RESEARCH ARTICLE Legume Research- An International Journal, Volume 44 Issue 7: 759-765 (July 2021)

Genetic Diversity and Population Structure of Two Faba Bean Mutant Populations Based on AFLP Markers

Nurmansyah[1,2], Hussein M. Migdadi[1], Salem S. Alghamdi[1], Muhammad A. Khan[1], M. Afzal[1]

10.18805/LR-594

ABSTRACT

Background: Although induced mutagenesis can rapidly generate genetic diversity, every genotype responds differently to different mutagen treatments in inducing genetic diversity. This study assesses and compares the genetic diversity of two mutant faba bean populations.

Methods: Two genotypes representing landrace (Hassawi 2) and inbred variety (ILB4347) were exposed to gamma radiation and diethyl sulfate (DES). Two hundred eighty-two samples derived from individual 140 M_2 mutant plants of Hassawi 2 and ILB 4347 and two parental lines were characterized using 11 Amplified Fragment Length Polymorphism (AFLP) primer combinations.

Result: 89,820 bands within 2,083 polymorphic alleles were generated from the samples. Genetic diversity comparison of the mutant populations revealed that each genotype had varying responses to different treatments. The two genotypes had a relatively similar response to gamma radiation, while different responses were recorded in DES-derived mutant plants. Based on the Nei's genetic distance, the populations were separated based on the genotypic origin. The population structure analyses showed that the Hassawi 2 and ILB4347 mutant populations were clustered into three and two groups, respectively. The difference in the number of clusters between the two mutant populations explains their breeding history. The present diversity considered as a valuable material used for breeding and conservation purposes.

Key words: Genetic diversity, Mutagenesis, Population structure comparison, *Vicia faba*

INTRODUCTION

Faba bean is the fourth most produced cool-season legume worldwide, with the total production of dry grains up to 4.92 million tons from 2.51 million ha-harvested areas in 2018 (FAOSTAT 2018). Faba bean has potency as a future protein supply for the human diet because of its chief protein source, fiber and other non-nutrient compounds (Multari *et al.*, 2015). Oliveira *et al.* (2016) stated that faba bean also has an essential role in soil fertility and nitrogen fixation. Despite its importance, Nedumaran *et al.* (2015) reported that the growth rate of faba bean's yield was the lowest among legume crops. Therefore, the development of new high-yielding faba bean varieties is needed to boost faba bean production. The development of high-yielding varieties depends on the availability of genetic diversity, which can be enriched by induced mutagenesis. The genetic diversity generated by induced mutagenesis has been reported in many studies that included faba bean (Nair and Mehta 2014; Shekar and Pushpendra 2017; Khursheed and Khan 2017; Singh and Sadhukhan 2019). However, most of these studies were based on morphology-based diversity. Plant breeders widely use morphology-based markers to determine genetic diversity because of their simplicity and low cost. However, only a few traits can be tested. Environmental factors also affected most economically important traits, making it difficult to test using morphological markers.

Molecular or DNA-based markers are abundant and are not influenced by the environment. Therefore, DNA-based markers serve as a powerful tool for characterizing genetic diversity in various crop species, including faba bean (Alghamdi *et al*. 2012a). One of the best molecular markers for studying genetic diversity among the faba bean populations is amplified fragment length polymorphism, AFLP (Zeid *et al.* 2003; Zong *et al*. 2010, Alghamdi *et al.* 2014; Nurmansyah et al. 2020a). The AFLP-based molecular marker significant advantage is the high reproducibility or the capacity to generate high polymorphism bands, which is useful for evaluating genetic diversity.

[1]Department of Plant Production, College of Food and Agriculture Sciences, King Saud University, P.O. BOX 2460, Riyadh 11451, Saudi Arabia.
[2]Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

Corresponding Author: Hussein M. Migdadi, Department of Plant Production, College of Food and Agriculture Sciences, King Saud University, P.O. BOX 2460, Riyadh 11451, Saudi Arabia.
Email: h.migdadi@gmail.com

How to cite this article: Nurmansyah, Migdadi, H.M., Alghamdi, S.S., Khan, M.A. and Afzal, M. (2021). Genetic Diversity and Population Structure of Two Faba Bean Mutant Populations Based on AFLP Markers. Legume Research. 44(7): 759-765. DOI: 10. 18805/LR-594.

