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The Dose Response and Mutation Induction by Gamma Ray in *Vicia Faba* Cv. Saraziri

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ABSTRACT

Introduction: Sufficient genetic variation is an essential source for having a successful breeding program. Mutation is known to be a high throughput technique to induce genetic variation in plants. Irradiation is the most common method of inducing mutations in plants mutation breeding programs leading to the production of mutants with superior genotypes. Faba bean is a crop whose diversity needs to be improved for breeding programs in Iran. Unfortunately, few researches have been carried out on mutagenesis of faba bean.
Materials and Methods: In the present study, the dose response and effects of gamma irradiation have been investigated by exposing the seeds of *Vicia faba* cv. Saraziri to doses of 25, 35, 45, 55, 100, and 120 Gray (Gy) in the Lab at the college of Agriculture, Shahid Chamran University of Ahvaz in 2013 to 2015. The implementation of the research was under the guidance and in cooperation with the Atomic Energy Organization of Iran. The experiments were assigned as a completed block design with four replications in both laboratory and the field.
Results and Discussion: The results of the analysis of the variance indicated that there was no significant difference in germination trait of M0 generation in the seeds at various gamma irradiation doses whereas, germination rate, seedling length, and weight were significantly altered at varied doses ($P \leq 0.05$), and further responses of these traits decreased as the dosage increased. Moreover, increasing the irradiation doses caused a delay in flowering, pod setting, and pod ripening period in M2 generation. A linear regression between different characters and gamma doses was detected. Lethal dose, 50% (LD50) for fertility and seed setting, was detected as between 60-65 Gy based on reproductive traits. The principal component analysis revealed information that the first two components within the traits at the different doses were accounted for approximately 71% of the total variance. Using the biplot diagram of the first two components, 320 mutant plants appeared and the superior one within plants was identified as compared to control.
Conclusion: This research is reported the optimum dose of gamma irradiation of 50 to 55 Gy to exert mutation induction in *Vicia Faba* based on the LD50 of vegetative and reproductive phases. The findings of the current work acquired several promising mutants that might be used as beneficial sources to develop new faba bean cultivars.

Introduction

Faba bean (*Vicia faba* L.) is an annual legume belonging to the family Fabaceae, the Papilionoideae sub-family of the Viceae tribe (Bond et al., 1985). Faba bean is a diploid plant with 6 pairs of chromosomes (2n= 12) and natural cross pollination between 20% and 60%. Faba beans are cultivated in a wide range of areas of the world including: Asia, Europe, Latin America, and Africa, in particular, Sudan, Ethiopia, Morocco, Egypt, and the Mediterranean region; China is the primary producer of faba beans. The cultivated land area under faba bean production is 35000 hectares in Iran. Golestan, Khuzestan, Lorestan, Hormozgan, and Darab are primary regions that contribute to the major Vicia faba production in the country. Zohre, Aljazayeri, Shami or Baghi, Saraziri, and Barakat are among highly productive Faba bean cultivars which predominately sown in these regions. Although, most of Faba bean cultivars are planted in Khuzestan, there is a native cultivar named *Saraziri* which most of farmers preferred to grow in contrast to other cultivars because, it is highly adapted and grew well with a high yielding potential (Parsa and Bagheri, 2006).

