



Original article

Morphological diversity of faba bean (*Vicia faba* L.) M₂ mutant populations induced by gamma radiation and diethyl sulfateNurmansyah^{a,b}, Salem S. Alghamdi^{a,*}, Hussein M. Migdadi^a^a Legume Research Group, Plant Production Department, College of Food and Agricultural Sciences, King Saud University, 2460, Riyadh 11451, Saudi Arabia^b Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia

ARTICLE INFO

Article history:

Received 14 July 2019

Revised 3 December 2019

Accepted 25 December 2019

Available online 16 January 2020

Keywords:

Faba bean

Gamma radiation

Diethyl sulfate

Mutant population

Genetic diversity

ABSTRACT

Induced mutagenesis is one of the most widely used breeding methods for rapidly increasing genetic diversity in many crops. This study used gamma radiation and diethyl sulfate (DES) to increase the genetic diversity in two faba bean genotypes. The genetic diversity assessment was based on 12 qualitative traits. The purpose of this study was to assess the genetic diversity in the M₂ faba bean mutant populations and to select promising mutant. A total of 5271 M₂ seeds generated from the M₁ generation of two genotypes—Hassawi 2 and ILB4347—were used in this study. The seeds of M₁ were obtained by gamma-ray exposure (25 and 50 Gy) and treatment of 0.01, 0.02, and 0.05% DES. A total of 4333 (82.2%) seeds germinated, of which 4102 plants survived and were screened for morphological diversity. A total of 36 mutant types are presented in this study. Determinate plant type, white flower color, white hilum, and black seed coat color were some of desirable traits that were considered for further generations. Multivariate-based principal component analyses showed that high genetic diversity was induced by gamma radiation and DES in faba bean mutant populations. This study revealed the potency of gamma radiation and DES on increasing genetic diversity and demonstrated the successful program of induced mutagenesis in the faba bean.

© 2020 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Faba bean (*Vicia faba* L.) is a multipurpose legume crop used in both human food and animal feed because of its high protein content (average of 25%) and biomass (Musallam et al., 2004). It also plays an important role in crop rotations, effective nitrogen fixation, and soil improvement (Ye et al., 2003). However, in the last three decades, very low growth rates of the faba bean's yield have been recorded because of the slow progress in increasing genetic diversity (Nedumaran et al. (2015). Selection and extensive faba bean cultivation using one particular cultivar, generally with high-yielding variety, leads to an increase faba bean production

worldwide, but on the other hand, it was narrowing faba bean genetic diversity. Therefore, enriching genetic diversity as an essential step for most breeding programs is required to raise faba bean's yield.

Many methods have been utilized to enrich genetic diversity, from a classical genetics approach to the recent transformation genetics methods. Induced mutagenesis is one such transformation method that has been successfully reported for many crops, including the faba bean (Sjödín, 1971). The efficiency of induced mutagenesis to generate mutations valuable for plant breeders has been widely proven and documented through the official release of 3,318 mutant varieties (IAEA, 2019), of which, there are 20 mutant varieties of faba bean. However, no released cultivar of faba bean was reported via induced mutagenesis in the mutant variety database of the International Atomic Energy Agency (IAEA) since 2008.

Induced mutagenesis can be carried out by using physical and chemical mutagens. Physical mutagens are physical agents that usually cause large mutations or large-scale deletions of DNA (Kozjak and Meglič, 2012). Among the physical mutagens, gamma radiation is the most widely used mutagen in mutation breeding (Kodym et al., 2012). Gamma radiation has been reported as an effective mutagen to induce diversity in several legume crops, such

* Corresponding author at: Plant Production Department, Faculty of Food and Agricultural Sciences, King Saud University, P.O. BOX 2460, Riyadh 11451, Saudi Arabia.

E-mail address: salem@ksu.edu.sa (S.S. Alghamdi).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

as pigeon pea (Desai and Rao, 2014), cowpea (Girija et al., 2013), chickpea (Wani and Anis, 2008), and mung bean (Sangsiri et al., 2005). Chemical mutagens are compounds that cause alterations in DNA molecules, such as transitions, transversions, deletions, insertions, inversions, DNA single and double strand breaks, and DNA recombination. One of the chemical mutagens that have been reported to be an efficient inducer of mutations is diethyl sulfate (DES). DES is classified as alkylating agent that react with the DNA structure by alkylating the phosphate group and nitrogen bases (Acquaah, 2007).

The studies on the effectiveness and efficiency of various physical and chemical mutagens to increase genetic diversity have been performed for many crops. Genetic diversity can be identified by using morphological, biochemical, and molecular markers. A morphological marker is a trait widely used by plant breeders that can be assessed by a simple and inexpensive technique for determining genetic diversity. Morphological traits can be divided into qualitative and quantitative traits and the best time for the identification of mutant plants is the M_2 generation. In the M_2 generation, mutations that possibly occurred in the M_1 generation segregate to create homozygotes recessive and/or dominant alleles (Page and Grossniklaus, 2002). Therefore, the present study aims to assess the genetic diversity of the M_2 faba bean mutant populations derived from exposure to gamma radiation and treatment with DES, in order to screen and select promising mutant plants based on qualitative traits.

2. Materials and methods

2.1. Plant materials

M_2 seeds generated from the M_1 generation of two genotypes, namely, Hassawi 2 and ILB4347, were used in this study. The M_1 seeds were produced by exposing separate 120 dry seed samples of each genotype to 25 or 50 Gy at a dose rate of 15.48 Gy/min of gamma radiation using a ^{60}Co (Cobalt 60) gamma source under ambient conditions at the Central Lab, College of Science, King Saud University (Nurmansyah et al., 2018), and three concentrations of DES solution (0.01%, 0.02%, and 0.05%). The dry seed were soaked in the DES solution for 12 h at room temperature, and then, they were thoroughly washed under running water thrice. The M_1 seeds were then planted and allowed to produce the M_2 seeds. The M_1 plants are individually harvested and sown as M_2 families.

2.2. Experimental site and crop cultivation

The experiments were carried out at Dirab Agricultural Research and Experiment Station, South Riyadh (24° 43' 34" N, 46° 37' 15" E). Faba bean M_2 lines from the two aforementioned

cultivars were grown in the field according to the Pedigree Method; the M_1 plants are individually harvested and sown as M_2 families. The net plot size was 4 m × 4 m, with nine rows (each 4 m long) with a 45 cm distance between two rows and 20 cm distance between two plants. The untreated seeds (control) were planted in the first row of each plot. All cultural practices were applied as recommended: diammonium phosphate (18% N_2 , 46% P_2O_5) was added at the rate of 300 kg/ha during seedbed preparation; plots were immediately irrigated after sowing; weeds were under control during the vegetative growth of the crop; and the plants were harvested individually at physiological maturity.

2.3. Field observations

2.3.1. Seedling emergence and plant survival

Seedling emergence (SE) was recorded three weeks after sowing, and the percentage of SE was measured on the basis of the total number of seeds sown. Plant survival (PS) was recorded at the maturity stage. The percentage of PS was estimated based on the total number of emerged seedlings.

2.3.2. Morphological characterization

Twelve qualitative traits were recorded throughout the mutant and control plants. Twelve qualitative traits were examined, based on faba bean descriptors from the International Board for Plant Genetic Resources (IBPGR, 1985) and International Union for the Protection of New Varieties of Plants (UPOV, 2003). The twelve qualitative traits include growth habit; flower characteristics that consist of the standard petal color, wing petal color, the intensity of streak, streak color, and level of anthocyanin coloration; seed characteristics consist of seed coat color, seed shape, and hilum color; leaf characteristics consist of leaf shape and size; and stem pigmentation.

Growth habit is characterized as determinate (with terminal inflorescence), semi-determinate (without terminal inflorescence), and indeterminate; standard petal color is characterized as white, violet, dark brown, light brown, pink, red, yellow, and others; wing petal color represents the color of the petal wing; the intensity of streaks is characterized by streaks or lines on a standard petal that is classified as no streaks, slight, moderate, and intense; streak color is the color of streak on the standard petal; and the level of anthocyanin coloration is the intensity of anthocyanin in the standard petal that is classified as absence, small, medium, and high intensity. The seed coat color is characterized by the color of the seed coat observed immediately after harvest; the shape of the seed is characterized as flattened, angular, and rounded; the hilum color is characterized as black, white, colorless, and others; and the leaflet shape is observed in the fully expanded leaves at the intermediate flowering node, and it is classified as narrow, intermedi-

Table 1
Seedling emergence and plant survival of the M_2 generations of mutagenized faba bean populations.