Submitted: 22-10-2020 **Accepted:** 22-03-2021 **Online:** 15-07-2021

Although induced mutagenesis can rapidly generate genetic diversity, every genotype responds differently to different mutagen treatments in inducing genetic diversity. Therefore, this study compares and tests the genetic diversity of the two genotypes of faba bean treated with two mutagens based on AFLP markers in the M_2 generation. The study is crucial to select the most diverse population and help reduce population size for further generations. The two genotypes were Hassawi 2, representing a faba bean landrace cultivar from Saudi Arabia and ILB4347 represents an inbred line from ICARDA. Hassawi 2 is reported drought stress tolerance and well adapted in arid conditions of Saudi Arabia (Migdadi *et al.* 2016), while ILB4347 is reported to have salt stress tolerance and resistance to broomrape (Alzahrani *et al.* 2019; Rubiales *et al*. 2016). The two mutagens were gamma radiation and diethyl sulfate (DES). We investigated the population structure within mutant populations to assess population change in the two genotypes, landrace and inbred populations.

MATERIALS AND METHODS

Plant Materials and DNA extraction

5271 M_2 seeds generated from the M_1 generation of two faba bean genotypes, Hassawi 2 and ILB4347, were used in this study. The original seeds of Hassawi 2 were obtained from the Legume Research Group, Department of Plant Production, College of Food and Agriculture Sciences, King Saud University, and the seeds of ILB4347 were obtained from ICARDA. The M_1 seeds were produced by exposing separate 120 dry seed samples of each genotype to 25 and 50 Gy at a dose rate of 15.48 Gy/min of gamma radiation using a ^{60}Co (Cobalt 60) gamma source (Gamma Chamber 900, Nordion, Canada) under ambient conditions at the Central Lab, College of Science, King Saud University, and three concentrations of DES 0.01%, 0.02% and 0.05% solution. The dry seeds were soaked in DES solution for 12 h at room temperature and then were thoroughly washed under running water three times (Nurmansyah *et al*., 2018).

Two hundred eighty of 4102 mutant plants, representing 280 mutant families, were chosen from the mutant populations. Selection criteria of selected mutants were based on survival rates at the M_1 and M_2 generations, followed by phenotypic mutants described by our previous study, including determinate growth habit (Nurmansyah *et al*. 2019), flower, leaf and seed character mutants (Nurmansyah *et al.* 2020b) and quantitative characters such as the number of branches, pod length, number of seeds per pod and 100 seed weight. Leaves of 280 M_2 mutant plants derived from 140 mutant plants of Hassawi 2 and 140 mutant plants of ILB 4347 and two parental lines were collected for DNA isolation. As described by Alghamdi *et al.* (2012b), a modified SDS protocol was used for DNA isolation.

AFLP procedure

The AFLP plant mapping protocol from (ABI) Applied Biosystems (Waltham, MA, USA) was used in this study with some modifications as described by Nurmansyah *et al.* (2020a). *EcoRI/MseI* and *T4DNA-ligase* enzymes used for digestion and ligation of DNA samples. The AFLP adapter/primer name and sequence used are shown in Table 1. The pre-selective amplification was performed using primers that matched the adapter sequence and had one additional 'selective' base (*Eco*RI + G and *Mse*I + C). Selective amplification used the diluted pre-selective amplification product as the new template. This reaction's primers had the same sequence as the pre-selective primers with two and three other selective bases in *Eco*RI and *Mse*I primers. Eleven AFLP primer combinations were used to estimate genetic diversity among faba bean mutant lines. AFLP fragments were performed using GeneMapper Analysis Software v3.7 (ABI) and the data assembled in a binary format, with 1 representing allele presence and 0 for absence. The threshold for allele calling is set at 100 relative fluorescence units (rfu). We assigned peaks at 100 rfu or higher assigned as one and those with lower 100 rfu a zero value. We performed fragment analysis for allele sizes between 100 and 500 bp, as recommended by Paris *et al*. (2010).

The total number of alleles, the number of bands, the average number of bands per sample, and the average number of bands per allele recorded for each primer. Polymorphism information content (PIC) estimated according to Botstein *et al.* (1980) and discrimination power (DP) calculated by dividing the number of polymorphic alleles amplified for each primer by the total number of polymorphic alleles recorded. Dendrogram using the Jaccard similarity coefficient and the unweighted pair group method with arithmetic average (UPGMA) constructed using Paleontological Statistics (PAST) v. 3.20 program (Hammer *et al.*, 2001).

Table 1: Adapter and primer sequence used for AFLP analysis.

