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High Quantitative Trait Variability in Faba Bean Mutagenized Population for High-yielding Breeding Program in Saudi Arabia

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ABSTRACT

Background: The narrow genetic base and environmental stresses are behind the low rate of faba bean yield in the past two decades. Drought stress is one of the most destructive abiotic stresses. Using induced mutagenesis on locally adapted landrace cultivar is proposed to cope with this problem.

Methods: This study was conducted on faba bean landrace cultivar of Saudi Arabia, namely Hassawi 2, treated by 25 and 50 Gray gamma radiation. The genetic diversity assessment of M_2 mutant populations was based on seven quantitative traits and nine Amplified Fragment Length Polymorphism (AFLP) primer combinations.

Result: A total of 3419 M_2 seeds were planted, of which 2782 (81.4%) seeds germinated and 2658 plants survived. A 5 to a 10-fold range of quantitative traits studied among mutant plants compared to control plants showed high variability. The number of pods per plant and seeds per plant was a valid selection criterion for a high-yielding faba bean breeding program. Nine AFLP primer combinations generated 1079 polymorphic alleles from 88 samples that comprised mutant and control plants. Shannon index (I) and expected heterozygosity (He) were 0.337 and 0.206, respectively. The AFLP results validated high variability in M_2 populations. These findings will assist faba bean breeders in developing high-yielding cultivars with drought stress tolerance.

Key words: AFLP, Genetic diversity, Landrace cultivar, Mutagenesis, *Vicia faba*.

INTRODUCTION

Faba bean (*Vicia faba* L.) is the fourth most produced cool-season legume worldwide with a total production of 5.432 million tons on a harvested area of 2.577 million ha (The Food and Agriculture Organization Corporate Statistical Database 2020). However, a meager growth rate of faba bean's yield recorded in the last three decades. Nedumaran *et al*. (2015) reported the growth rate of faba bean's production and harvested from 1994 to 2010 was 1.4 and 0.6%. The growth rate of faba bean's yield was only 0.8%, which was the lowest among legume crops. This low rate maybe because of the narrow genetic basis of faba bean, in China, as the largest producer (Gong *et al*. 2011) and the unstable faba bean yield is also because of abiotic and biotic stresses (Maalouf *et al*. 2018).

Drought stress is one of the most destructive abiotic stresses in arid and semi-arid regions. It decreased crop production more than any other abiotic stresses (Lambers *et al*. 2008). The Kingdom of Saudi Arabia (KSA) is classified as a semi-arid region with low annual rainfall, high temperature and limited water reserves (Choudhury and Al-Zahrani 2013) that make drought stress significant in KSA. Faba bean is the third most imported pulse crop in the KSA after chickpea and lentil. It cost 61 million SAR for 48,888 tons of dried faba bean in 2017 (General Authority for Statistics 2017). Faba bean cultivation in KSA faces more challenges because of faba bean sensitivity to water stress (Migdadi *et al*. 2016). Therefore, developing a high-yielding faba bean cultivar with drought stress tolerance is the crucial goal for faba bean breeders in KSA and worldwide to increase drought events as an impact of climate change.

Crop wild relatives and landraces reported having better abiotic stress tolerance and biotic resistance to assist plant breeders in developing better cultivars (Redden 2013). Landrace cultivars will play a significant role in the breeding program because of their adaptation to specific edaphic and climatic conditions (Casañas *et al*. 2017). However, as there is no successful interspecific crossing between cultivated species of faba bean and its wild relatives as it was the case of other legume crops (Maalouf *et al*. 2018). The exploitation of landrace cultivars is a workable option for developing abiotic stress tolerance cultivar is. Hassawi 2 is a faba bean landrace cultivar from KSA that reported having drought stress tolerance and adapting to the KSA environment (Migdadi *et al*. 2016).

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The present study was conducted to broaden the genetic diversity of faba bean, using induced mutagenesis in Hassawi 2 and to assess genetic diversity for quantitative traits and Amplified Fragment Length Polymorphism (AFLP)-based molecular marker. Induced mutagenesis has its utility in increasing genetic diversity and shortening breeding programs compare to conventional crossing-based breeding.

