective approach in legume crop improvement (Mba et al., 2010). Mutations may occur naturally or can be induced artificially (Singh et al., 2006; Wani, 2006 and Tulmann Neto et al., 2011). The natural mutation rate occurs rarely and difficult to identify. The induced mutations have been successfully used in breeding of seed propagated crops since 1940s (Gnanamurthy et al., 2012). In recent years, induced mutations through physical and chemical mutagens have received great attention as a powerful method to enhance genetic variation for desirable traits in self-pollinated crops (Sharma and Mishra 2007; Dewanjee and Sarkar, 2018 and Olaolorun et al., 2019). To date, more than 431 varieties of legumes developed through artificial mutations as listed in the FAO/IAEA Mutant Varieties Database (MVD) (FAO, 2017). Physical mutagens are physical agents that usually cause alterations in DNA molecules, such as DNA double strand breaks and deletions (Kozjak and Meglič, 2012). Among physical mutagens, gamma-ray is most widely used in plants for the development of large mutant populations in comparison to other rays such as alpha and X- rays, about 90% of the obtained mutant cultivars were obtained with the gamma-rays method, and 22% with X-rays (Jain, 2010; Kodym et al., 2012). Gamma rays can be exploited to generate favorable changes in plant's morphology, anatomy, biochemistry, and physiology in a short period of time without any negative impact on the environment, and this depended on plant species or varieties and the dose of irradiation used (Gunkel, 1957; Mokobia and Anomohanran, 2005 and Maluszynski et al., 2009). Several studies on the effectiveness and efficiency of gamma-ray to increase genetic diversity have been performed in several legume crops, such as cowpea (Metwally et al., 1998 and Girija et al., 2013), chickpea (Wani and Anis, 2008 and Amri-Tiliouine et al., 2018), mung bean (Sangsiri et al., 2005), common beans (Villavicencio, et al., 2000), and pigeon pea (Desai and Rao, 2014).

Developing improved selections with higher seed yield and protein content are the major breeding objectives to develop improved varieties in Egypt. Numerous studies showed the effect of gamma rays on the plant development of common bean is quite limited. Therefore, the objective of this study was to increase genetic variability in Nebraska bean cultivar through mutation breeding by gamma-ray to improve seed yield and protein content for development of a new improved cultivar and adapted to biotic and abiotic stress.

MATERIALS AND METHODS

This study was carried out in three consecutive growing seasons (2016, 2017 and 2018) at Sakha Experimental Station, Horticulture Research Institute (HRI) at Kafr El-Sheikh governorate, Egypt. Bean seeds of Nebraska cultivar were obtained from HRI and exposed to three doses of gamma-rays 10, 20 and 30 Kr with a dose level of 7.69 rad/sec at the Nuclear Research Center, Atomic Energy Establishment, in Cairo. The source of gamma-rays was produced from Cobalt 60. Un-irradiated seeds served as a comparative control. A total of 500 seeds per dose of Nebraska cultivar (together with control) were sown separately in April 2016 in hills of 30 cm apart and 70 cm row width. A total of 537 M1-plants were obtained from three different gamma ray treatments and evaluated for plant height (cm), number of branches per plant, number of pods per plant, number of seeds per plant, pods length, pod width, leaf area per plant (cm^2), fresh weight per plant, and dry weight per plant. The seeds of M1-plants were separately harvested to generate the M2-seeds.

M2-seeds from the individual M1-plants were sown in April 2017 and 250 families were evaluated for some characters in open field condition i.e. plant height (cm) , number of branches per plant, number of pods and seeds for each plant from each family to determine the differences of M2-micro mutations. According to performance data of M2 generation, 25 M2-plants were selected with vigorous growth and higher seed yield than original cultivar (Nebraska) and seeds were harvested separately to generate M3-seeds.