Adapter/primer name	(5′-3′)
*Eco*R1 adapter 1	CTCGTAGACTGCGTACC
*Eco*R1 adapter 2	AATTGGTACGCAGAGTCTAC
*Mse*I adapter 1	GACGATGAGTCCTGAG
*Mse*I adapter 2	TACTCAGGACTCAT
*Eco*R1 pre-selective primer (G)	GACTGCGTACCAATTCG
*Mse*I pre-selective primer (C)	GATGAGTCCTGAGTAAC
*Eco*R1 selective primer (TG)	GACTGCGTACCAATTCTG
*Eco*R1 selective primer (TC)	GACTGCGTACCAATTCTC
*Eco*R1 selective primer (TA)	GACTGCGTACCAATTCTA
*Eco*R1 selective primer (TT)	GACTGCGTACCAATTCTT
*Eco*R1 selective primer (AA)	GACTGCGTACCAATTCAA
*Eco*R1 selective primer (CC)	GACTGCGTACCAATTCCC
*Mse*I selective primer (CTT)	GATGAGTCCTGAGTAACTT
*Mse*I selective primer (CCA)	GATGAGTCCTGAGTAACCA
*Mse*I selective primer (CAG)	GATGAGTCCTGAGTAACAG
*Mse*I selective primer (CAC)	GATGAGTCCTGAGTAACAC
*Mse*I selective primer (CCC)	GATGAGTCCTGAGTAACCC
*Mse*I selective primer (CCT)	GATGAGTCCTGAGTAACCT

Genetic diversity parameters and population structure

The presence/absence binary matrix was used for further analysis with GenAlEx 6.503 complement for MSExcel (Peakall and Smouse 2012). The total number of different alleles (Na), number of effective alleles (Ne), Shannon's information index (I), expected heterozygosity (He), percentage of polymorphic loci (% P) and private alleles per population estimated using this approach. Pair-wise, Jaccard genetic similarity using Paleontological Statistics (PAST) v. 3.20 program used to assess genetic diversity among the 280 mutant and control plants. Analysis of molecular variance (AMOVA) was performed using GenAlEx 6.503 with 999 permutations. Using STRUCTURE 2.3.4 program, the population structure was tested and supported by Principal Coordinates Analysis (PCoA) using the PAST program based on Jaccard's similarity index.

RESULTS AND DISCUSSION

Assessment of genetic diversity based on AFLP-molecular marker

The characteristics of 11 AFLP primer combinations are presented in Table 2. 2083 alleles were generated from 282 samples (280 mutant plants and two control plants). The number of alleles ranged from 97 for primer combination (*Eco*RI/*Mse*I) TT/CTT to 305 for primer combination TG/CTT. The 11 primer combinations generated 89,820 bands with an average of 8,165.45 bands per primer combination. The average bands recorded across the samples were 28.96 bands per sample, ranged from 15.48 to 60.63 bands. Simultaneously, the average bands per allele ranged from 31.57 to 56.06 with an average of 42.70 bands.

The analysis of genetic diversity parameters is presented in Table 3. The analysis was divided based on the initial genotype and the combination between the initial genotype and treatment, gamma radiation (physical mutagen) and DES (chemical mutagen). The genetic diversity based on the initial genotype revealed that the mutant population of ILB 4347 was more diverse than the mutant population of Hassawi 2, as shown by the higher value in the genetic diversity parameters. Based on the combination between the initial genotype and treatment, four subpopulations were analyzed and revealed that the highest genetic diversity was found in the mutant population of ILB 4347 induced by DES. The lowest genetic diversity was recorded in the mutant population of Hassawi 2 induced by DES. Genetic diversity comparison among the mutant populations revealed that each genotype responds differently to different treatments in inducing genetic diversity.

Based on genetic diversity parameters, treatments with gamma radiation generated the maximum genetic diversity in Hassawi 2, while in ILB4347 mutant populations, DES generated higher genetic diversity than the gamma radiation. It showed that gamma radiation is more efficient to enrich genetic diversity than DES in Hassawi 2. In ILB4347, DES is more efficient to enrich genetic diversity than gamma radiation. Therefore, for further generation, gamma radiation-induced and DES-induced populations should be selected in Hassawi 2 and ILB4347, respectively.