MATERIALS AND METHODS

Plant materials

Two batches were prepared from Hassawi 2 seeds to generate M_1 seeds, each batch comprised 120 healthy dried seeds. The two batches irradiated by 25 and 50 Gray (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, Saudi Arabia (Nurmansyah *et al*. 2018). The M_1 seeds were planted, harvested and allowed to generate the M_2 seeds that were then planted as M_2 families.

Experimental site and crop cultivation

The experiments were carried out at the Education Farm, King Saud University, Riyadh, Saudi Arabia for M_1 generation and at Dirab Agricultural Research and Experiment Station, South Riyadh (24° 43′ 34″ N, 46° 37′ 15″ E) for M_2 generation during 2019 growing season. The M_1 seeds were planted in two plots that represented two different treatments and untreated seeds (control) were planted in a separate plot. In the M_2 generation, the M_2 seeds are sown as M_2 families in 22 plots. The plot size was 4 m × 4 m, with nine rows with a 45 cm distance between two rows and a 20 cm distance between two plants. The crop cultivation followed, as described by Nurmansyah *et al*. (2019).

Data collection of quantitative traits

Seedling emergence (SE) and the SE percentage recorded three weeks after sowing, based on the total number of seeds sown. Plant survival (PS) is recorded at the maturity stage and the percentage of PS estimated based on SE's total number. The survived mutant plants measured and recorded for seven quantitative traits *viz.* number of branches per plant, number of pods per plant, number of pods per branch, number of seeds per plant, number of seeds per pod, 100-seed weight (g) and seed weight per plant (g).

Molecular characterization

Leaves of 88 plants, derived from 65 mutant plants induced by 25 Gy, 22 mutant plants induced by 50 Gy and one control plant, were collected and stored at -80°C until DNA extraction. DNA extraction was performed using an SDS protocol. Nine AFLP primers combination was used to estimate genetic diversity among faba bean mutant's lines. The AFLP plant mapping protocol from Applied Bio-systems (ABI) (Waltham, MA, USA) used in this study with some modification as described by Nurmansyah *et al*. (2020).

Genetic diversity parameters

The total number of alleles, the number of bands, the average number of bands per sample and the average number of bands per allele were recorded for each primer. The presence/absence binary matrix was used for further analysis with GenAlEx 6.503 complement for MSExcel (Peakall and Smouse 2012). 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 (Hammer *et al*. 2001) used to assess genetic diversity among the 88 mutant plants and the control plants.

RESULTS AND DISCUSSION

Seedling emergence and plant survival

It was observed in M_1 generation that exposing seeds to gamma radiation reduced seedling emergence (SE) and plant survival (PS). The percentage of SE 75.8% and 22.5% was recorded in seeds treated by 25 Gy and 50 Gy gamma radiations, respectively. Plant survival percentage was 84.6% and 81.5% for 25 Gy and 50 Gy gamma radiations treatments, respectively. In M_2 generation, 3419 M_2 seeds were planted, of which 2782 (81.4%) seeds germinated and 2658 (95.5%) plants survived. Based on the treatments used, 25 Gy generated 79.9% of SE and 95.9% of PS, while 50 Gy showed SE and PS of 87.3% and 94.0%, respectively (Table 1).

Quantitative trait variations

Mean performance and range of the mutant plants compared to the control plants for seven quantitative traits present in Table 2. The mean performance for these traits in the mutant plants decreased compared to the control plants, except for the number of seeds per pod and 100-seed weight. However, the mean values of the number of seeds per pod and 100-seed weight were not different from the control plant. The mean performance of quantitative traits in mutant plants compared to control plants varied. Some traits had lower mean values than the control plants, while the others did not differ. Similar results in variation by comparing mean values for some quantitative traits between mutant populations and control plants in the M2 and M3 generation of lentils (Laskar and Khan 2017), in Mungbam (Dewanjee and Sarkar 2018), in cowpea (Nair and Mehta 2014; Bind and Dwivedi 2014) and in grasspea (Singh and Sadhukhan 2019) induced by gamma radiation.