M3 selected mutant lines along with un-irradiated seeds (control) were sown in open field trials as indicated above in a randomized complete blocks design (RCBD) with three replications in April 2018. At vegetative growth stage, plant height (cm), number of branches per plant and number of days to first flower were recorded. Chlorophyll content was measured by Soil Plant Analysis Development (SPAD) chlorophyll meter (Minolta, Co., Ltd, Japan), on fully expanded leaves without destroying them. At harvest stage, number of pods per plant, pods length and number of seeds per plant, number of seeds per pods, seed index per plant and seed weight per plant (g) were recorded. The total protein percentage 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

Statistical procedures were performed using the statistical software SAS (version 9.1; SAS Institute, Cary, NC). Data of M1 and M3 generations were subjected to one-way analysis of variance (ANOVA) and mean comparisons were made using Duncan's multiple range test. In addition, minimum and maximum values, means of treatments and coefficient of variation (C.V.%) were calculated in M2 generation to determine the relative genetic variability induced by gamma rays.

RESULTS AND DISCUSSION

Effect of gamma-rays on seed germination and morphological changes in M_1-plants

Germination percentages of un-irradiated and irradiated bean seeds of Nebraska cultivar are presented in Fig. 1. Germination percentage of Nebraska cultivar was significantly decreased in irradiated seeds compared to un-irradiated seeds (control). Seed germination percentage was recorded 85% in un-irradiated seeds. The maximum inhibition in germination percentage was observed at 30 Kr with lower values than 6%. Lesser decreases in seed germination were observed at 10 and 20 Kr with germination percentages 52.4% and 47.5%, respectively. A total of 262, 241 and 34 M1-plants were obtained from gamma irradiation treatments 10, 20 and 30 Kr, respectively. In general, it was observed that exposure to gamma ray produces morphological and physiological changes compared to the control. These changes are largely relying on the dose of gamma irradiation (Wi et al., 2005 and Gnanamurthy et al., 2012). The germination percentage of seeds is a good indicator of the effectiveness of gamma rays (Ulukapi and Ozmen, 2018). Our results revealed that the germination percentage of seeds was significantly decreased at higher doses of gamma irradiation. Similarly, Ulukapi and Ozmen (2018) also observed that a high dose of gamma irradiation reduced the germination percentage of seeds in common bean. These results have also been reported in other crops such as chickpea (Toker et al., 2005), cowpea (Ezzat et al., 2019), Pea (Masry et al., 2019), soybean (Khan and Tyagi, 2010), mungbean (Wani et al., 2011) and Faba bean (Khursheed et al., 2016). However, other studies reported that gamma irradiation have no significant influence on germination percentages in cowpea and faba bean (Mejri et al., 2015; Horn et al., 2016), suggesting differential responses of crop species for the tested irradiation doses.

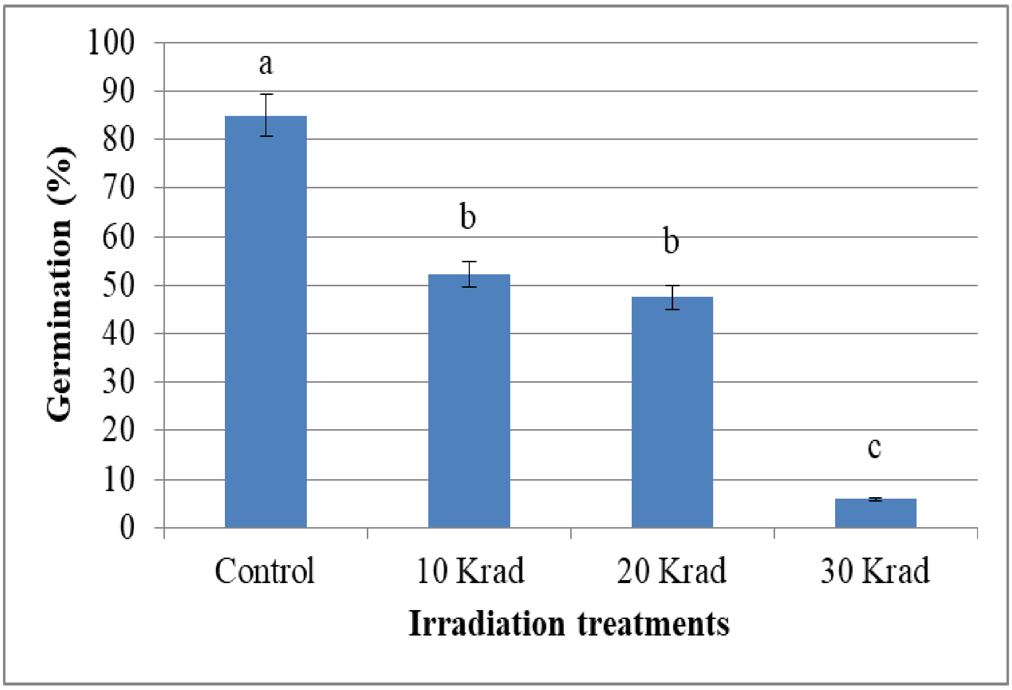

Fig. 1. The effect of different gamma radiation doses on the germination of common bean Nebraska cultivar compared to un-irradiated seeds (control)