The AMOVA analyses showed that the maximum genetic variation is found within the populations. These results align with other faba bean studies (Wang *et al.*, 2012; Göl *et al.*, 2017; El-Esawi, 2017). The two-level analysis of AMOVA based on the initial genotype showed that 94% of the variation was concentrated within genotypes, and the variations between the initial genotype population contributed to the remaining 6% (Table 4). All the mutant plants from both genotypes subjected to the same treatments may have equal chances to contribute to both genotypes. A similar likelihood of inducing diversity in both genotypes was supported by a relatively similar value seen in the pair-wise Jaccard's genetic similarity index between populations. The similarity index within Hassawi 2 population ranged from 0.05 to 0.65, whereas the similarity index within

Table 2: Characteristics of 11 AFLP primers selected of 282 samples.

Primer combination *Eco*R1/*Mse*I	Total alleles	Total no. of bands	Average bands per sample	Average bands per allele	PIC	DP (%)
TG/CTT	305	17097	60.63	56.06	0.99	19.03
TC/CCA	220	9630	34.15	43.77	0.99	10.72
TA/CCA	221	9096	32.26	41.16	0.99	10.13
TA/CAG	171	8377	29.71	48.99	0.99	9.33
TC/CAC	204	7477	26.51	36.65	0.99	8.32
TT/CAC	180	7635	27.07	42.42	0.99	8.50
AA/CCC	198	7666	27.18	38.72	0.99	8.53
AA/CCT	178	7497	26.59	42.12	0.99	8.35
TC/CAG	203	6409	22.73	31.57	0.99	7.14
CC/CCA	106	4366	15.48	41.19	0.98	4.86
TT/CTT	97	4570	16.21	47.11	0.97	5.09
Total	2083	89820	318.51	469.75	-	100
Average	189.36	8165.45	28.96	42.70	0.99	9.09

PIC: polymorphism information content, DP: discrimination power.

the ILB4347 population ranged from 0.05 to 0.67. The 6% variation was observed, possibly because of the different genetic makeup between the genotypes or random mutation events.

Cluster analysis

Cluster analysis of populations based on Nei's genetic distance revealed that two clusters could be formed based on the initial genotype if the populations were grouped by genotype-treatment combinations (Fig 1). Fig 1A shows two clusters formed with a genetic similarity value of 0.54 (50% of genetic similarity). Cluster one and two represent ILB 4347 and Hassawi 2 populations, respectively. The PCoA analysis also clearly divided the populations into two clusters through the first and second principal coordinates that accounted for 73.6% of the total variations (Fig 1B). Cluster analysis-separated faba bean mutant populations based on genotypic origin. The population could be divided into two clusters; cluster one for the ILB 4347 population and cluster two for the Hassawi 2 population. This grouping was based on a comparison of four mutant populations based on combinations between genotypes and treatments. This grouping revealed the pedigree of the populations. Fig 2B shows a clear separation of the clusters, but the distance between the mutant populations in genotype Hassawi 2 is higher than that in ILB4347 mutant populations. Based on the AMOVA analysis, in Table 5 and 6, 10% variation is observed between the populations induced by gamma radiation and DES in Hassawi 2, whereas only 3% variation was observed between the treatments ILB4347 mutant populations.

Population structure

The first panel of the population structure examined the population structure within the Hassawi 2 mutant population. Evanno's ΔK peaked at K = 3 (Fig 2A), showing that mutant plants within the Hassawi 2 population could be divided into three clusters. Fig 2B shows the results of STRUCTURE on Hassawi 2 mutant populations. Red lines represent cluster one and green and blue lines represent clusters two and three, respectively. The second panel of population structure tested the ILB 4347 mutant population structure. Evanno's ΔK value peaked at K = 2 (Fig 2C), showing that the mutant plants can be divided into only two clusters. Fig 2D shows the STRUCTURE analysis of ILB 4347 mutant populations categorized as major and minor

Table 3: Diversity parameters of mutant populations got from the analysis of 2083 AFLP alleles.

Mutant population	No. of mutants	Na	Ne	I	He	% P	Private alleles
Genotype							
Hassawi 2	140	1.883	1.181	0.217	0.125	94.14	59
ILB 4347	140	1.942	1.191	0.236	0.135	97.12	121
Mean	140	1.913	1.187	0.227	0.130	95.63	
Genotype × treatment							
Hassawi 2 (Gamma Radiation)	110	1.852	1.196	0.227	0.132	92.61	41
ILB 4347 (Gamma Radiation)	110	1.892	1.185	0.228	0.130	94.72	37
Hassawi 2 (DES)	30	1.172	1.119	0.149	0.085	58.57	0
ILB 4347 (DES)	30	1.677	1.215	0.247	0.146	83.68	1
Mean	70	1.649	1.178	0.213	0.123	82.39	

Na: no. of different alleles, Ne: no. of effective alleles, I: Shannon index, He: expected heterozygosity, % P: percentage of polymorphic loci, Private alleles: no. of alleles unique to a single population.