Sufficient genetic variation is an essential source for having a successful breeding program (Hamid et al., 2014; Omidi et al., 2015). The literature is replete with significant numbers of reports considering the efficacy of mutation in affecting the genetic diversity of the plant species. Many breeders believed that mutation agents were keys to the past and will be considered as significant tools for inducing the genetic variation in the plant species (Saha et al., 2005). Nowadays, due to vast selection processes, a large number of economical crops became intensely uniform; such trend caused a limiting genetic makeup and genetic variation in most of crops. Thus, beside hybridization, the application of mutation could be a noble approach in plant breeding to develop desirable genotypes with improved traits (Ashraf et al., 2003; Melki and Salami, 2008). Irradiation is the most common method of inducing mutations in plants (Jain, 2005). It is estimated that approximately 89% of mutant cultivars have been developed via irradiation procedures. Plant breeders proclaimed that 265 grain cultivars were released in 32 countries, which the majority of them have attributed to the effect of induced mutations procedures and showed that China owned the maximum number of cultivars released (Bhatia et al., 2001). In 1986, it was reported that among 100 mutant cultivars of grain legumes released, two of them were faba beans. In another investigation, via utilizing varied sources of mutations, it has been shown that out of 13 faba bean cultivars that currently released 6 cultivars were derived under direct mutants following by gamma or x-ray irradiation, 4 when applied chemical mutagen treatments, and 3 when received a combination of treatments of physical and chemical mutagens (Bhatia et al., 2001).

Gamma rays are the main physical mutagens which are used to study mutation influences on plant species behavior (Melki and Dahmani, 2009). These rays are widely used in nuclear programs because of their ease of application, reproducibility, high mutation frequency, and less disposal problems (Chung et al., 2006). Gamma rays are a form of ionizing radiation that produce free radicals in cells when interacting with atoms and molecules. These radicals can damage or modify important components within plant cells and have also been claimed to affect morphology, anatomy, biochemistry, and physiology of plants differentially. These effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system, and accumulation of phenolic compounds. These performances are simply depending on the plant type and the radiation doses (Wi et al., 2005; Dhanavel et al., 2012). Moreover, ionizing radiation passes through the tissues and cells, and physically affects the genetic material, that is, the genetic material targeted by mutagenic agents undergoes changes. A report is stated that positive or negative changes which occur in the morphological, physiological, and biochemical processes of plants are simply depending on the intensity of the radiation, (Ashraf et al., 2003; Wi et al., 2005). In an investigation, the application of gamma irradiation of 0, 25, 50, 75, 100, and 125 Gray was assigned to assess some of the physiological characteristics of two wheat varieties. The results showed that there were no significant differences in the yield components and grain yield at the 25 and 50 Gray (Gy) between irradiation and the control treatments. Whereas, at the high rate of gamma ray doses, these traits decreased as compared to rein. Although the findings revealed information in which the 25 and 50 Gy irradiations were the best rate of doses to increase all the traits, at the high doses of gamma irradiation, all the traits decreased (Rahimi and Bahrani, 2011). In the study of the effect of gamma irradiation on yield and yield components of barley, it was observed that except for 1000-grain weight, there was a significant decrease in the grain yield and yield components; however, 1000-grain weight showed an increase as the radiation dose was increased (Khan et al., 2003). In another research, genetic variability induced by gamma rays of 750 and, 1000 Gy and ethylmethane sulphonate (EMS) (0.75%, 1.00% solution) were utilized to evaluate the economic characters in rapeseed (*Brassica napus* L.) cv. Waster. The results indicated that the mutagen treatments showed to enhance the effect of siliquas per plant and deteriorate the effect of grains per siliqua and oil (Siddiqui et al., 2009). Moreover, the study of radiation-induced effects on some quantitative and qualitative characteristics in rice (*Oryza sativa* L.) and mung (*Phaseolus mungo* L.) revealed information that irradiation at the lower doses of gamma rays effectively influences the improvement of the morphological traits such as seedling/plant height, tiller number, panicle number, panicle length, seed per panicle, seed per pod, pod length, and pod number; this is while exposure to high doses results in a depletion of these parameters (Maity et al., 2005). In a study by Wani and Anis (2008), it was documented that three high-yielding cultivars of chickpea (*Cicer arietinum* L.) were treated with gamma rays and EMS; a significant increase in quantitative traits, such as the size of leaflets, flowers, pods, seeds and a considerable improvement in yield were observed. More Plant breeding approaches are required to boost the varieties of faba bean. So far, not many researches have been implemented on the effect of mutagens on faba bean plants in Iran. The purpose of this study was to investigate the effects of different doses of gamma irradiation on the germination traits in M0 generation and agronomic traits of M2 generation in faba bean.