The results also showed a wide range of variation in the mutant plants' seven quantitative traits compared to those of the control plants. Hassawi 2 mutant population had a more comprehensive range of all quantitative traits than control plants; the number of branches per plant (18 vs. 3), number of pods per plant (71 vs. 12), number of pods per branch (20.90 vs. 2.14), number of seeds per plant (220

Table 1: Seedling emergence and plant survival of the M_1 and M_2 generations of mutagenized faba bean populations.

Generation	Treatment	TS	SE	% SE	PS	% PS
M_1	25 Gy Gamma	120	91	75.8	77	84.6
	50 Gy Gamma	120	27	22.5	22	81.5
	Total	**240**	**118**	**49.2**	**99**	**83.9**
M_2	25 Gy Gamma	2748	2196	79.9	2107	95.9
	50 Gy Gamma	671	586	87.3	551	94
	Total	**3419**	**2782**	**81.4**	**2658**	**95.5**

TS: Total seed planted, SE: Seedling emergence, PS: Plant survival.

Table 2: Mean performance and range of seven quantitative traits in control and mutant population.

Quantitative traits	Control				Mutants			
	$\bar{x}\pm s$	Min	Max	Range	$\mu \pm \sigma$	Min	Max	Range
NOB	5.29±1.25	4.00	7.00	3.00	$5.22^{ns}\pm2.35$	1.00	19.00	18.00
NOP	18.71±4.15	13.00	25.00	12.00	13.66*±9.60	1.00	72.00	71.00
NPB	3.61±0.75	2.86	5.00	2.14	2.82*±1.85	0.10	21.00	20.90
NOS	47.86±8.63	37.00	61.00	24.00	37.13*±26.81	1.00	221.00	220.00
NSP	2.60±0.33	2.00	3.00	1.00	$2.73^{ns}\pm0.54$	1.00	6.00	5.00
100-SW	84.28±11.27	70.84	99.16	28.32	$85.98^{ns}\pm19.32$	26.00	254.88	228.88
SWP	40.24±8.74	30.46	56.52	26.06	31.68*±23.46	0.45	176.03	175.58

$\bar{x}$: sample mean of control plant, s: standard deviation of control plant, μ: population mean, σ: standar deviation of population, NOB: number of branches, NOP: number of pods, NPB: number of pods per branch, NOS: number of seed per plant, NSP: number of seeds per pod, 100-SW: 100 seed weight, SWP: seed weight per plant, ns: no significant difference, *: significant at $p \leq 0.05$, **: significant at $p \leq 0.01$ using Mann Whitney U test.

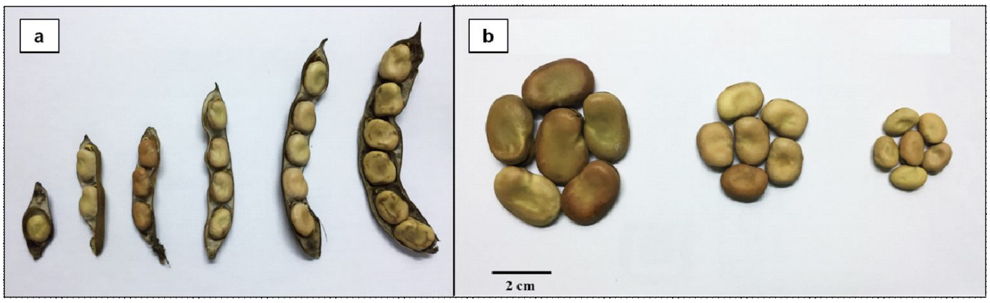

Fig 1: (a) Number of seeds per pod variations, (b) Seed size variations.

vs. 24), number of seeds per pod (5.00 vs. 1.00), 100-seed weight (228.88 g vs. 28.32 g) and seed weight per plant (175.58 g vs. 26.06 g). Fig 1 shows the variation in the number of seeds per pod and seed size.