Genotype	Treatment	TS	SE	% SE	PS	% PS
Hassawi 2	25 Gy Gamma	2748	2196	79.91	2107	95.95
	50 Gy Gamma	671	586	87.33	551	94.03
	0.01% DES	173	130	75.14	88	67.69
	0.02% DES	23	19	82.61	18	94.74
	0.05% DES	16	14	87.50	14	100
Total		3631	2945	81.11	2778	94.33
ILB4347	25 Gy Gamma	1309	1112	84.95	1059	95.23
	50 Gy Gamma	170	138	81.18	128	92.75
	0.01% DES	123	106	86.18	106	100
	0.02% DES	24	21	87.50	21	100
	0.05% DES	14	11	78.57	10	90.91
Total		1640	1388	84.63	1324	95.39

TS: total seed planted, SE: seedling emergence, PS: plant survival.

ate, and rounded. The leaflet size is characterized as small, medium, and large, and stem pigmentation at the flowering time indicates the level of pigmentation on the stems.

2.4. Morphological mutant frequency and qualitative trait diversity

In the field observation, morphological mutant characteristics for inflorescence variation, leaf, seed, flower, and other mutant morphological phenotypes were recorded and calculated as a frequency of mutation (F). The frequency of the morphological mutant was calculated as

$$F = \frac{\text{Total number of mutants}}{\text{Total number of plant studied}} \times 100.$$

3. Results

3.1. Seedling emergence and plant survival

A total of 5271 M₂ seeds were obtained after the mutagenesis of the M₁ generation from two cultivars, Hassawi 2 and ILB4347, which were planted in this study. A total of 4333 (82.2%) seeds ger-

Table 2

Summary of mutant plant types based on qualitative traits compare to control traits.

No	Mutant Characteristics	Hassawi 2		ILB 4347		Control traits
		Number of mutants	Freq (%)	Number of mutants	Freq (%)	
1	Inflorescence					
	<i>Growth Habit or Inflorescence Architecture*</i>					
	Determinate	14	0.50	7	0.53	Indeterminate
	<i>Peduncle or Inflorescence Stem*</i>					
	a. Long peduncle	1	0.04	–	–	Single short
	b. Double peduncle	–	–	1	0.08	
2	Flower Characteristics					
	<i>Wing Petal Color*</i>					
	a. Yellow spotted	–	–	27	2.04	Black spotted
	b. Light yellow spotted	–	–	20	1.51	
	c. White	3	0.11	9	0.68	
	d. Faded black	1	0.04	2	0.15	
	<i>Intensity of Streak*</i>					
	a. Absence	–	–	30	2.27	Medium
	b. Slight	–	–	79	5.97	
	c. Intense	–	–	110	8.23	
	<i>Color of Streak*</i>					
	Purple	3	0.11	5	0.38	Brown
	<i>Standard Petal Color*</i>					
	a. White	380	13.68	84	6.34	Purple spotted
	b. Violet spotted	3	0.11	11	0.83	
	c. Brown spotted	8	0.29	56	4.23	
d. Pink spotted	–	–	9	0.68		
<i>Level of Anthocyanin Coloration**</i>						
a. Absence	380	13.68	108	8.16	Small	
b. Medium	–	–	280	21.15		
c. High	–	–	54	4.08		
3	Seed Characteristics					
	<i>Seed Coat Color*</i>					
	a. Yellow	29	1.04	7	0.53	Beige
	b. Brown	480	17.28	163	12.31	
	c. Green	–	–	1	0.08	
	d. Black	–	–	1	0.08	
	e. Darken beige	–	–	9	0.68	
	<i>Hilum Color*</i>					
	White	18	0.65	17	1.28	Black
	<i>Seed Shape*</i>					
Flattened	7	0.25	124	9.37	Angular	
4	Leaf Characteristics					
	<i>Leaf Shape*</i>					
	a. Narrow leaflet	274	9.86	21	1.59	Intermediate leaflet
	b. Broad leaflet	231	8.32	–	–	
	c. Rounded leaflet	2	0.07	–	–	
	<i>Leaf Size*</i>					
a. Small	135	4.86	100	7.55	Medium	
b. Large	184	6.62	36	2.72		
5	Stem Pigmentation*					
	a. Absence	564	20.3	100	7.55	Small
	b. Medium	163	5.87	155	11.71	
	c. Intense	15	0.54	33	2.49	
6	Other mutants*					
	Unifoliate	2	0.07	–	–	
	Giga plant	–	–	2	0.15	
	Branching pattern	2	0.07	3	0.23	

*: classification based on faba bean descriptor (IBPGR, 1985), **: classification based on UPOV (2003), *: author additional classification, Freq: frequency.

minated, of which 4102 (94.67%) plants survived and produced pods. The statistics for Hassawi 2 mutants consisted of 3631 seeds planted, of which 2945 seeds (81.11%) germinated, and 2778 plants (94.33%) survived, whereas for ILB4347 mutants, 1640 seeds were planted, from which 1388 seeds (84.63%) germinated, and 1324 plants (95.39%) survived (Table 1). Each of the survived mutant plants was recorded for 12 qualitative traits.

3.2. Qualitative trait variations

Mutant populations were screened for 12 qualitative traits. A total of 4102 mutant plants were evaluated for inflorescence, flower characteristics, seed characteristics, leaf characteristics, stem pigmentation, and other features. We observed 36 types of mutant plants that exhibited traits different from the control plants (Table 2). The Hassawi 2 mutant population accounted for 23 out of the 36 mutant types, whereas the ILB4347 mutant populations generated 32 mutant types. The frequency of traits ranged from 0.04 to 21.15%. Seed coat color was the most diverse trait, which generated five novel mutant types: yellow, brown, green, black, and darkened beige, instead of the beige color for the control plant seeds.

3.2.1. Inflorescence variations

Inflorescence is a group of flowers that spread on the flower stem called peduncle. In the legume plants, raceme as well as panicle inflorescence are common. Variations in the inflorescence architecture and peduncle were observed in this study (Fig. 1).

Both control plants, Hassawi 2 and ILB4347, and most of the faba bean cultivars are classified as compound indeterminate inflorescence (Fig. 1a). Twenty-one compound determinate mutants were found in the mutant populations (Fig. 1b). The ILB4347 mutant population exhibited more frequent determinate type inflorescence than the Hassawi 2 mutant population with a frequency of 0.53% and 0.50%, respectively. The control plants are also characterized by a single short peduncle. Long peduncles and double peduncles were found in the mutant populations (Fig. 1c, 1d). The long peduncle mutant reached a length of 5 cm, while the control plant peduncle only reached 2 cm in length.

3.2.2. Variations in flower characteristics

The faba bean flower consists of several parts such as the standard petal, wing petals, keel petals, pistil, and stamens. Based on the faba bean descriptors in the [International Board for Plant Genetic Resources \(IBPGR\) \(1985\)](#), there are several characteristics of the faba bean flower that can be observed, mainly in the standard and wing petals. Five traits, namely, wing petal color, standard petal color, streak intensity, streak color, and the level of anthocyanin coloration were characterized throughout the mutant plants, which are presented in Fig. 2.