Significant and highly significant differences were observed among irradiation treatments for all vegetative growth characters (Table, 1). Taller plants were observed in control and M1-plants at 10 Kr with values 40.5 and 40.4 cm, respectively. Higher numbers of branches per plant (4.3) were observed in M1-plants at 20 Kr. M1-plants at 10 Kr were recorded the highest leaf area per plant, fresh and dry weight per plant with values 176.8, 94.7, 15.5, respectively. In contrast, M1-plants at 30 Kr were recorded the lowest values of vegetative growth characters. Highly significant differences were observed among irradiation treatments for number of pods and seeds per plant and pod length (cm) (Table 2). Higher numbers of pods and seeds per plant, and pod length were observed in control and M1-plants at 10 and 20 Kr, where 30 Kr gave the lowest values. On the other hand, gamma radiation had no significant impact on pod width (Table, 2). Reduced growth caused by radiation may be attributed to decrease in internal growth regulators and increase in the production of reactive oxygen species (ROS) in plant cell that are responsible for lethality or due to the increase in gross structural chromosomal changes induced by radiation (Kiong et al., 2008, Sharma et al., 2012, Jan et al., 2012). The reduction in vegetative growth parameters may be attributed to the damage to the process of cell division and cell elongation or destructions to apical meristem or reduction in the level of amylase activity that generally result after mutagenic treatment (Iqbal, 1969, Esnault and Chenal, 2010 and Jan et al., 2012).

Table 1. Means of vegetative traits of M1-plants and control evaluated in summer season of 2016 under open field conditions

Treatment	Plant height (cm)	No. of branches/ Plant	Leaf area/ plant (cm^2)	Fresh weight/ Plant (g)	Dry weight/ Plant (g)
Control	40.5 a	3.6 b	122.5 c	78.0 b	12.5 b
10 KR	40.4 a	3.2 b	176.8 a	94.7 a	15.5 a
20KR	36.8 b	4.3 a	145.9 b	79.7 b	12 b
30KR	30.7 c	3.7 ab	66.0 d	30.0 c	4.3 c
F test	**	*	**	**	**

* and ** indicate significance at $P \leq 0.05$ and $P < 0.001$, respectively

Table 2. Means of seed yield components and pod characteristics evaluated on control and M1-plants in summer season of 2016 under open field conditions

Treatment	No. of pods/plant	No. of seeds/plant	Pod length (cm)	Pod width (cm)
Control	13.7 a	31.4 a	9.2 a	1.0
10 Kr	13.4 a	29.7 a	9.4 a	1.0
20Kr	16 a	26.3 a	9.0 a	1.1
30Kr	2.7 b	1.5 b	4.2 b	0.9
F test	**	**	**	ns

** indicate significance at $P < 0.001$, ns indicates not significant at $P \leq 0.05$

Evaluation of M$_2$-generation for vegetative growth and seed yield traits

The morphological and agronomical alterations in M2-generation were observed and characterized (Table, 3). Results show that 10 Kr was significantly induced higher values in plant height, number of pods per plant, number of seeds per plant and seed index than control. However, the number of branches per plant at 30Kr dose showed noticeable increases despite the appearance of some differences among the treatments. Furthermore, C.V% values were increased at irradiation treatments compared to control for tested traits plant height as well as numbers of branches, pods and seeds per plant. Higher values were observed at 20 Kr for plant height and numbers of pods and seeds per plant. Higher values were observed at 30 Kr for number of branches per plant. The present study demonstrated that high doses of gamma radiation had negative effects on the plant height, leaf area per plant, fresh and dry weight per plant, and pod length. The reduction of plant height with increased irradiation doses was reported by Wani (2011) and Kozgar (2014) in chickpea. Furthermore, Bader et al (2014) reported that the high levels of irradiation doses adversely reduced the fresh and dry weight per plant in Egyptian varieties of cowpea the reduction in fresh and dry weights might be attributed to the decrease in shoot moisture contents due to radiation stress (Hanafy and