Correlation among quantitative traits

Character association through correlation analysis was done to determine effective selection criteria for further generation. Selection criteria are importantly needed for successful long-term breeding programs. In this study, the pedigree method was used and massive data was collected. Significant positive correlation values were detected for seed weight per plant and six quantitative traits (Table 3). However, based on rs' value, a strong positive correlation was recorded between seed yield per plant with the number of pods per plant and number of seeds per plant. These results are also in line with the results reported in other faba bean studies where the number of pods per plant has a significant positive correlation with seed yield (Hamza 2017). Therefore, selection based on these traits can be valid for developing a high-yielding cultivar.

Assessment of genetic diversity based on AFLP molecular marker

Using nine AFLP primer combinations, 1079 alleles were generated from 88 samples (Table 4). Number of alleles ranged from 68 for primer combination (*Eco*RI/*Mse*I) TC/CAG to 210 for primer combination TG/CTT. Nine primer combinations generated 24,946 bands across the mutants and control with an average of 2,771.78 bands per primer combination. An average of 31.50 bands per sample recorded across the samples ranged from 16.48 bands for the primer combination TC/CAG to 58.42 bands for the primer combination TG/CTT. Simultaneously, the average bands per allele ranged from 18.23 to 30.24, with an average of 23.07 bands. All the primer combinations had an average

Table 3: Spearman's coefficient correlation of seven quantitative traits in two mutant populations.

Characters	NOB	NOP	NPB	NOS	NSP	100-SW
NOP	0.390**	-				
NPB	-0.252**	0.751**	-			
NOS	0.371**	0.962**	0.725**	-		
NSP	-0.011	-0.002	0.002	0.239**	-	
100-SW	0.039*	-0.003	-0.030	-0.025	-0.085**	-
SWP	0.367**	0.922**	0.688**	0.951**	0.207**	0.250**

*: Correlation is significant at the 0.05 level, **: Correlation is significant at the 0.01 level, NOB: number of branches, NOP: number of pods, NPB: number of pods per branch, NOS: number of seed per plant, NSP: number of seeds per pod, 100-SW: 100 seed weight, SWP: seed weight per plant.

Table 4: Characteristics of nine AFLP primers selected of 88 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	210	5141	58.42	24.48	0.99	19.46
TC/CCA	138	2516	28.59	18.23	0.99	12.79
TA/CCA	130	3531	40.13	27.16	0.99	12.05
TA/CAG	123	2697	30.65	21.93	0.99	11.40
TC/CAC	121	2678	30.43	22.13	0.98	11.21
TT/CAC	116	2680	30.45	23.10	0.98	10.75
AA/CCC	87	1652	18.77	18.99	0.98	8.06
AA/CCT	86	2601	29.56	30.24	0.98	7.97
TC/CAG	68	1450	16.48	21.32	0.97	6.30
Total	1079	24946	-	-	-	100
Average	119.89	2771.78	31.50	23.07	0.98	11.11

PIC: Polymorphism Information Content, DP: Discrimination Power.