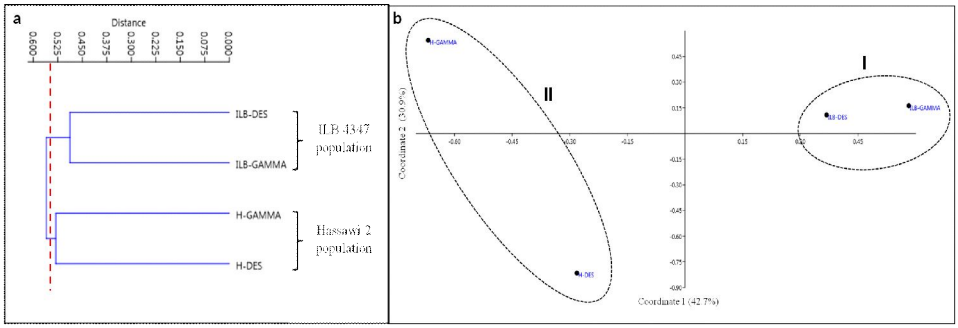

Fig 1: Cluster analysis of four mutant populations based on Nei's genetic distance. A. the UPGMA dendrogram generated by AFLP data using Gower similarity index, B. PCoA of four mutant populations for the first and second coordinates estimated for AFLP data using Gower similarity index.

H-GAMMA: Hassawi 2 mutant population induced by gamma radiation, H-DES: Hassawi 2 mutant population induced by DES, ILB-GAMMA: ILB 4347 mutant population induced by gamma radiation, ILB-DES: ILB 4347 mutant population induced by DES.

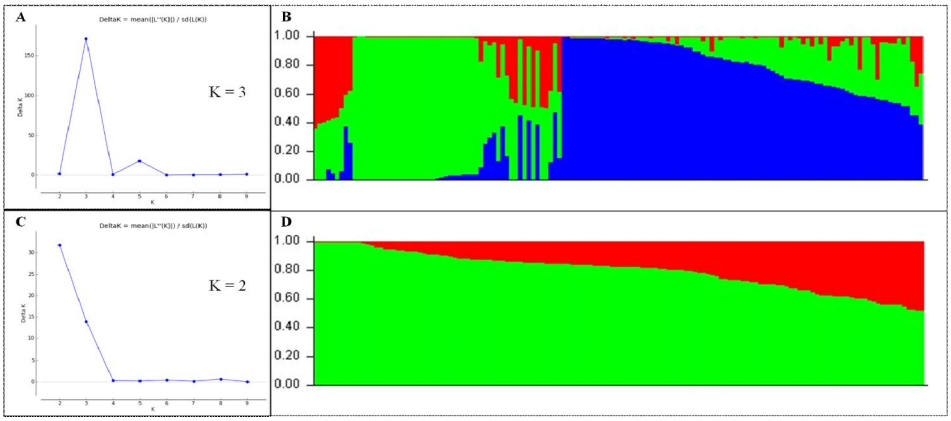

Fig 2: Comparison of population structure of two mutant populations based on genotypical origin (Hassawi 2 and ILB 4347). A, B belongs to Hassawi 2 mutant population, C, D belongs to the ILB4347 mutant population. A. Evanno's ΔK value with K = 1-10, B. Genetic structure inferred by STRUCTURE program with K = 3, C. Evanno's ΔK value with K = 1-10, D. Genetic structure inferred by STRUCTURE program with K = 2.

Table 4: Summary of AMOVA of mutant population-based on initial genotype.

Source	df	SS	MS	Est. var.	%	Phi statistics
Between genotypes	1	1847.91	1847.91	11.79	6	0.057**
Within genotypes	278	54677.11	196.68	196.68	94	
Total	279	56525.03		208.47	100	

Table 5: Summary of AMOVA of Hassawi 2 mutant population-based on treatment.