Table 3. Means of morphological and seed traits in M2-plants and unirradiated plants of Nebraska cultivar evaluated during summer season of 2017

Irradiation dose (Kr)	Plants (no.)	Minimum	Maximum	Mean ± S.E	C.V. %	Minimum	Maximum	Mean ± S.E	C. V.%
		Plant height (cm)				Number of branches/ plant			
0 (Control)	500	30	52	40.13 ± 0.17	10.92	2	4	2.65 ± 0.03	29.22
10	262	22	170	48.73 ± 0.22	36.84	1	6	3.78 ± 0.01	29.91
20	241	30	132	49.35 ± 0.60	41.56	1	7	3.55 ± 0.03	35.4
30	34	30	50	38.50 ± 0.82	17.22	2	6	4.25 ± 0.20	39.27
		Number of pods/plant				Number of seeds/plant			
0 (Control)	500	10	20	13.68 ± 0.12	23.14	12	81	36.82 ± 0.45	30.94
10	262	6	52	20.86 ± 0.12	49.1	9	132	59.37 ± 0.35	47.55
20	241	4	47	16.23 ± 0.25	53.26	10	119	47.50 ± 0.82	58.88
30	34	7	34	21.00 ± 1.12	42.74	21	86	55.00 ± 2.47	35.97

Akladious, 2018). Aney (2013) also observed reduction in pod size in gamma irradiated plants of pea. In the present study the irradiated dose 10 Kr had a stimulatory effects on vegetative growth parameters, but reduced at higher doses. However, number of branches per plant increased at irradiated dose of 30 Kr in M_2 generation. Similar results have been reported in different crop legumes (Tah 2006; Velu et al., 2012; Horn and Shimelis, 2013; Bader et al., 2014; Roslim *et al.,* 2015; Olasupo et al., 2018 and Ezzat et al., 2019).

Evaluation of M_3–generation for vegetative growth and agronomical traits

A total of 25 M_3-lines were characterized for nine quantitative phenotypic traits (plant height (cm), number of branches per plant, number of days to first flower, number of pods per plant, number of seeds per plant, number of seeds per pods, seed index, pod length (cm), pod width (cm)), in addition to total protein and chlorophyll contents (Tables 4 and 5). Significant differences among M_3-lines and the control (Nebraska cultivar) were detected for plant height and number of branches per plant (P < 0.05). The tallest plants (68.3 cm) were observed in Mutant line 8, while the shortest plants (47 cm) were observed in Mutant line 17. Highest numbers of branches per plant (6.7) were observed in Mutant line 23, whereas the lowest numbers of branches (3.3) were observed in mutant line 14.

Highly significant differences among M_3-lines and the control were detected for total chlorophyll and protein content (P < 0.01). The maximum total chlorophyll content was observed in mutant line 16, while minimum content was observed in mutant line 25. The chlorophyll mutants are used for the evaluation of genetic effects of various mutagens (Khan and Tyagi, 2009; Goyal and Khan, 2010 and Tulmann Neto et al., 2011). Here, we observed that the chlorophyll content was increased at 20 Kr and decreased significantly at 30 Kr dose in the M_3 lines. This result is consistent with that obtained by Ulukapi and Ozmen (2018) and Singh et al. (2013). They indicated that chlorophyll mutants were also decreased in the common beans and cowpea when higher doses of gamma irradiation used. The reduction in the chlorophyll content caused by the high doses of radiation applied may be related to alteration in photosynthesis, changes in the plant cell structure and metabolism (Ashraf 2009; Jan et al., 2012). Furthermore, it is stated that the high doses of irradiation may cause inhibition of senescence and dedifferentiation into agranal stage in plastid (Kim et al., 2004).