Materials and Methods

Seeds and gamma irradiation

The seeds of *Sarziri* cultivar were obtained from Agricultural Research Center of Safi-Abad, Dezful. Digital SINAR moisture analyzer and dry oven method were used to eliminate the effects of free radicals and adjust the seed moisture content to 10-13% moisture in order to obtain repeatable results prior to irradiation. The seeds were subjected to seven doses of gamma rays (0, 25, 35, 45, 55, 100, and 120 Gray (Gy)) at a dose rate of 0.159 Gy/s. A Gamma-cell irradiator was utilized to apply the irradiation, at the Agricultural, Medical, and Industrial Research Institute of the Atomic Energy Organization, Iran.

A series of experiments were assigned as a randomized completed block design using seven irradiation treatments with three replications (unless stated otherwise) in the field and/or laboratory at the Department of Production Engineering and Plant Genetic, College of Agriculture, Shahid Chamran University of Ahvaz in 2013-2014. The treatments included 25, 35, 45, 55, 100, and 120 Gray of gamma rays, as well as a control dose (seeds not irradiated).

Assessment of germination traits in M0 generation

The experiment was performed as three independent tests in phytotron at a temperature of 30 °C, with 35% humidity, and day/night length of 16/8 hours in the vases containing coco peat soil. Finally, a combined analysis of the obtained data was conducted for the three experiments. Prior to the germination process, the seeds were sterilized in order to reduce the contamination. The seeds of each treatment were washed individually via distilled water and placed in a steam bath at 50 °C for 7 minutes. Then, they were washed again with distilled water, placed in 10% sodium hypochlorite solution for 15 minutes, and rinsed with distilled water. When the seedlings were initiated after seven days, germination characteristics of the treated seeds were recorded according to the performance of the seed growth as described below: to determine the germination percentage (GP), the number of seeds germinated 7 days after the start of the experiment was counted in each treatment, and the percent of GP was calculated using equation (1). Germination rate was obtained via regression analysis, in which the slope of the curve between the number of seeds germinated against the days showed the germination rate. A ruler was used to measure the plumule and radicle length of seedlings 10 days after the germination. The average weight in grams was obtained at the end of the test via weighing the plumule and radicle of the treated seedlings in each treatment, then the total of weight was divided by the weight of each plant.

Equation (1)

$$GP = \frac{\text{number of germinated seeds}}{\text{total number of seeds}} \times 100$$

Field cultivation and agronomic traits evaluation

The irradiated M0 seeds were cultivated on the experimental research farm in the following year. At the maturity stage, the seeds of the treated plants were harvested as M1 generation, and then used to produce M2 generation. The plants which received 100 and 120 Gy treatments were unable to produce any seeds, perhaps due to a higher radiation effect. The experiment was conducted as an unbalanced completely randomized design with 4 replications. Each experimental plot consisted of 4 stacks of 2 m long and 75 cm apart, with 30 cm spacing between the plants. From each replication, ten treated plants were randomly selected to measure the following plant characters: pods per plant, number of seeds per pod, number of seeds per plant, seed yield per plant, the percentage of pod sterility, and the weight of 100 seeds. For a better comparison of mutant plants with control cultivars, the control plants were cultivated on the borders of the plots. All the other plant characters were monitored and the data were recorded on the individual plant according to the growth behavior during the growing season.

Data analysis was performed using SAS software. Prior to the analysis of the variance (ANOVA), two main assumptions underlying ANOVA, including the normal distribution of the data and the homogeneity of variance in experimental errors, were examined. And, if necessary, the square root transformation was also carried out. Duncan's multiple range test was applied to compare the means, and Excel software was used to depict the biplot graph.