Source	df	SS	MS	Est. var.	%	Phi statistics
Between treatments	1	1119.71	1119.71	19.93	10%	0.100**
Within treatments	138	24865.87	180.19	180.19	90%	
Total	139	25985.58		200.12	100%	

Table 6: Summary of AMOVA of ILB 4347 mutant population-based on treatment.

Source	df	SS	MS	Est. var.	%	Phi statistics
Between treatments	1	525.42	525.42	6.82	3%	0.032**
Within treatments	138	28166.12	204.10	204.10	97%	
Total	139	28691.54		210.92	100%	

clusters. A significant cluster or cluster two is represented in green and a minor cluster or cluster one is represented in red.

The population structure analyses showed a different population structure between Hassawi 2 and ILB4347 mutant populations. Hassawi 2 mutant populations were clustered into three groups, whereas ILB4347 mutant populations were clustered into two groups based on the best Evanno's ΔK inferred by STRUCTURE HARVESTER software. The different number of clusters between the two mutant populations shows their breeding history. Sim *et al.* (2011) revealed that population structure data positively correlated with the population's breeding history. Based on this knowledge, distinct patterns of population structures between Hassawi 2 and ILB4347 mutant populations could be because of differences in their breeding history. Hassawi 2 is a landrace cultivar and it is probably genetically more diverse than the inbred line (ILB4347). Therefore, the number of gene pools influencing the population structure was higher in the Hassawi 2 population than in the ILB4347 population.

CONCLUSION

This study showed that each genotype had a unique response to different treatments. Gamma radiation is efficiently generating genetic diversity in Hassawi 2, while

DES is preferable in ILB4347. Therefore, for further generation, gamma radiation-induced and DES-induced populations should be selected in Hassawi 2 and ILB4347, respectively. The grouping of mutant plants can be separated regarding mutant populations based on Nei's genetic distance. It can be separated based on the genotypic origin. The population structure analyses showed that Hassawi 2 mutant populations were clustered into three groups, while ILB4347 mutant populations were clustered into two groups. The different number of clusters between two mutant populations explained their breeding history. Hassawi 2 is a landrace cultivar; it is probably genetically more diverse than the inbred line (ILB4347). Thus, the number of gene pools influenceing the population structure was higher in the Hassawi 2 population than in the ILB4347 population.

ACKNOWLEDGEMENT

This work is supported by the NSTIP strategic technologies program number (11-AGR1861-02) in Saudi Arabia.

REFERENCES

Alghamdi, S.S., Migdadi, H.M., Ammar, M.H., Paull, J.G. and Siddique, K.H.M. (2012a). Faba bean genomics, current status, and future prospects. Euphytica. 186 (3): 609-624.

Alghamdi, S.S., Al-Faifi, S.A., Migdadi, H.M., Khan, M.A., EL-Harty, E.H. and Ammar, M.H. (2012b). Molecular diversity assessment using Sequence Related Amplified Polymorphism (SRAP) markers in *Vicia faba* L. International Journal of Molecular Sciences. 13 (12): 16457-16471.

Alghamdi, S. S, Al-Shameri, A.M., Migdadi, H.M., Ammar, M.H., El-Harty, E.H., Khan, M.A. and Farooq, M. (2014). Physiological and molecular characterization of faba bean (*Vicia faba* L.) genotypes for adaptation to drought stress. Journal of Agronomy and Crop Science. 201 (6): 401-409.

Alzahrani, S.M., Alaraidh, I.A., Khan, M.A., Migdadi, H.M., Alghamdi, S.S. and Alsahli, A.A. (2019). Identification and characterization of salt-responsive microRNAs in *Vicia faba* by high-throughput sequencing. Genes. 10 (303): 1-20.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics. 32: 314-331.

El-Esawi, M.A. (2017). SSR analysis of genetic diversity and structure of the germplasm of faba bean (*Vicia faba* L.). Comptes Rendus Biologies. 340: 474-480.

Food and Agriculture Organization of the United Nations. (2018). FAOSTAT Statistical Database. Available at: http://www.fao. org/faostat/en/#compare. Accessed on October 17, 2020.

Gö l, S., Doganlar, S. and Frary, A. (2017). Relationship between geographical origin, seed size and genetic diversity in faba bean (*Vicia faba* L.) as revealed by SSR markers. Molecular Genetics and Genomics. 292 (5): 991-999.

Hammer, Ø., Harper, D.A.T. and Ryan, P.D. (2001). Past: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica. 4(1): 1-9.