For the wing petal color, four new mutant colors were identified, namely, faded black, white, yellow, and light yellow, instead of the black spotted wing petal color observed in the control plants. For the standard petal color, the mutants showed brown-spotted, violet-spotted, pink-spotted, and white-colored petals, instead of the purple-spotted color seen in control plants. The intensity and

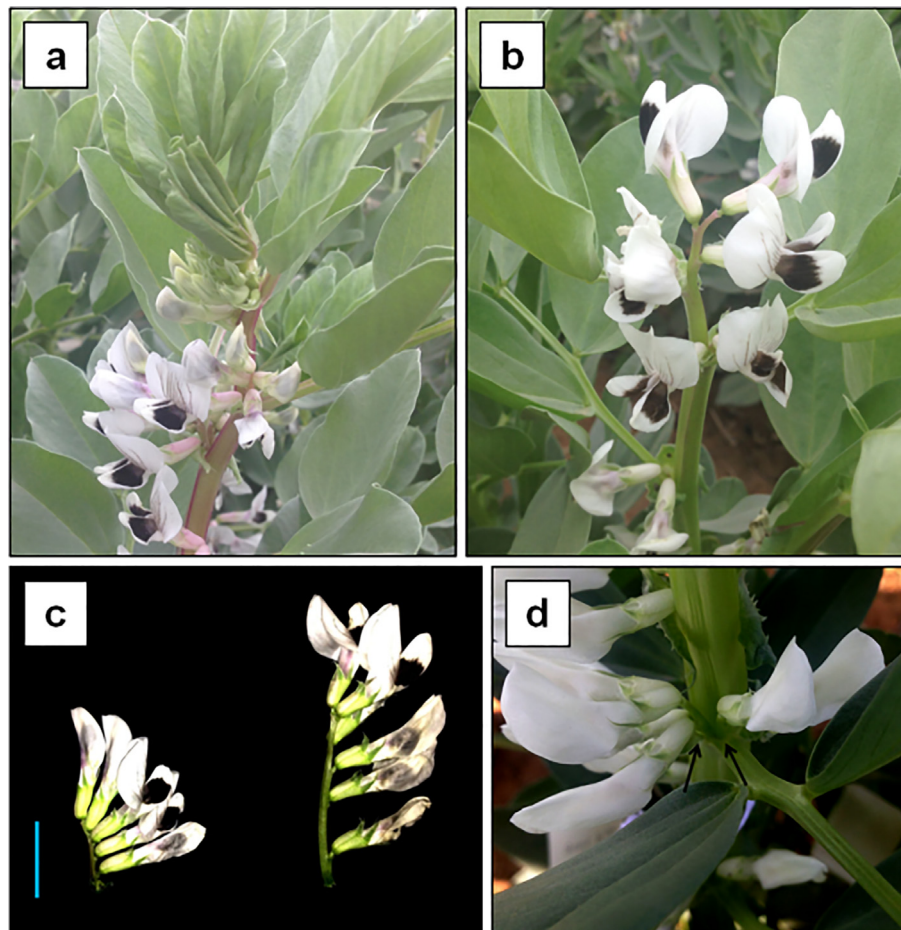


Fig. 1. Inflorescence variations. **a** control indeterminate type without terminal inflorescence. **b** mutant determinate type with terminal inflorescence. **c** comparison between control (left) and mutant long peduncle (right), blue bar = 2 cm. **d** double peduncle mutant.

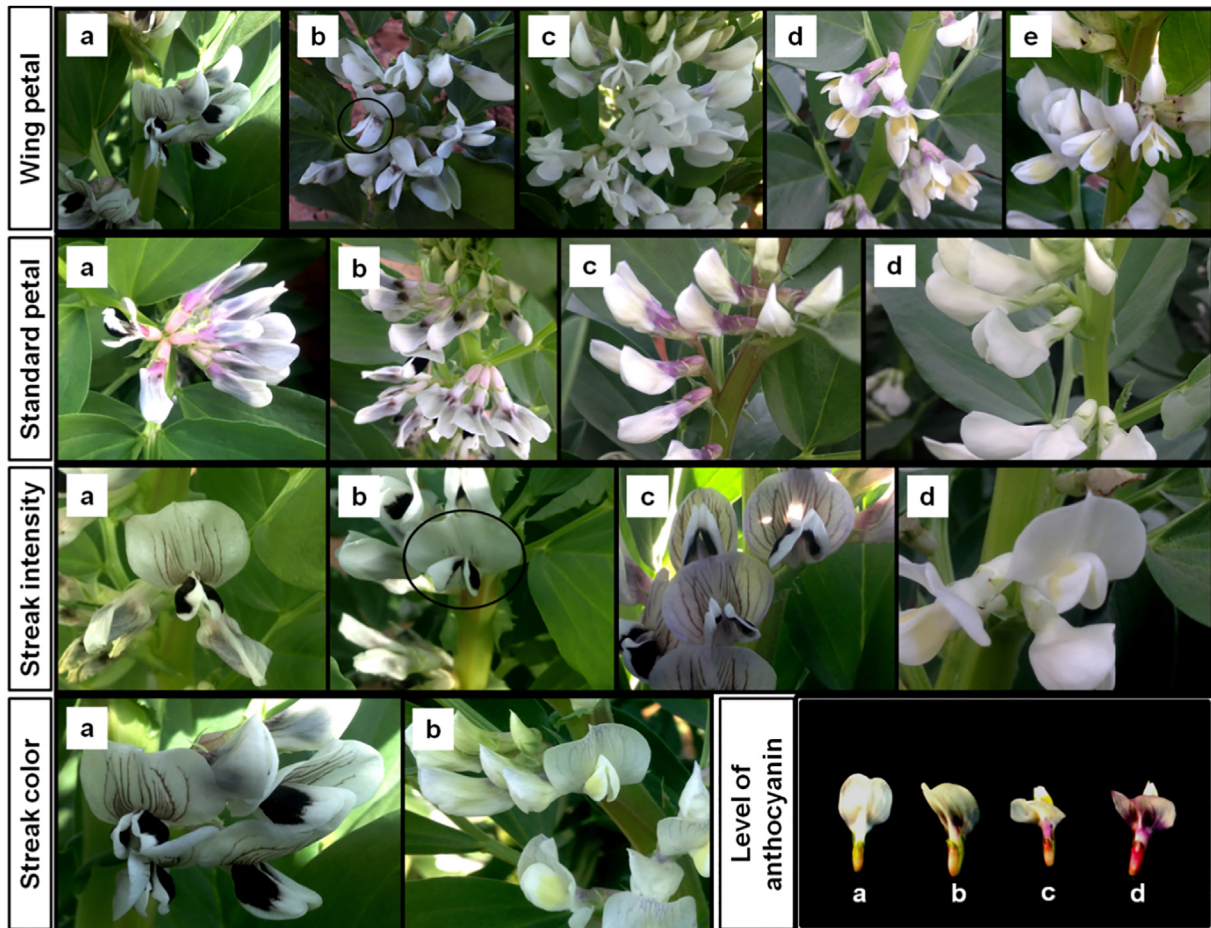


Fig. 2. Flower characters variation. Wing petal color variations, **a** black spotted (control), **b** faded black, **c** white, **d** yellow spotted, **e** light yellow spotted. Standard petal color variations, **a** purple spotted (control), **b** brown spotted, **c** violet spotted, **d** white. Intensity of streak variations, **a** medium intensity (control), **b** slight, **c** intense, **d** absence or without streak. Streak color variations, **a** brown (control), **b** purple. Level of anthocyanin coloration variations, **a** absence or without anthocyanin coloration, **b** small coloration (control), **c** medium coloration, **d** intense coloration.

color of streak or lines on the standard petals were also evaluated throughout mutant plants. There are four different types of streak intensities observed, namely medium intensity (control), slight, intense intensity, and absence or without streak. Only one new type of mutant was observed for streak color, which showed a purple color. Another trait that was evaluated for the standard petal was the level of anthocyanin coloration. In the control plants, the levels of anthocyanin coloration are small; however, there were three different variations in anthocyanin coloration observed in mutant plants, which included an absence of anthocyanin coloration, medium, and intense coloration intensity, respectively.

3.2.3. Seed characteristics

Three seed characteristics were evaluated throughout the mutant plants: seed color, seed shape, and hilum color. There were five different seed coat colors, namely, yellow, brown, green, black, and darkened beige instead of the beige generally observed in the control seeds (Fig. 3a). The brown seed coat color showed the highest frequency of appearance in mutants, with 643 mutants out of 4102 plants, followed by yellow, darkened beige, green, and black seed coat colors with 36, 9, 1, and 1 mutants, respectively.

The relationship between the white flower mutants and the seed color mutants was studied. We found 12 white flower mutants, with 3 mutants showing low anthocyanin coloration in the standard petal. All white flower mutants without anthocyanin coloration showed a darkened seed color. However, mutants with

anthocyanin coloration prevented seed color from becoming dark, and the seed coats remained beige colored, which is the same as the control plant, as indicated in Fig. 3b.

Hilum color and seed shape were also evaluated in the mutant plants. The mutant population showed a white hilum color instead of the black color observed in the control plant (Fig. 3c). Seed shape mutants showed a flattened or flat seed instead of the angular seed observed in the control plants (Fig. 3d).