Results and Discussion

Germination traits in the M0 generation

The analysis of variance of germination traits indicated significant differences among the treated seedlings on the all traits except for the germination percentage in the laboratory (Table 1). As irradiation dose increased, germination rate, plumule and radicle length, as well as plumule and radicle weight decreased. So, the lowest values of the seedlings' performance were attributed to the treatments which meant receiving high doses of 100 and 120 Gy., & it elucidated that there was a significant difference between these two radiation treatments and control in relation to the germination rate which prohibited seed germination by a 35% reduction at 100 Gy. Although there was a significant difference between plumule and radicle growth weights in associated with the radiation treatments and control, the differences among treatments were not significant (Table 2). Research reports stated that the inability of seeds to germinate at higher doses of gamma rays has been attributed to several reasons: a change in α-amylase activity, a decrease in lipase activity in oily seeds, and an increased membrane permeability (Thanki et al., 2007). Moreover, Majeed et al. (2010) concluded that the decrease in the plumule and radicle length, especially at higher radiation doses could be ascribed to the reduction of mitotic activity in the meristematic tissue, and the decrease in seedling weight which simply caused a decrease in plant height and organs growth development. Furthermore, Borzouei et al. (2010) investigated the effects of gamma radiation on the germination and the physiological characteristics of two wheat genotypes and found out that the mean germination time, root and shoot length, and the seedling dry weight decreased as radiation doses increased. Similar observations were also reported by Hameed et al. (2008) and Momeni et al. (2011) in chickpea and rapeseed, respectively.

Table 1. Analysis of variance for germination traits of plants in M0 generation

S.O.V.	df	Mean of squares					
		Germination (%)	Germination rate	Rootlet length	Plumule length	Rootlet weight	Plumule weight
Experiment (Exp)	2	97.75^{ns}	0.42^{ns}	0.01^{ns}	0.01*	0.003^{ns}	0.11*
Repeat (Exp)	3	337.12	0.12	0.005	0.001	0.003	0.0008
Treatment (Treat)	6	113.88^{ns}	0.27*	0.02*	0.09*	0.008*	0.03**
Exp × Treat	12	83.50^{ns}	0.13^{ns}	0.006^{ns}	0.01^{ns}	0.001^{ns}	0.005^{ns}
Error	7	115.00	0.07	0.004	0.01	0.003	0.002
C.V. (%)	-	11.94	24.80	7.76	18.11	16.39	14.59

* and **: Significant at 0.05 and 0.01 respectively; ns: Non significant.
The table is combined analysis of three independent experiments.

Table 2. Means comparison for germination traits of plants in M0 generation

Treatment (Gy)	Germination rate	Root length	Plumule length	Rootlet weight	Plumule weight
0	1.19^{ab}	0.94^{a}	0.83^{a}	0.41^{a}	0.48^{a}
25	1.18^{ab}	0.95^{a}	0.72^{abc}	0.36^{ab}	0.40^{b}
35	1.34^{a}	0.91^{ab}	0.74^{ab}	0.3^{4b}	0.33^{cd}
45	1.04^{bc}	0.89^{abc}	0.61^{bcd}	0.32^{b}	0.33^{cd}
55	1.04^{bc}	0.86^{abc}	0.69^{bc}	0.35^{ab}	0.35^{c}
100	0.84^{c}	0.84^{c}	0.60^{cd}	0.32^{b}	0.34^{cd}
120	0.90^{c}	0.82^{c}	0.53^{d}	0.32^{b}	0.28^{d}

Different letters in each column shows significant difference (P < 0.05).