Khursheed, S. and Khan, S. (2017). Genetic improvement of two cultivars of *Vicia faba* L. using gamma irradiation and ethyl methanesulphonate mutagenesis. Legume Research. 40 (2): 338-344.

Migdadi, H.M., El-Harty, E.H., Salamh, A. and Khan, M.A. (2016). Yield and proline content of faba bean genotypes under water stress treatments. The Journal of Animal and Plant Sciences. 26: 1772-1779.

Multari, S., Stewart, D. and Russell, W.R. (2015). Potential of fava bean as future protein supply to partially replace meat intake in the human diet. Comprehensive Reviews in Food Science and Food Safety. 14 (5): 511-522.

Nair, R. and Mehta, A.K. (2014). Induced mutagenesis in cowpea [*Vigna unguiculata* (L.) Walp] var. Arka garima. Indian Journal of Agricultural Research, 48 (4): 247-257.

Nedumaran, S., Abinaya, P., Jyosthnaa, P., Shraavya, B., Rao, P. and Bantilan, C. (2015). Grain Legumes Production, Consumption and Trade Trends in Developing Countries. ICRISAT, Telangana, India. pp. 64.

Nurmansyah, Alghamdi, S.S., Migdadi, H.M. and Farooq, M. (2018). Morphological and chromosomal abnormalities in gamma radiation-induced mutagenized faba bean genotypes. International Journal of Radiation Biology. 94: 174-185.

Nurmansyah, Alghamdi, S.S., Migdadi, H.M. and Farooq, M. (2019). Novel inflorescence architecture in gamma radiation-induced mutagenized faba bean genotypes. International Journal of Radiation Biology. 95(12): 1744-1751.

Nurmansyah, Alghamdi, S.S., Migdadi, H.M., Khan, M.A. and Afzal M. (2020a). AFLP-based analysis of variation and population structure in mutagenesis induced faba bean. Diversity. 12 (8): 1-14.

Nurmansyah, Alghamdi, S.S. and Migdadi, H.M. (2020b). Morphological diversity of faba bean (*Vicia faba* L.) M_2 mutant populations induced by gamma radiation and diethyl sulfate. Journal of King Saud University-Science. 32: 1647-1658.

Oliveira, H.R., Tomás, D., Silva, M., Lopes, S., Viegas, W. and Veloso, M.M. (2016). Genetic diversity and population structure in *Vicia faba* L. landraces and wild related species assessed by nuclear SSRs. Plos One. 11 (5): 1-18.

Paris, M., Bonnes, B., Ficetola, G.F., Poncet, B.N. and Després, L. (2010). Amplified fragment length homoplasy, *in silico* analysis for model and non-model species. BMC Genomics. 11 (287): 1-13.

Peakall, R. and Smouse, P.E. (2012). GenAlEx 6.5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research-An Update. Bioinformatics. 28 (19): 2537-2539.

Rubiales, D., Rojas-Molina, M.M. and Sillero, J.S. (2016). Characterization of resistance mechanism in faba bean (*Vicia faba*) against Broomrape species (*Orobance* and *Peliphance* spp.). Frontiers in Plant Science. 7 (1747): 1-8.

Shekar, G.C. and Pushpendra. (2017). Induced mutations in soybean (*Glycine max* L.). Legume Research. 40 (6): 1012-1019.

Sim, S.C., Robbins, M.D., Van Deynze, Michel, A.P. and Francis, D.M. (2011). Population structure and genetic differentiation associated with breeding history and selection in tomato (*Solanum lycopersicum* L.). Heredity. 106(6): 927-935.

Singh, P. and Sadhukhan, R. (2019). Ems and gamma radiation induced mutation in grasspea (*Lathyrus sativus* L.). Legume Research. 42(3): 300-307.

Wang, H., Zong, X., Guan, J., Yang, T., Sun, X., Ma, Y. and Redden, R. (2012). Genetic diversity and relationship of global faba bean (*Vicia faba* L.) germplasm revealed by ISSR markers. Theoretical and Applied Genetics. 124(5): 789-797.

Zeid, M., Schon C.C. and Link W. (2003). Genetic diversity in recent elite faba bean lines using AFLP markers. Theoretical and Applied Genetics. 107(7): 1304-1314.

Zong, X., Liu, X., Guan, J., Wang, S., Liu, Q., Paull, J.G. and Redden, R. (2009). Molecular variation among Chinese and global winter faba bean germplasm. Theoretical and Applied Genetics. 118(5): 971-978.