3.2.4. Leaf characteristics

Leaf characteristics were observed in fully expanded leaves at the intermediate flowering nodes. Leaf shape and size variations were observed in the mutant populations. Compared to the control leaves, the mutants showed narrower or broader leaf shapes (Fig. 4a). There were also two different leaf sizes, namely small or a larger size, instead of an intermediate size observed for the control leaves.

3.2.5. Stem pigmentation

Stem pigmentation was observed at the time of flowering. Stem pigmentation variations were seen in both the mutant populations. Four different stem pigmentations were found, namely an absence of pigmentation, low, medium, and intense pigmentations (Fig. 4b, c).

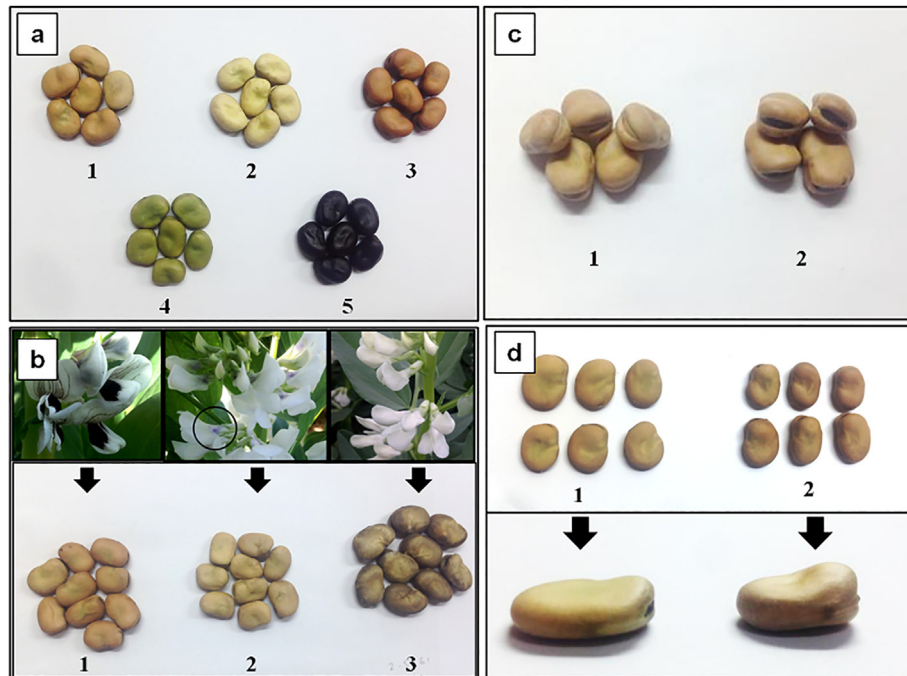


Fig. 3. Seed characters variation. **a** seed coat color variations, 1: beige (control), 2: yellow, 3: brown, 4: green, 5: black. **b** Comparison of flower colors and their seed colors. 1: black spotted control flower has beige seed, 2: white flower with anthocyanin coloration (black circle) has beige seed, 3: white flower has darken-beige in seed color. **c** hilum color variations, 1: white, 2: black (control). **d** Seed shape variations, 1: flattened, 2: angular (control).

3.2.6. Unifoliate mutants

A unifoliate mutant is characterized by a single leaflet instead of the compound leaf found in the control plants. Two unifoliate mutants were found in this study and both of them were observed in the Hassawi 2 mutant population induced by 25-Gy gamma radiation. Another characteristic of the unifoliate mutant was rounded leaflets instead of the intermediate leaflets found in the control plants (Fig. 5a, b, c).

3.2.7. “Giga” mutants

Giga mutants are characterized as very large plants. Two giga mutants were isolated from the ILB4347 mutant population induced by 0.02% DES. Fig. 5d shows the difference between giga mutants and the other normal plants in the field. The difference in the leaf sizes of the normal plant and giga mutant is also shown in Fig. 5e.

3.2.8. Branching pattern variations

Branching in most faba bean cultivars is usually classified by a lower axillary branching pattern or branching that occurs under or close to the ground. However, mutant branching patterns have been found in this study, which are classified as the homeostasis pattern, upper axillary branch pattern, dichotomous branch pattern, and multi-branch pattern (Fig. 6). The homeostasis pattern is characterized by a plant without branches; the upper axillary branch pattern is characterized by branches that appear on the upper node of the main stem; the dichotomous branch pattern is characterized by the main stem that is divided into two branches with similar sizes; and the multi-branch pattern is characterized by the development of several primary and secondary branches. The primary branch directly generates from the main stem, while the secondary branch develops from the primary branch.

3.3. Assessment of genetic diversity and principal component analysis (PCA)

The assessment of genetic diversity based on 12 qualitative traits was performed by evaluating every plant in the mutant populations as indicated in Table 3. In the Hassawi 2 mutant population, 1278 plants (46% plants) showed no significant differences in the traits compared with the control plants, and 1500 plants (54% plants) exhibited variation in at least one trait compared to the control plants. The number of different traits among these mutant plants ranged from one to five traits out of 12 quantitative traits, and 50 different mutant classes of plants were found. In the ILB4347 mutant population, 877 plants or 66.2% plants had at least one different trait from the control plants. The number of different traits among these mutant plants ranged from one to seven traits compared to the control plants, and 110 different mutant classes were found among mutant plants.

Principal component analysis (PCA) was performed for 50 mutant classes of the Hassawi 2 mutant population and 110 mutant classes of the ILB4347 mutant population, as presented in Supplementary Table S1. The eigenvalue, variability, and cumulative variability were described. The first five principal components (PCs) showed 91.44% diversity in the Hassawi 2 mutant population and 86.54% diversity in the total variance in the ILB4347 mutant population.

The 12 trait loadings based on the Euclidean distance for five PCs from two mutant populations are presented in Supplementary Tables S2 and S3. In the Hassawi 2 mutant population, the first principal component (PC1) that accounted for 50.92% of the total variation, associated positively with stem pigmentation (0.96), whereas the second principal component (PC2) that demonstrated 16.93% of the total variation was related to the leaf shape (0.72), leaf size (0.51), flag petal color (0.31), and anthocyanin pigmentation (0.25). The maximum Euclidean value for the third principal component (PC3) was recorded for seed color (0.92), followed by leaf size (0.23), and leaf shape (0.22).