Detecting the optimum dose of gamma irradiation

Regression analysis was applied to detect the optimum dose of gamma irradiation for mutation induction in V. faba. The best fitted regression for all traits against gamma ray doses was obtained as a linear model (Figure 1). The same linear model was reported in previous researches. Singh et al. (1997) detected a linear model of regression for the germination percentage and survival of seedlings on gamma ray doses in the M1 generation in mung bean (Vigna radiate L.). Besides, according to the investigations which led by Ellyfa et al. (2007) and Parvan Kumar et al. (2013), a linear regression was discovered between reproducibility traits and gamma ray doses in Long bean (Vigna sesquipedalis) and Lima bean (Phaseolus lunatus L.) crops, respectively. The regression coefficients and coefficient of determination for each model are presented in the Table (3), which indicates that all the regression coefficients were significant and the coefficient of determination was more than 84% for all the regression models. This obviously manifests the best fitted models for different traits in the experiment. Based on the regression models, the lethal dose of 50% (LD50) i.e. the doses of gamma radiation which induce 50% reduction in each trait was estimated. Variation in LD50 values obviously depends on the sensitivity of different traits to gamma irradiation, that is, the germination rate (LD50=45) and the number of seeds per pod (LD50=76) showed the maximum and minimum sensitivity, respectively. In mutation breeding, the appropriate mutagens should make the maximum genetic variation whilst the physiological effects on plant should be very low (Cheema and Atta, 2003; Hallajian et al. 2011). Therefore, in the crop prior to the reproductive initiation, the vegetative traits have the major role in estimating the optimum dose of the irradiation. In the present study, based on the LD50 of vegetative and reproductive traits, the optimum dose of gamma irradiation in V. faba was estimated as 50-55 Gy.

Comparison of mutant plants compared with control plants in the M2 generation

In order to investigate the importance of the traits in yield increment and identify the superior mutant plants, principal component analysis was used. Based on the results, the first two components accounted for 71% of the total variation. The first principal component explained 53.46% of the total data variation (Table 4).