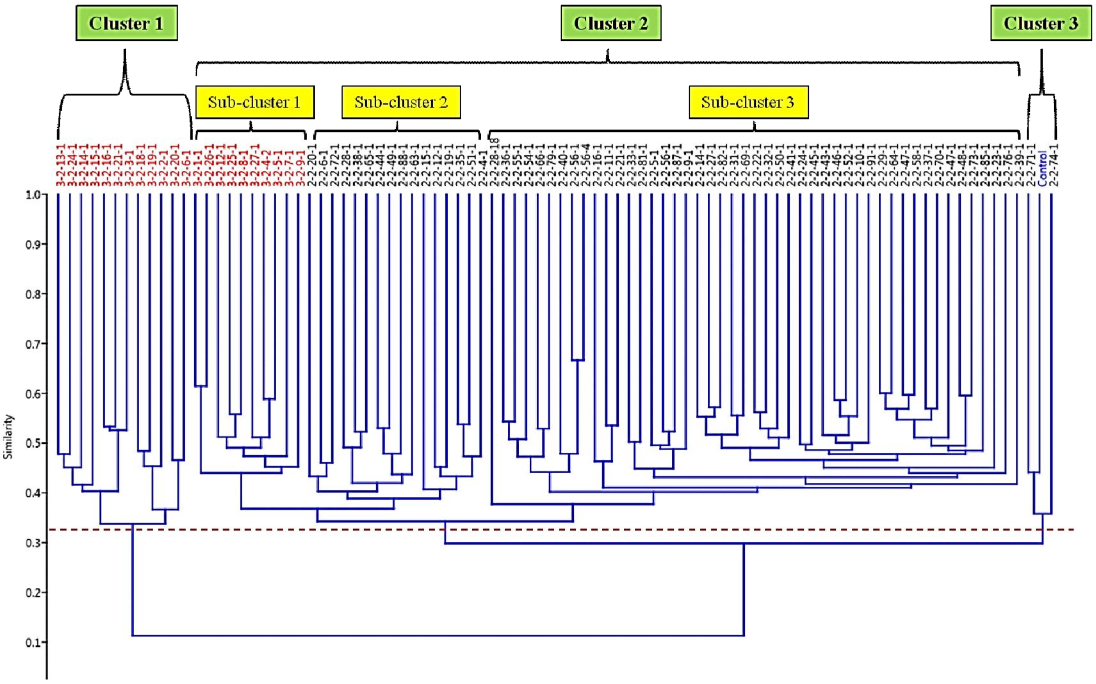

Fig 2: Dendrogram of 88 samples from AFLP markers by Jaccard's coefficient and the UPGMA clustering method. Red font color: 50Gy-gamma radiation-induced mutant plant, black font color: 25Gy-gamma radiation-induced mutant plant, blue font color: control plant.

of 0.98 of polymorphism information content (PIC). Discrimination power (DP) of all the primer combinations ranged from 6.30% for TC/CAG primer combination to 19.46% for TG/CTT primer combination.

Analysis of genetic diversity parameters showed the mean number of different alleles (Na) and the number of effective alleles (Ne) was 1.998 and 1.319, respectively. The mean values of Shannon index (I) and expected heterozygosity (He) were 0.337 and 0.206, respectively. Jaccard's genetic similarity pair-wise among the 88 mutant plants, along with control plants, ranged from 0.06 to 0.67, with an average value of 0.32. UPGMA dendrogram (Fig 2) showed the genetic relationship among tested lines using nine AFLP primers. Three clusters were generated with a mean genetic similarity value of 0.32. They considered cluster one as a 50Gy-gamma radiation-induced group that comprised 12 mutant plants. Cluster two was the central cluster with 73 mutant lines and cluster three compassed the control plant and two mutant plants of 25Gy-gamma radiation-induced.

The genetic diversity assessment using AFLP markers showed mutant lines generated by gamma radiation had high diversity. The mean value of expected heterozygosity (He) was 0.206, which was lower than the value reported by El-Esawi (2017) with He = 0.271. El-Esawi (2017) assessed the genetic diversity of 35 faba bean germplasm originated from six countries. Based on the pair wise Jaccard's genetic similarity index, the average genetic similarity value (0.32) was lower than that of Ammar *et al*. (2015) with 0.37. It showed that our mutant lines are more diverse than faba bean germplasm tested by Ammar *et al*. (2015). The UPGMA dendrogram showed that the 88 samples were divided into three clusters.