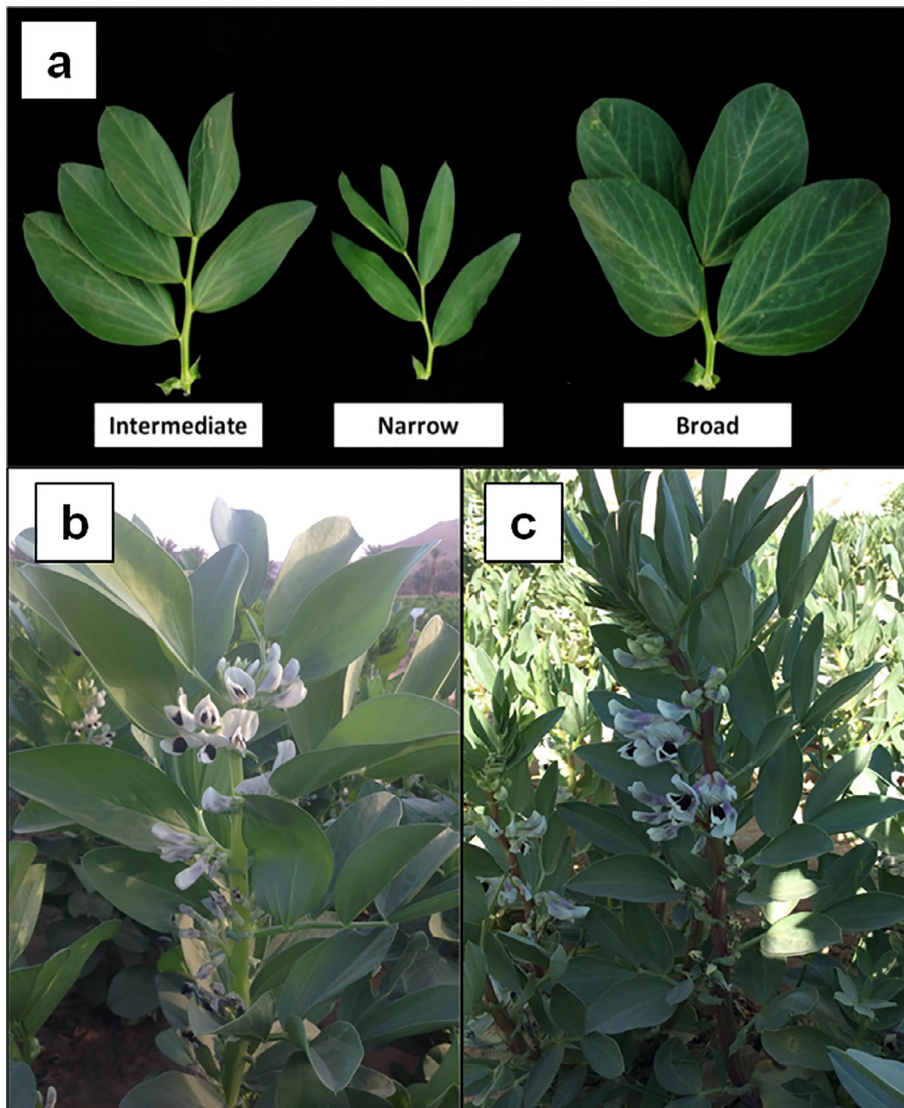


Fig. 4. Leaf shape and stem pigmentation variations. **a** three different leaf shape. **b** stem without pigmentation (absence). **c** stem with intense pigmentation.

In the ILB4347 mutant population, PC1 that revealed 34.22% of the total variation was associated with wing petal color (0.45), stem pigmentation (0.45), and seed color (0.43), whereas, PC2 that accounted for 24.42% of the total variation was related to seed color (0.65). The maximum Euclidean value for PC3 was recorded for flag petal color (0.79), followed by anthocyanin pigmentation (0.44), seed color (0.29), and stem pigmentation (0.25).

The scatter biplot of the first two PCs of the two mutant populations is presented in Fig. 7. The two PCs distributed the mutant plants into four quarters, indicating that the mutation induced by gamma radiation exposure and DES treatment succeeded in increasing the genetic diversity in both genotypes. Moreover, there are differences in the main traits involved in the diversity between the two mutant populations. The main traits in the first quarter (positively correlated to PC1 and PC2) of the Hassawi 2 mutant population were stem pigmentation, leaf shape, and growth habit, whereas the main traits in the first quarter of the ILB4347 mutant population were seed color, wing petal color, and streak color.

4. Discussion

Induced mutagenesis has been proposed to rapidly increase genetic diversity in many crops. This study evaluated two doses of gamma radiation and three concentrations of DES in inducing genetic diversity in two faba bean genotypes. The genetic diversity was assessed in the M_2 generation of faba beans, which was the most diverse population obtained after mutation breeding in terms of morphological markers. Twelve qualitative traits were used to determine the genetic diversity.

The effect of mutagens on SE and PS was recorded and calculated for each treatment. SE decreased by 17.8% in all mutant plants; a total of 18.9% and 15.4% reduction in SE was recorded in the Hassawi 2 and ILB4347 mutant populations, respectively. This considerable reduction indicated that the effect of gamma radiation and DES on SE also continued in the M_2 generation. However, the reduction on SE in the M_2 generation was not as markedly observed as in the M_1 generation. This could be due to a reduction in the mutagenic effect in the second generation as well as the DNA repair mechanisms operating in the plant cells (Curtis, 2011).

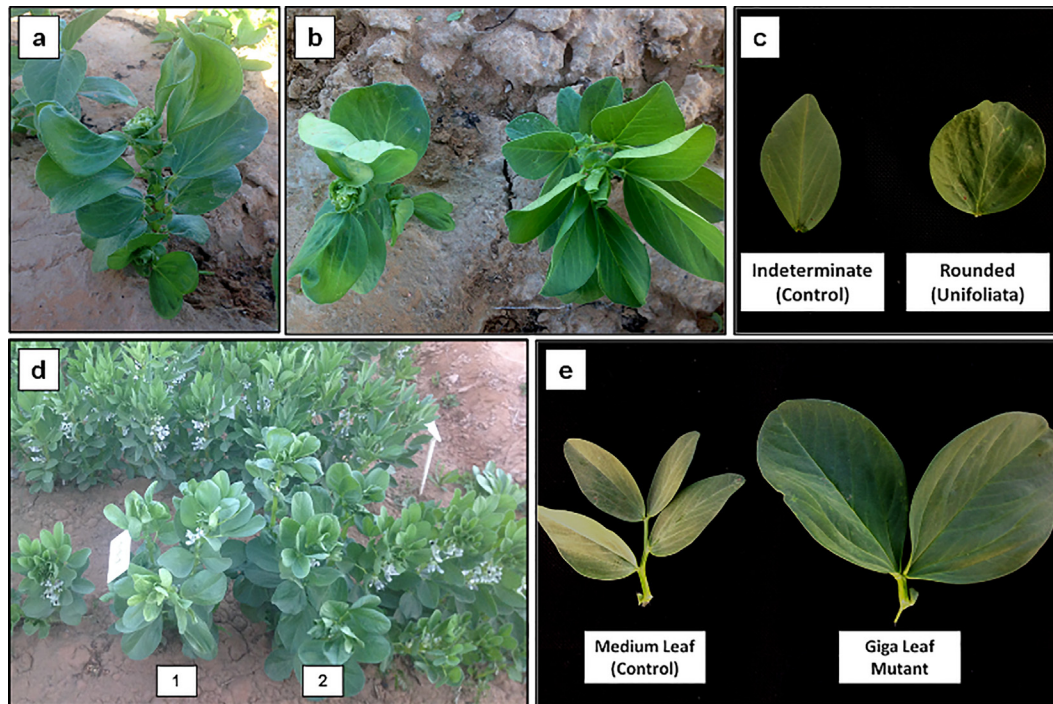


Fig. 5. Unifoliolate and giga mutant characters. **a** unifoliolate plant structure. **b** comparison between unifoliolate (left) and control plant (right) from above. **c** comparison between control and unifoliolate leaf. **d** two giga plants in the field. **e** comparison between control and giga mutant leaf (taken from node number 8).

Previous studies conducted by [Filippetti and De Pace in 1986](#) reported that SE in the M_1 generation of faba bean var. Manfredini decreased by 34% when exposed to 50 Gy of gamma radiation, and [Bhat et al. \(2007\)](#) reported a reduction of 25.3% in seed germination when the faba bean seeds of var. Major were exposed to 0.05% DES. On the other hand, only a 4.6% reduction was recorded in PS; however, most of this reduction in PS was due to environmental effects, pests, and diseases.

This study confirmed the potent effects of gamma radiation and DES in increasing the genetic diversity in the faba bean. The effects were further confirmed by the derivation of 36 new mutants types throughout the mutant plants. The mutants showed variations in determinate growth habit, flower characteristics, leaf characteristics, stem pigmentation, and seed characteristics.

The determinate growth habit has several advantages over indeterminate type in terms of crop management and fruit set production. In crop management, the determinate type facilitates large-scale mechanical harvesting because of the shorter height and higher compactness, and its relatively synchronous maturation ([Tian et al., 2010](#)). Furthermore, [Avila et al. \(2007\)](#) stated that the reduction in plant height in the determinate type prevents plants from lodging and promotes a better partitioning of assimilates between vegetative and reproductive growth compared to the indeterminate plants. In the present study, a total of 21 determinate mutants were found in the two populations and most of them were derived from gamma radiation-induced mutants. The detail characteristics of these mutants were described by [Nurmansyah et al. \(2019\)](#). Gamma radiation has also been reported to induce determinate mutants in several legume crops such as urad bean ([Vanniarajan et al., 2019](#)), cowpea ([Dhanasekar and Reddy 2015](#)), and chickpea ([Van Rheenen et al., 1994](#)). The other variation in inflorescence observed in the mutant population was in the peduncle. Long and double peduncles were found in this study; the long peduncle mutant was also observed by [Filippetti and De Pace in 1986](#) upon induced mutagenesis by gamma radiation and EMS.

Flower characteristic variations were also observed in the standard and the wing petal. Flower mutants were identified because of the different colors and the aggregation of pigmented cells in the petals. [Jiang and Rausher \(2018\)](#) found that the different petal pigmentation pattern in the genus *Clarkia* was due to two simple mutations in a regulatory stretch of DNA that caused a change in the transcription factor binding site. Therefore, the flower mutants in this study may also be derived due to the same mutations that change the transcription factor binding.