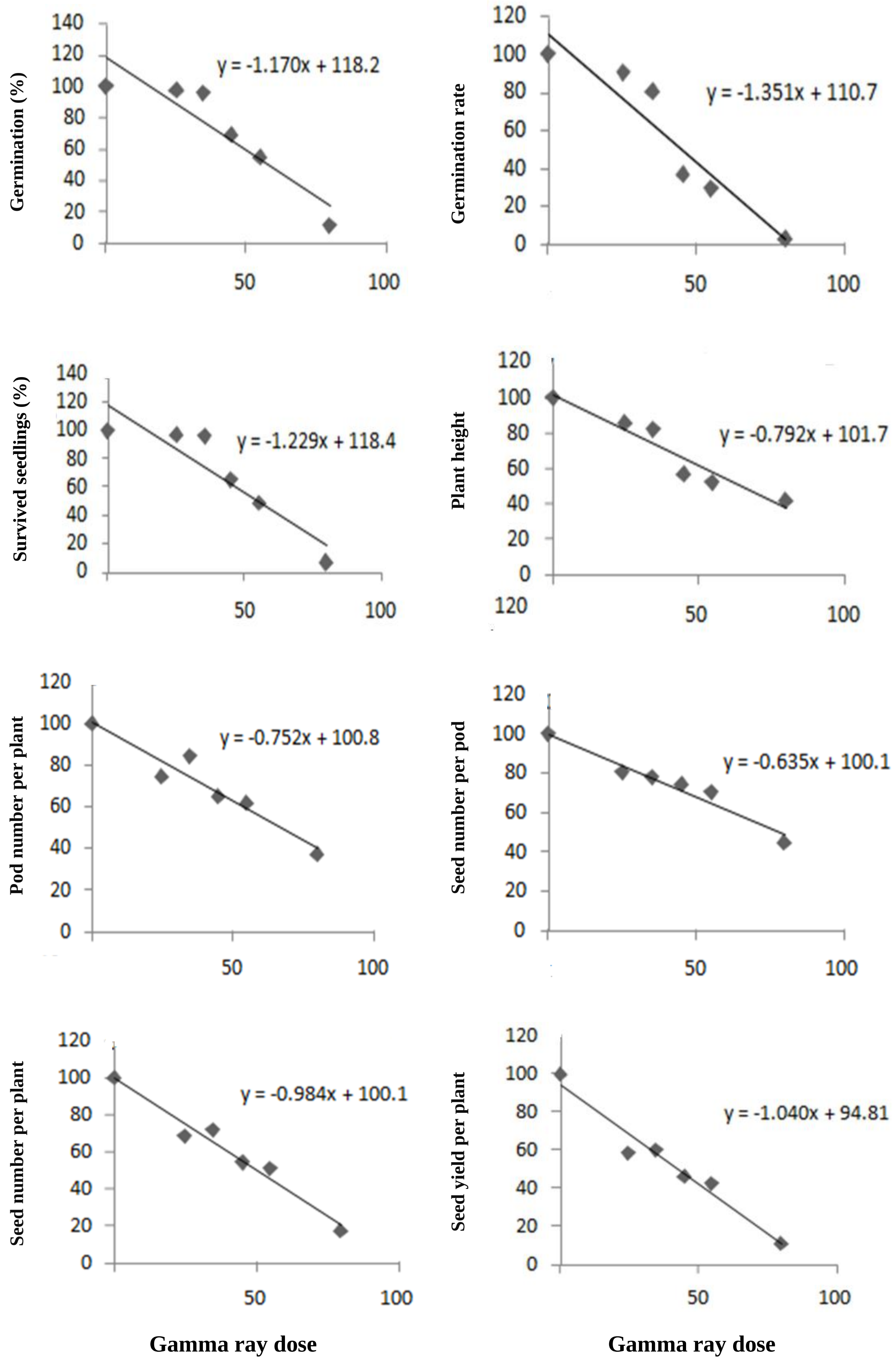

Figure 1. Linear regression of different traits against gamma ray doses

Table 3. Regression parameters and estimated LD50 for different traits

Traits	b_0	b_1	R^2	LD50
Germination percentage	-1.18**	118.28**	0.84	58
Germination rate	-1.35**	110.1**	0.89	45
Survived seedlings	-1.22**	118.4**	0.85	56
Plant height	-0.79**	101.7**	0.91	65
Pod number per plant	-0.75*	100.8**	0.92	68
Seed number per pod	-0.63**	100.8**	0.95	76
Seed number per plant	-0.98**	100.1**	0.96	51
Seed yield in plant	-1.04**	94.81**	0.96	43

* and **: Significant at 0.05 and 0.01 respectively.

Table 4. Principal component analysis parameters

Principal components	Eigen values	Explained variance	Cumulative variance
1	3.21	53.46	53.46
2	1.06	17.74	71.20
3	0.83	13.76	84.96
4	0.70	11.7	96.66
5	0.18	3	99.66
6	0.02	0.34	100

In this component, positive coefficients for seed yield per plant, number of seeds per plant, number of pods per plant, weight of 100 seeds, and the number of seeds per pod, as well as a negative coefficient for the pod sterility percentage were estimated. As the seeds fertility traits increased, the first component increased; therefore, the first component was entitled as the plants fertility competency component. That is, high plant yield was noticed in plants with high values for this component. The second principal component, accounting for 17.77% of the total variance, was significantly associated with the pod sterility percentage and negatively correlated with the number of pods per plant, number of seeds per pod and plant were estimated. Hence, the second component can be considered as the infertility component; so that, high values of this component for each plant showed high sterility in the corresponding plant.

The biplots of the first and second components were constructed to screen the plants and identify the superior mutant plants (Figure 2). The plants treated with different doses were clearly segregated on the biplot, especially the control plants compared with gamma-ray induced mutant plants. At 25 and 35 Gy doses, mutant plants showed higher yields than the control plants. At 25 Gy dose, the plants with the minimum number of pods per plant (7), number of seeds per pod (6), number of seeds per plant (21), and seed yield per plant (20.5 g) were selected. These plants showed superiority over the control for the mean of these traits. Among treatments with 35 Gy, plants with the minimum number of seeds per plant (17) and the minimum seed yield per plant (18.2 g) were the most favorable plants compared with the control. Moreover, at 45 Gy dose, low-yield mutants which were superior in some traits such as the number of seeds per pod, were identified. These mutant plants probably express the recombinant genes that increase the number of seeds per pod and can be indirectly used in breeding programs to improve faba bean cultivars. However, at the highest irradiation dose (55 Gy), no superior plant over the control was observed, which could be attributed to the destructive effect of 55 Gy gamma rays on plants.