Results show that total soluble protein content is also affected by irradiation. The mutant line 5 gave the highest protein content (18.30%) followed by mutant line 6 and 8, whereas the mutant line 20 gave the lowest content (14.90%). The application of gamma irradiation also affects proteins by causing conformational changes, oxidation of amino acids, and formation of protein free radicals (Lee et al., 2005). The present study demonstrated that gamma radiation has a significant impact on the protein content compared to the control in M_3 generation. Our results agree with the findings described by several reports. Mahmoud et al. (2016) reported that total protein content was slightly higher than control in millet grains. Abu et al., (2006) reported also similar results in cowpea. Increasing of protein content after radiation may be due to the reduction in the anti-nutrients particularly tannin content of seeds (Osman et al., 2014; Ahmed et al., 2018).

Table 4. Means of vegetative traits, number of days to first flower and chemical analyses of M3-lines evaluated in summer season of 2018

Line code	Plant height (cm)	No. of branches/plant	Number of days to 1st flower	Chlorophyll	Protein (%)
1	55.2 b-e	4.7 b-e	45	21.0 a-d	16.34 e-i
2	63.3 abc	4.7 b-e	45	16.8 def	16.87 d-g
3	61.2 a-d	4.0 cde	45	16.9 def	17.02 c-f
4	58.8 a-e	5.7 abc	45	18.5 b-f	16.90 c-g
5	52.7 cde	6.3 ab	46	19.2 a-f	18.30 a
6	56.0 a-e	5.0 a-e	45	21.4 abc	18.07 ab
7	56.7 a-e	4.3 cde	45	19.7 a-f	16.19 f-i
8	68.3 a	5.3 a-d	45	20.2 a-e	18.29 ab
9	57.3 a-e	4.7 b-e	45	19.4 a-f	16.05 f-i
10	66.7 ab	4.7 b-e	45	22.0 ab	15.98 ghi
11	61.0 a-d	5.3 a-d	45	17.3 c-f	17.30 b-e
12	53.3 cde	4.0 cde	45	18.5 b-f	15.32 ij
13	57.0 a-e	3.7 bc	45	20.1 a-f	18.23 ab
14	60.3 a-d	3.3 e	45	20.5 a-e	15.70 hij
15	61.7 a-d	4.3 cde	45	20.0 a-f	15.91 ghi
16	52.0 cde	5.0 a-e	45	23.1 a	16.12 f-i
17	47.0 e	4.7 b-e	46	19.2 a-f	16.64 d-f
18	57.0 a-e	5.3 a-d	45	20.9 a-d	17.37 a-d
19	50.5 de	4.3 cde	45	21.1 a-d	16.77 d-g
20	54.0 b-e	4.7 b-e	46	20.1 a-f	14.90 j
21	58.3 a-e	5.7 abc	45	17.5 c-f	15.58 i-j
22	64.7 abc	5.3 a-d	45	17.1 c-f	17.86 abc
23	63.0 a-d	6.7 a	45	18.1 b-f	16.87 d-g
24	56.0 a-e	4.3 cde	45	16.2 ef	17.39 a-d
25	56.7 a-e	5.3 a-d	46	15.8 f	15.71 hij
Nebraska CV.	55.0 b-e	5.0 a-e	45	18.5 b-f	15.50 ij
F-Test	*	*	ns	**	**

* and ** indicate significance at $P \leq 0.05$ and $P < 0.01$, respectively, and ns indicates not significant at $P \leq 0.05$

Table 5. Means of seed yield components and pod characteristics in M3-lines evaluated in summer season of 2018