The importance of the screening and selection of flower characteristics is associated with the relationship between the flower color and the tannin content—an anti-nutritional factor found in faba bean. [Martin et al. \(1991\)](#) reported that white flowers have the lowest tannin content compared to flowers with other colors. Furthermore, [Duc et al. \(1999\)](#) reported a zero-tannin cultivar called Gloria that had recessive monogenetic segregation and a pure white flower as a pleiotropic effect of this gene. Therefore, the easiest way to produce a tannin-free faba bean is choosing the white-flowered faba bean. On the other hand, the importance of flower characteristics, especially petal pigmentation, is to attract bees as pollinators because the faba bean is a partially cross-pollinated crop with an average 35% outcrossing rate ([Bond and Poulsen, 1983](#)). As a consequence of this partially outcrossing nature, the optimal seed set depends on the wild bees for pollination, and a pollinator insufficiency has been reported to decrease the grain yield up to 64% ([Nayak et al., 2015](#)).

Seed characteristics are traits important for both the quality and quantity of the seed yield. Different seed coat colors possess different nutritional and anti-nutritional factors. [Shazali \(1990\)](#) reported that the darker color of the faba bean seed corresponded with a lower tannin content and a higher resistance to bruchids, the most detrimental storage pest for pulses. Moreover, [Kovinich et al. \(2011\)](#) indicated that an overaccumulation of anthocyanins altered procyanidin, and reduced flavonol, benzoic acid, and isoflavone content in black seeds compared to the brown seeds of the soybean observed phenotypically as a result of the altered transcription of

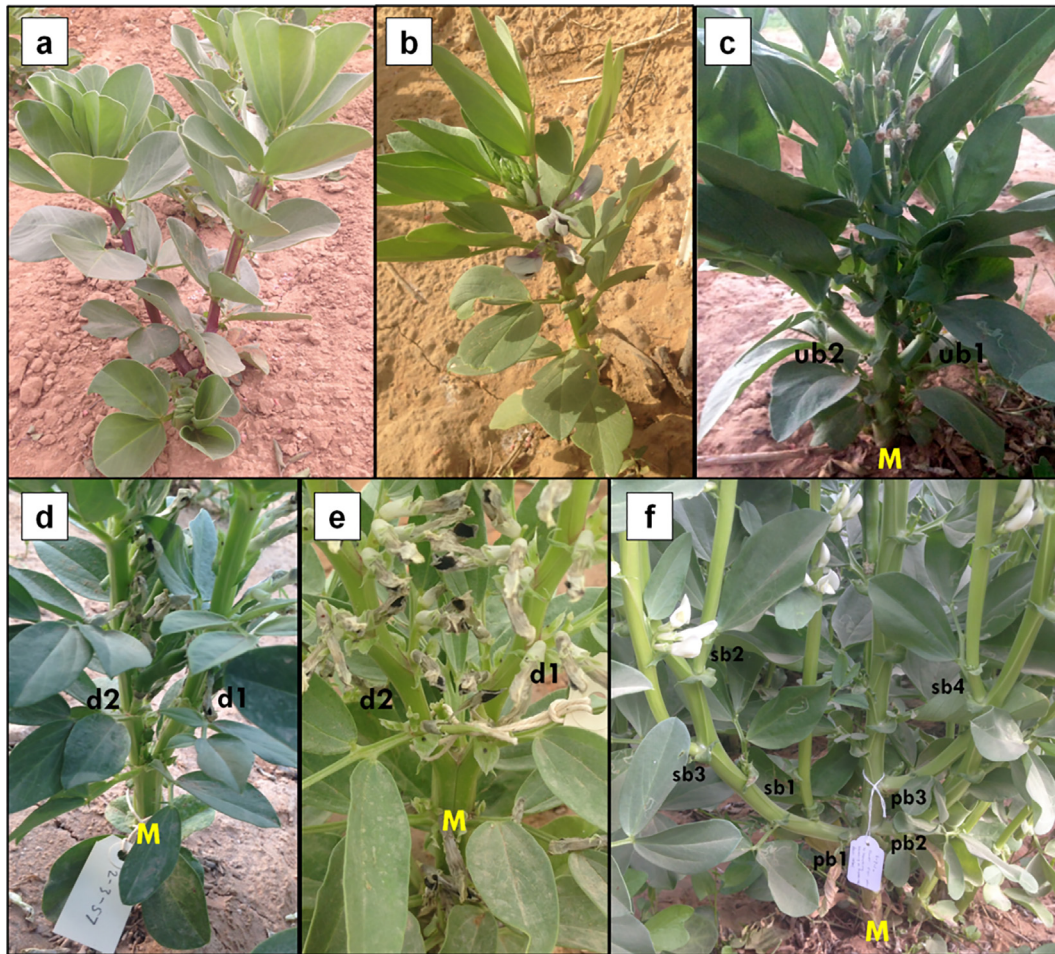


Fig. 6. Branching pattern variations. **a** control lower axillary branch pattern, **b** homeostatis pattern, **c** upper axillary branch pattern, **d-e** dichotomous branch pattern, **f** multi-branch pattern. M: main stem, ub: upper branch, d: dichotomous branch, pb: primary axillary branch, sb: secondary axillary branch.

Table 3
Number of different mutant trait and total number of mutant class in two mutant populations.

No. of different trait	Hassawi 2			ILB4347		
	Total no. of plant	%	Total no of mutant class	Total no. of plant	%	Total no of mutant class
0	1278	46.0	–	447	33.8	–
1	795	28.6	14	489	36.9	21
2	246	8.9	19	232	17.5	34
3	304	10.9	9	72	5.4	25
4	145	5.2	6	57	4.3	14
5	10	0.4	2	9	0.7	4
6	–	–	–	14	1.1	9
7	–	–	–	4	0.3	3
Total	2778	100	50	1324	100	110

numerous biosynthetic pathway genes. Another important character in seed is the hilum color. Duc et al. (1999) reported a low vicine cultivar that had monogenetic recessive and white hilum as a morphological marker for this trait.

Seed mutants were also found in different legume crops induced by physical and chemical mutagens. Wani and Anis (2008) found three mutants in chickpea by exposure to gamma radiation and EMS induction, which yielded different seed shapes and showed higher production compared to the control seeds. Similar results were also observed by Barshile (2018), where sodium azide was used to induce bold-seeded mutants in chickpea. Wani

et al. (2017) also reported bold seed and long pod mutants in mung bean induced by EMS.

Leaf mutants were also recorded in the present study. The leaves of the control plant were categorized as intermediate in shape with a medium size. Mutants with narrow and broad leaf shapes as well as small and large sizes were found. Moreover, a round leaf shape was also found in the unifoliate mutant. The importance of these leaf characteristics is associated with the efficiency and effectiveness of photosynthesis. Large and broad leaves cause a higher shading effect than small and narrow leaves. Furthermore, these leaf mutants can be used to identify genes that