Conclusion

The results indicated that increasing the irradiation dose reduced the germination rate and plumule and radicle weight; however, the germination percentage was not affected by gamma rays. Moreover, based on the results, gamma rays significantly decreased the yield and yield component traits and high gamma ray doses had a severe destructive effect on the seed yield per plant. Regarding the phenological traits, despite the observation of a delay in flowering and pod setting characteristics, mutant plants were slightly sensitive to irradiation.

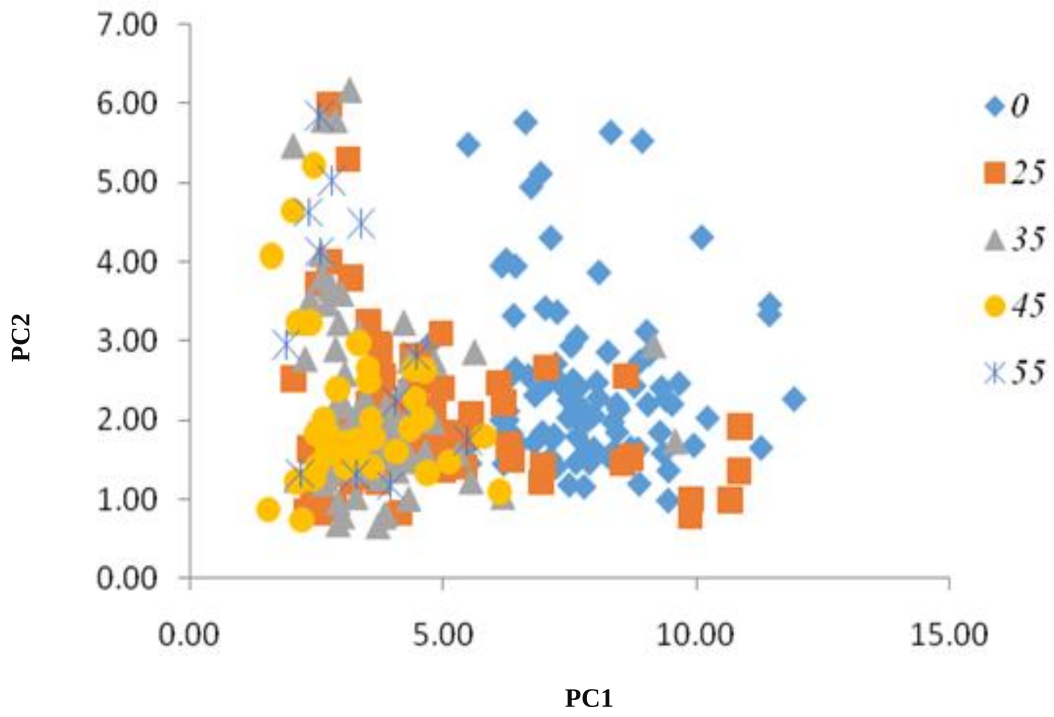

Figure 2. Biplot of first two components to identify superior mutants based on different gamma ray doses (0, 25, 35, 45 and 55 Gy)

On the other hand, pod ripening was significantly delayed in gamma ray treatments as compared with the control. Interestingly, radiation sensitivity was more observed in the seeds fertility traits than their phenological traits. In this respect, radiation sensitivity was higher in traits such as sterility and seed production, and less in the weight of 100 seeds. In this regard, LD50 for fertility and seed setting was reported as 60-65 Gy. Meanwhile, based on the vegetative and reproductive traits, the optimum LD50 for mutation induction in faba bean var. Saraziri was estimated as 50-55 Gy. High correlation of traits in M2 population showed eligibility of the principal component analysis in this experiment. The biplot presentation, based on the first and second components, made it possible to identify the superior mutant plants compared with the control plants, which can be used directly or indirectly in the advancement of breeding programs in the next generations to produce commercial faba bean cultivars.

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