Interestingly, it also revealed that the 50 Gy-gamma radiation-induced mutant plants gathered in two groups separated from the 25 Gy-gamma radiation-induced mutant plants. The first group was in cluster 1 and the second group was in the subcluster of cluster 2. This separation explained that the number of mutations that occurred depends on the level or dose of the treatment and the mutant lines treated with the same level of treatment had a relatively similar number of mutations. Li *et al*. (2016) assessed the mutational rate of M_2 rice plants treated by a different dose of gamma rays using whole-genome sequencing. He found that the mutational rate was relatively similar to plants irradiated with different gamma rays' doses, suggesting that a lower dose than that commonly used could be applied.

CONCLUSION

Induced mutagenesis using gamma radiation increased the genetic diversity for the quantitative traits in the population developed from faba bean landrace cultivar, Hassawi 2. It was validated by genetic diversity assessment using AFLP markers. The quantitative traits with a wide range of genetic variation are valuable resources for the further breeding program for developing high yielding cultivar with drought stress tolerance.

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REFERENCES

Ammar, M.H., Alghamdi, S.S., Migdadi, H.M., Khan, M.A., El-Harty, E.H. and Al-Faifi, S.A. (2015). Assessment of genetic diversity among faba bean genotypes using agro-morphological and molecular markers. Saudi Journal of Biological Sciences. 22: 340-350.

Bind, D., Dwivedi, V.K. (2014). Effect of mutagenesis on germination, plant survival and pollen sterility in M1 generation of cowpea [*Vigna unguiculata* (L.) Walp]. Indian Journal of Agricultural Research. 48(5): 398-401.

Casañas, F., Simó, J., Casals, J. and Prohens, J. (2017). Toward an evolved concept of landrace. Frontiers in Plant Science. 8: 1-7.

Chowdhury, S. and Al-Zahrani, M. (2013). Implications of climate change on water resources in Saudi Arabia. Arabian Journal for Science Engineering. 38: 1959-1971.

Dewanjee, S., Sarkar, K.K. (2018). Evaluation of performance of induced mutants in mungbean [*Vigna radiata* (L.) Wilczek]. Legume Research. 41(2): 213-217.

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.

General Authority for Statistics. (2017). Kingdom of Saudi Arabia Imports Statistics 2017. Available at: https://www.stats.gov.sa/sites/default/files/imports_bulletin_2017.pdf. Accessed on October 18, 2020.

Gong, Y., Xu, S., Mao, W., Li, Z., Hu, Q., Zhang, G. and Ding, J. (2011). Genetic diversity analysis of faba bean (*Vicia faba* L.) based on EST-SSR markers. Agricultural Sciences in China. 10: 838-844.

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

Hamza, F.E.A. (2017). Performance assessment, genetic variability, heritability, genetic advance and correlation coefficient analysis for yield and some agro-morphological traits in faba bean (*Vicia faba* L.) genotypes in the Northern State, Sudan. International Journal of Current Microbiology and Applied Sciences. 6: 1206-1214.

Laskar, R.A. and Khan, S. (2017). Assessment on induced genetic variability and divergence in the mutagenized lentil populations of microsperma and macrosperma cultivars developed using physical and chemical mutagenesis. Plos ONE. 12: 1-18.

Li, S., Zheng, Y., Cui, H., Fu, H., Shu, Q. and Huang, J. (2016). Frequency and type of inheritable mutations induced by γ rays in rice as revealed by whole genome sequencing. Journal of Zhejiang Univ-Sci B (Biomed and Biotechnol). 17: 905-915.

Maalouf, F., Hu, J., O'Sullivan, D.M., Zong, X., Hamwieh, A., Kumar, S. and Baum, M. (2018). Breeding and genomics status in faba bean (*Vicia faba* L.). Plant Breeding. 138: 465-473.

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.

Nair, R., 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, 64pp

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. and Migdadi, H.M. (2019). 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.

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

Peakall, R. and Smouse, P.E. (2012). GenAlEx 65: Genetic Analysis in Excel Population Genetic Software for Teaching and Research-An Update. Bioinformatics. 28: 2537-2539.

Redden, R. (2013). New approaches for crop genetic adaptation to the abiotic stresses predicted with climate change. Agronomy. 3: 419-432.

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