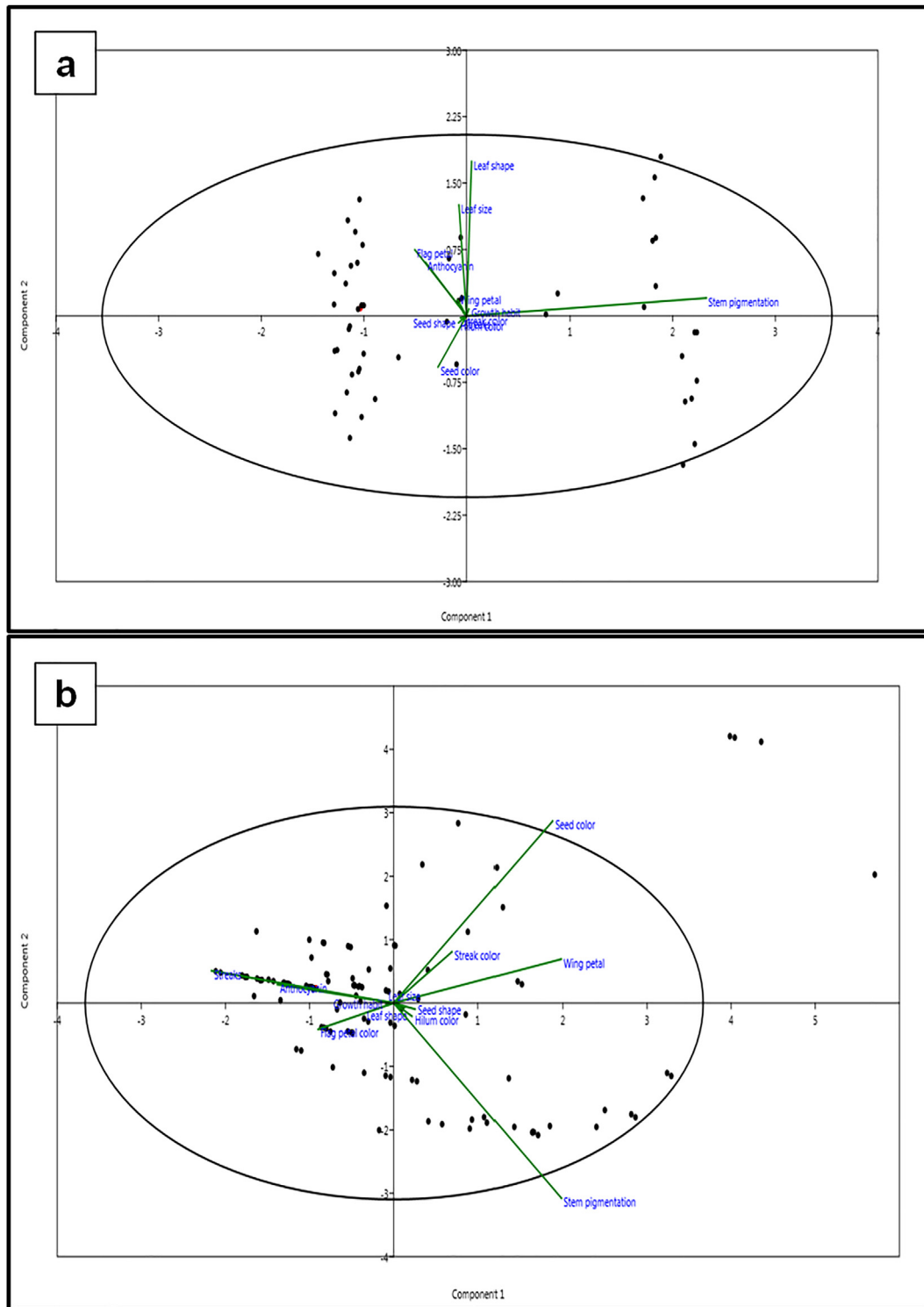


Fig. 7. Principle components analysis of 12 qualitative traits in two mutant populations. **a** two dimensional principle components analysis (PCA) of 12 qualitative traits in Hassawi 2 mutant population. **b** two dimensional principle components analysis (PCA) of 12 qualitative traits in ILB4347 mutant population.

are involved in regulating the leaf morphology in the faba bean. This approach has been used to study genes controlling leaf morphology in pea (*Pisum sativum*). Moreau et al. (2018) have characterized *Stipule reduce* (St) as gene controlling cell division and expansion in stipule from fast neutron irradiated pea mutant.

Stem pigmentation is another trait that was found to vary in the mutant populations. Goyal (1965) reported that a green stem was dominant among the pigmented stems. Furthermore, the inheri-

tance of stem pigmentation was also studied in chickpea by Yasar et al. (2014) who found that a single recessive gene is responsible for stem pigmentation in chickpea.

The other mutants found in the present study were the unifoliate and giga plant mutants. Sjödin (1971) reported that some of the unifoliate mutants originated from spontaneous mutations and some from induced mutagenesis using X-rays, neutrons, acetone, and methyl methanesulphonate (MMS). We speculate that the giga

mutants are caused due to polyploidy. A polyploid mutant is characterized by larger leaves, flowers, and other gigantisms. Baker et al. (2017) studied the relationship between ploidy level and leaf structure in genus of *Brassica*. They concluded that there were correlation among leaf morphology, anatomic structures, and physiological function to ploidy level. Colchicine is a mutagen that is widely used to induce polyploidy in many crops. However, because the other mutagens can cause chromosome aberrations, the chances of other mutagens inducing polyploidy in plants are considerably high. Four branching pattern mutants were also found in this study. Fujita and Kawaguchi (2011) suggested that the branching pattern of a plant is determined by the mode of stem cell proliferation and stem cell containment in the shoot apical meristem (SAM).

The assessment of genetic diversity based on 12 qualitative traits in two mutant populations, Hassawi 2 and ILB4347 was performed, and we concluded that more than 50% of the mutant plants have at least one different trait compared to the control plants. Multivariate-based PCA was also performed in this study. Karakoy et al. (2013) reported that multivariate analysis can be used to measure the contribution of relative variation in various traits to the total variability in a crop germplasm collection. The PCA results for two mutant populations explained that different traits have different contributions to the variation and the total variability within the population, which explains the random mutation induced by gamma radiation and DES.

5. Conclusion

This study revealed the potency of gamma radiation and DES on increasing genetic diversity and demonstrated the successful program of induced mutagenesis in the faba bean. A total of 36 mutant types were identified throughout the mutant populations. These results are valuable resources for further faba bean breeding programs. For future research, the mutant plant with desirable traits should be planted in an isolated area to prevent cross-pollination. Selection also should be conducted in several generations due to the chance of segregation. The true breeding behavior of mutant plant was obtained after several generations of selection usually in M_6 generation. In this stage, the mutant plant can be used to study gene and gene function of mutant traits. Moreover, the mutant plant can also be used to develop marker related to a particular trait to support marker-assisted selection (MAS).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank Deanship of scientific research for funding and supporting this research through the initiative of DSR Graduate Students Research Support (GSR).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jksus.2019.12.024>.

References

Acquaah, G., 2007. Principles of Plant Genetics and Breeding. Blackwell Publishing, Oxford.