Line code	No. of pods/ plant	No. of seeds/ plant	No. of seeds/ pod	Seed weight/plant (g)	Seed index	Pod length (cm)	Pod width (cm)
1	33.3 a-b	73.7 b-f	3.6	31.00 b-f	42.0 d-h	14.7 b-f	1.1
2	28.3 a-d	64.0 b-i	3.7	31.67 b-e	49.5 a-d	12.8 b-i	1.0
3	22.0 c-g	57.3 c-i	3.5	26.33 c-h	46.2 a-e	11.5 c-i	1.0
4	28.0 a-d	67.3 b-h	3.2	29.00 c-g	42.8 c-h	13.5 b-h	0.9
5	25.7 b-e	80.7 bcd	3.7	28.67 c-g	35.5 ghi	16.1 bcd	1.0
6	19.0 d-g	47.0 e-i	3.0	20.00 e-i	44.4 b-g	9.4 e-i	1.0
7	21.3 d-g	70.7 b-h	3.7	34.00 bcd	48.1 a-e	14.1 b-h	0.9
8	25.0 b-f	70.3 b-h	3.3	29.00 c-g	41.3 d-h	14.1 b-h	1.0
9	22.3 c-g	69.0 b-h	3.8	27.00 c-h	38.9 e-h	13.8 b-h	0.9
10	24.7 b-f	75.7 b-e	3.7	41.00 ab	53.9 a	15.1 b-e	1.0
11	27.7 a-d	77.3 bcd	3.1	27.33 c-h	36.3 f-i	15.5 bcd	1.0
12	21.3 b-g	72.3 b-g	3.9	33.00 bcd	45.5 a-f	14.5 b-g	0.9
13	12.7 g	42.7 ghi	3.7	22.67 d-i	53.1 ab	8.5 ghi	1.0
14	13.0 g	44.3 f-i	3.6	19.00 f-i	42.9 c-h	8.9 f-i	0.9
15	19.7 d-g	52.3 d-i	3.5	24.00 d-i	45.1 a-g	10.5 d-i	0.9
16	19.3 d-g	59.0 c-i	3.3	27.33 c-h	45.2 a-g	11.8 c-i	1.0
17	14.7 fg	42.3 hi	3.3	15.33 hi	36.1 f-i	8.5 hi	0.9
18	19.0 d-g	53.7 d-i	3.2	21.67 d-i	40.3 d-h	10.7 d-i	0.9
19	12.3 g	45.7 f-i	3.7	18.33 ghi	40.1 d-h	9.1 f-i	1.0
20	13.0 g	34.7 i	3.1	13.33 i	38.6 e-h	6.9 i	0.9
21	37.0 a	92.0 ab	3.5	48.00 a	52.2 abc	18.4 ab	0.9
22	32.3 abc	114.0 a	3.7	38.33 abc	34.4 hi	22.8 a	1.0
23	25.0 b-f	83.3 bc	3.0	33.00 bcd	39.6 e-h	16.7 bc	0.9
24	20.3 d-g	65.3 b-h	3.3	18.33 ghi	28.1 i	13.1 b-h	0.9
25	16.7 efg	47.0 e-i	3.5	18.67 f-i	39.7 e-h	9.4 e-i	0.9
Nebraska cv.	18.0 d-g	57.7 c-i	3.5	23.00 d-i	39.9 d-h	11.5 c-i	1.0
F-Test	**	**	ns	**	**	**	ns

* and ** indicate significance at $P \leq 0.05$ and $P < 0.01$, respectively, and ns indicates not significant at $P \leq 0.05$

Highly significant differences were observed among M_3-lines and the control for all seed yield traits (P < 0.01), except number of seeds per pod (Table, 5) the highest numbers of pods per plant (37), numbers of seeds per plant (114), seed weight per plant (48g) and seed index (53.9) were obtained by the mutant lines 21, 22, 21 and 10, respectively. Results in Table (5) showed that pod length varied significantly from 6.9 to 22.8 cm. Maximum pod length was obtained by mutant line 22, followed by mutant line 21, while minimum pod length was recorded for mutant line 20. On the other hand, the number of seeds per pods showed no significant differences among M_3-lines and the control. Interestingly,