- Avila, C.M., Atienza, S.G., Moreno, M.T., Torres, A.M., 2007. Development of a new diagnostic marker for growth habit selection in faba bean (*Vicia faba* L.) breeding. *Theor. Appl. Genet.* 115 (8), 1075–1082. <https://doi.org/10.1007/s00122-007-0633-y>.
- Baker, R.L., Yarkhunova, Y., Vidal, K., Ewers, B.E., Weinig, C., 2017. Polyploidy and the relationship between leaf structure and function: implications for correlated evolution of anatomy, morphology, and physiology in *Brassica*. *BMC Plant Biol.* 17 (3), 1–12. <https://doi.org/10.1186/s12870-016-0957-3>.
- Barshile, J.D., 2018. Characterization of bold seed mutant in chickpea cultivar Vijay. *JRBAT IV* (2), 17–20.
- Bhat, T.A., Sharma, M., Anis, M., 2007. Comparative analysis of mitotic aberrations induced by Diethyl sulphate (DES) and Sodium Azide (SA) in *Vicia faba* L. (Fabaceae). *Pak. J. Biol. Sci.* 10 (5), 783–787. <https://doi.org/10.3923/pjbs.2007.783.787>.
- Bond, D.A., Poulsen, M.H., 1983. Pollination. In: Hebblethwaite, P.D. (Ed.), *The Faba Bean* (*Vicia faba* L.). Butterworths, London, pp. 77–101.
- Curtis, M., 2011. DNA repair pathways and genes in plant. Shu Q, Forster, BP and Nakagawa, H (Eds.), *Plant Mutation Breeding and Biotechnology*. Rome (Italy): FAO, pp. 57–69. doi: 10.1079/9781780640853.0057.
- Desai, A.S., Rao, S., 2014. Effect of gamma radiation on germination and physiological aspects of pigeon pea (*Cajanus cajan* (L.) Mill sp) seedlings. *Int. J. Res. Appl. Nat. Soc. Sci.* 2 (6), 47–52.
- Dhanasekar, P., Reddy, K.S., 2015. A novel mutation in TFL1 homolog affecting determinacy in cowpea (*Vigna unguiculata*). *Mol. Genet. Genom.* 290, 55–65. <https://doi.org/10.1007/s00438-014-0899-0>.
- Duc, G., Marget, P., Esnault, R., Guen, J.L., Bastianelli, D., 1999. Genetic variability for feeding value of faba bean seeds (*Vicia faba*): comparative chemical composition of isogenics involving zero-tannin and zero-vicine genes. *J. Agric. Sci.* 133, 185–196. <https://doi.org/10.1017/S0021859699006905>.
- Filippetti, A., De Pace, C., 1986. Improvement of seed yield in *Vicia faba* L. by using experimental mutagenesis II Comparison of gamma-radiation and ethyl-methane-sulphonate (EMS) in production of morphological mutants. *Euphytica* 35, 49–59. <https://doi.org/10.1007/bf00028540>.
- Girija, M., Dhanavel, D., Gnanamurthy, S., 2013. Gamma rays and EMS induced flower color and seed mutants in cowpea (*Vigna unguiculata* L Walp). *Adv. Appl. Sci. Res.* 4, 134–139. <https://doi.org/10.18052/www.scipress.com/ilns.22.33>.
- Goyal, R.D., 1965. Breeding behaviour of anthocyanin pigmentation in flower and stem of broad beans (*Vicia faba* L.). *Sci. Cul.* 31, 147–148.
- IAEA. 2019. FAO/IAEA Mutant Varieties Database. <http://www.mvdiAEA.org> (Accessed 30 November 2019).
- IBPGR. 1985. Faba bean descriptors. IBPGR Secretariat, Rome, Italy, p. 17.
- Jiang, P., Rausher, M., 2018. Two genetic changes in cis-regulatory elements caused evolution of petal spot position in Clarkia. *Nat. Plants* 4, 14–22. <https://doi.org/10.1038/s41477-017-0085-6>.
- Karakoy, T., Baloch, F.S., Toklu, F., Özkan, H., 2013. Variation for selected morphological and quality-related traits among 178 faba bean landraces collected from Turkey. *Plant Genet. Resour.-Character. Util.* 12 (1), 5–13. <https://doi.org/10.1017/S1479262113000208>.
- Kodym, A., Afza, R., Forster, B.P., Ukai, Y., Nakagawa, H., Mba, C., 2012. Methodology for physical and chemical mutagenic treatments. In: Shu, Q., Forster, B.P., Nakagawa, H. (Eds.) *Plant Mutation Breeding and Biotechnology*. FAO, Rome, pp. 169–180. doi: 10.1079/9781780640853.0169.
- Kovnich, N., Saleem, A., Arnason, J.T., Miki, B., 2011. Combined analysis of transcriptome and metabolite data reveals extensive differences between black and brown nearly-isogenic soybean (*Glycine max*) seed coats enabling the identification of pigment isogenes. *BMC Genom.* 12 (381), 1–18. <https://doi.org/10.1186/1471-2164-12-381>.
- Kozjak, P., Meglič, V., 2012. Mutagenesis in plant breeding for disease and pest resistance. In: Rajnikant, M., (Ed.) *Mutagenesis*. InTech, pp. 195–220. doi: 10.5772/50332.
- Martin, A., Cabrera, A., Medina, J.L., 1991. Antinutritional factors in faba bean Tannin content in *Vicia faba*: possibilities for plant breeding. *Options Mediterraneennes* 10, 105–110.
- Moreau, C., Hofer, J.M.I., Eléouët, M., Sinjushin, A., Ambrose, M., Skot, K., Blackmore, T., Swain, M., Hegarty, M., Balanzà, V., Ferrándiz, C., Ellis, T.H.N., 2018. Identification of *Stipules reduced*, a leaf morphology gene in pea (*Pisum sativum*). *New Phytologist* 220 (1), 288–299. <https://doi.org/10.1111/nph.15286>.
- Musallam, I.W., Al-Karaki, G.N., Ereifei, K.I., Rahman, A., Tawaha, A.R.M., 2004. Chemical composition of faba bean genotypes under rainfed and irrigation conditions. *Int. J. Agr. Biol.* 6, 359–362.
- Nayak, G.K., Roberts, S.P., Garratt, M., Breeze, T.D., Tschulin, T., Harrison-Cripps, J., Vogiatzakis, I.N., Stirpe, M.T., Potts, S.G., 2015. Interactive effect of floral abundance and semi-natural habitats on pollinators in field beans (*Vicia faba*). *Agr. Ecosyst. Environ.* 199, 58–66. <https://doi.org/10.1016/j.agee.2014.08.016>.
- Nedumaran, S., Abinay, P., Jyosthnaa, P., Shraavya, B., Parthasarthy, R., Bantilan, C., 2015. Grain legumes production, consumption and trade trends in developing countries. Working Paper Series No 60. ICRIASAT Research Program, Markets, Institutions and Policies. Patancheru 502 324, Telangana, India.
- Nurmansyah, Alghamdi, S.S., Migdadi, H.M., Farooq, M., 2018. Morphological and chromosomal abnormalities in gamma radiation-induced mutagenized faba bean genotypes. *Int. J. Rad. Biol.* 94 (2), 174–185. <https://doi.org/10.1080/09553002.2018.1409913>.
- Nurmansyah, Alghamdi, S.S., Migdadi, H.M., Farooq, M., 2019. Novel inflorescence architecture in gamma radiation-induced faba bean mutant populations. *Int. J. Rad. Biol.* 95 (12), 1744–1751. <https://doi.org/10.1080/09553002.2019.1665205>.

- Page, D.R., Grossniklaus, U., 2002. The art and design of genetic screens: *Arabidopsis thaliana*. *Nat. Rev. Genet.* 3, 124–136.
- Sangsiri, C., Sorajjapinun, W., Srinives, P., 2005. Gamma radiation induced mutations in mungbean. *Sci. Asia* 31, 251–255. <https://doi.org/10.2306/scienceasia1513-1874.2005.31.251>.
- Shazali, M.H., 1990. Faba bean (*Vicia faba* L.) seed coat colour, tannin content, and susceptibility to Bruchids. *Int. J. Trop. Insect Sci.* 11 (6), 855–859. <https://doi.org/10.1017/s1742758400010742>.
- Sjodin, J.A.N., 1971. Induced morphological variation in *Vicia faba* L. *Hereditas* 67, 155–180. <https://doi.org/10.1111/j.1601-5223.1971.tb02371.x>.
- Tian, Z., Wang, X., Lee, R., Li, Y., Specht, J.E., Nelson, R.L., McClean, P.E., Qiu, L., Ma, J., 2010. Artificial selection for determinate growth habit in soybean. *Proc. Natl. Acad. Sci.* 107 (19), 8563–8568. <https://doi.org/10.1073/pnas.1000088107>.
- UPOV, 2003. Broad Bean (*Vicia faba* var major Harz). International Union for the Protection of New Varieties of Plants Geneva, Switzerland, p. 29.
- Vanniarajan, C., Ganeshram, S., Souframanien, J., Veni, K., Lavanya, S., Kuralarasan, V., 2019. Gamma rays induced urdbbean (*Vigna mungo* L. Hepper) mutants with YMV resistance, good batter quality and bold seeded type. *Legume Res.* 3824, 1–7. <https://doi.org/10.18805/lr-3824>.
- Van Rheenen, H.A., Pundir, R.P.S., Miranda, J.H., 1994. Induction and inheritance of determinate growth habit in chickpea (*Cicer arietinum* L.). *Euphytica* 78, 137–141. <https://doi.org/10.1007/bf00021409>.
- Wani, A.A., Anis, M., 2008. Gamma ray and EMS induced bold seeded high yielding mutants in chickpea (*Cicer arietinum*). *Turk. J. Biol.* 32, 1–5.
- Wani, M.R., Dar, A.R., Tak, A., Amin, I., Shah, N.H., Rehman, R., Baba, M.Y., Raina, A., Laskar, R., Kozgar, M.I., Khan, S., 2017. Chemo-induced pod and seed mutants in mungbean (*Vigna radiata* L. Wilczek). *SAAARC. J Agri.* 15 (2), 57–67. <https://doi.org/10.3329/sja.v15i2.35161>.
- Yasar, M., Ceylan, F.O., Ikten, C., Tokar, C., 2014. Comparison of expressivity and penetrance of the double podding trait and yield components based on reciprocal crosses of kabuli and desi chickpeas (*Cicer arietinum* L.). *Euphytica* 196, 331–339. <https://doi.org/10.1007/s10681-013-1036-6>.
- Ye, Y., Lang, L., Xia, M., Tu, J., 2003. *Faba Beans in China*. China Agriculture Press, Beijing, China.