ten mutant lines were produced significantly higher seed yield per plant (4.3 to 108.6%), seed index (3.5 to 35%) and protein content (0.08 to 2.79%) than original parent Nebraska cultivar. Mutant lines 21 and 22 were superior in seed yield components as well as contained high protein content. The results of the present study on days to flowering and number of seeds per pods revealed non-significant changes between different irradiation treatments compared to the control in M3 generation. These findings are in agreement with the studies of Mudibu et al., (2012) and Khan et al., (2018), they found that mutation treatments did not significantly affect days to flowering in soybean and pea. Khan *et al*. (2005) reported an increase of pod number per plant at 50 Gy without a change in the number of seed per pod. However, some reports observed that days to flowering and number of seeds per pods increased with the application of gamma irradiation (Amri-Tiliouine et al., 2018). These differences could be due to the different genetic material and environmental conditions. Among the various traits studied in common bean, the most important traits in breeding programs are grain yield. The dose of gamma rays radiation is important for inducing genetic variation that can lead to quantitative and qualitative changes (Kiong et al., 2008). The results of the present study revealed that the irradiated dose of 30 Kr decreased significantly the number of pods per plant, number of seed per plant, pod length in M_1 and M_2 generations. On the contrary, some lines resulted from irradiation dose 30 Kr increased significantly seed yield traits in M_3 generations. Similarly, Mudibu et al., (2012) reported a significant increase in soybean grain yield using high doses of gamma irradiation. Gopinath and Pavadai (2015) observed higher number of seeds per plant and grain yield in soybean at high gamma irradiation dose 50 Kr when compared to control plants. Comparatively, data on seed index revealed that that the irradiated dose of 30 Kr reduced significantly the seed index in M_2 and M_3 generations. Horn et al., (2016) reported that seed index reduced significantly with increased concentration of irradiation doses in cowpea. Lande et al., (2018) also reported that seed index decreases in soybean with higher doses of gamma rays.

Conclusion

Common bean is one of the most important grain legumes in Africa including Egypt, and has been recognized as a "nearly perfect food" (Welch, 2002 and Broughton et al., 2003). The development of high yielding varieties with high protein content in common bean is an important achievement for food security, especially in Africa, where consumption of the crop is high (Abera et al., 2020). Induced mutation is a valuable breeding tool for inducing new genetic variation and select novel lines with desired traits in common bean. Mutation breeding has proven to be the rapid, effective and coherent method for improving agronomic characteristics of various crops (FAO, 2017). Here, genetic variations were induced in common bean Nebraska cultivars by different gamma irradiation doses, and continuous selections from M_2 to M_3 generations which enabled us to select promising mutant lines with higher seed yield and protein content. These lines could be valuable genetic resources for genetic improvements and breeding of common bean in Egypt. Further selections and improvements are needed for testing mutant lines for adaptability and stability under different environments under large-scale production for releasing new improved common bean cultivars.

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تحسين المحصول البذري والمحتوى البروتيني للفاصوليا من خلال التربية بالطفرات

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الملخص العربى

تعتبر الفاصوليا محصول غذائى اساسى ومصدر للبروتين في كثير من الدول الأفريقية. والتحسين الوراثي في الفاصوليا في مصر مازال متأخراً مقارنة بالمحاصيل البقولية الأخرى وذلك لمحدودية الموارد الوراثية. وتهدف الدراسة التي أجريت هذه الدراسه خلال موسمي 2016 ,2018,2017 بمحطه البحوث الزراعيه بسخا بمحافظه كفرالشيخ على الفاصوليا صنف نبراسكا إلى استحداث الاختلافات الوراثية من خلال الطفرات لانتخاب سلالات محلية ذات محصول مرتفع ومحتوى بروتيني عالي. تم استخدام ثلاثه جرعات مختلفة من أشعة جاما (10و20و30 كيلو راد) حيث استخدم تصميم القطاعات الكامله العشوائيه وتم إجراء التقييم لنباتات الجيل المطفر الأول والثاني لبعض الصفات . أظهرت النتائج تأثر هذه الصفات المورفولوجية والمحصولية معنويا بمعاملات الإشعاع في الجيل الأول وكانت الاختلافات مرغوبة في الجيل الثاني 2M بالنسبة لارتفاع النبات , التفريع وعدد القرون والبذور /نبات عن معاملة الكنترول. وايضا أظهر تحليل التباين في الجيل الثالث 3M وجود اختلافات ما بين السلالات و معاملة الكنترول بالنسبة لصفات النمو الخضري والصفات المحصولية ما عدا عدد الأيام حتى ظهور أول زهره وعدد البذور /قرن . تفوقت 10 سلالات من بين 25 سلاله في الجيل الثالث 3Mعن الصنف الأصلى نبراسكا المحلي فى محصول البذور الجافة (4,3 – 108,6%) ومحتوى بروتين (0,08– 35%).

وتوصى الدراسة بتقييم هذه السلالات المتفوقة على نطاق واسع وتحت ظروف اجهادات حيوية وبيئية مختلفة للحصول على سلالات متفوقة متأقلمه محلية للظروف المصرية.

أسماء السادة المحكمين

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