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Research Article
Evaluation of Yield Attributing Variants Developed Through Ethyl Methane Sulphonate in an Important Proteinaceous Crop-*Vicia faba*

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Abstract

Background and Objective: In present situation of variable natural environment and high population, sustainable increase in agricultural productivity is highest preference. Induced mutagenesis known to be significant tool for generating gene variation without affecting genomic make up of crop and provide cogent accomplishment in crop improvement programme. This study was aimed to find out the response of Ethyl Methane Sulphonate (EMS) on *Vicia faba* with a view to determine the effect of chemical mutagen on biological and cyto-morphological parameters in M_1 generations. **Material and Methods:** The effect of Ethyl Methane Sulphonate (EMS) was studied in *Vicia faba* L (2n = 12). Seeds were subjected to mutagenic treatments with 0.1, 0.25, 0.50 and 0.75% dose of ethyl methane sulphonate to develop viable variants in M_1 generation. Different traits of variant were screened time to time and contrasted with untreated plants. Statistical analysis viz, mean ($\bar{x}$), Standard Deviation (SD), Coefficient of Variation (CV %) were done to assess the intra and inter-population (mutagen) variations in different quantitative traits. **Results:** Three variants viz dwarf (0.75% EMS), tall variant with high yield (0.50% EMS) and bushy variants with high yield (0.25% EMS) were isolated from the M_1 progeny of *Vicia faba*. Comparative observations were recorded for bio-physiological damages, morphological variations in shape, height and quantitative traits to assess the genetic response of the variants plants toward the different concentrations of chemicals. Different cytological anomalies such as univalents, multivalents, stickiness, laggards, bridges, stray, multinucleate condition were also observed in all variants. **Conclusion:** The moderate dose of EMS showed notable diminution in the biological damages while accelerating the rate of desirable high-yielding variants had proved to be economical. The segregate of the selected variants in future generations will definitely contribute to the improvement of *Vica faba* genotype and these variants may be used as valuable breeding stocks for *Vicia faba* breeding.

Key words: Chemical mutagenesis, *Vicia faba*, quantitative traits, high yielding variants, ethyl methane sulphonate, cytological aberrations

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Demand on mutation breeding to contribute to sustainable global food security and livelihood is increased tremendously in recent times. Several morphological mutants have been found and utilized in faba bean improvement as well as in linkage studies[1]. It is investigated that legumes generally loose different alleles for high productivity, seed quality, pest and disease resistance during the processes of adaptation to environmental stress.

In most of the pulse crops, the variability found is considerably low due to the self-pollination[2]. To manage this problem, mutation breeding is one of the most effective and important tools to induce desirable variability in different qualitative and quantitative characters of crop species. Several researchers demonstrated that genetic variability for many desired characters can be successfully induced through mutations and its practical value in plant improvement programs has been well established. For enhancing genetic variability, Seed mutagenesis has been used for yield parameters[3,4] and the induction of chlorophyll mutations[5] and morphological mutations[6-8]. Mutagenesis is a potent method which has been widely used for improving and enhancing genetic variability of crops and the main advantage of this, to improve one or a few characters without changing the rest of the genotype.

Vicia faba 2n = 12 (fabaceae) valuable protein-rich food and animal feed in developing countries[9], native to North Africa, South West and South Asia and extensively cultivated else and common breakfast food in Mediterranean region, Middle region, China and Ethiopia. Inspite of protein, *V. faba* contain various types of vitamins, ions like calcium, iron, magnesium, potassium and phosphorus and are rich in tyramine and thus should avoid by those taking monoamine oxidase inhibitor. Raw broad beans also contain vicine and convicine which can induce hemolytic anemia in patients.

Unique defense responses are produced by the plants when it is subjected to any type of chemical stress (Alkylating agent) to counter balance the effects of that mutagen aggression for its normal growth and development[10]. In broad sense the higher concentrations of various toxic chemicals lead to the enhanced stimulatory production of oxidants, Reactive Oxygen Species (ROS) in plants which are highly reactive, toxic and halt the normal processes in plants like, synthesis of proteins, carbohydrates, lipids and DNA ultimately resulting in oxidative stress[11]. Biological effects which are induced by moderate doses of mutagens can be decreased by treating a cell with mild doses of the same or even other chemicals. In crop improvement, chemical mutagenesis is a valuable tool and Ethyl Methane Sulphonate (EMS) is a powerful mutagen that causes point mutations in the genomes of plants by producing AT>GC base pair transition. The chemo mutagens obtained from EMS show broad variation in morphological, cytological and yield characters as compared to normal plants[12].

From this background idea, the present investigation was undertaken to identify the effect of different concentrations of Ethyl Methane Sulphonate (EMS) causing maximum morphological variants in M_1 generation of *Vicia faba* and to identify the expression level of induced novel genes or new null alleles of genes concern in the morphogenesis of plant and to obtain the feasible morphological mutants in relation to other agronomic traits in the screened M_2 faba individuals from the progeny of M_1 parents

MATERIALS AND METHODS

A variety of *Vicia faba* germplasm was obtained from IARI New Delhi, India. Fresh aqueous stock solutions of EMS (1% v/v, respectively) manufactured by Sissco Research Laboratories Pvt. Ltd, Mumbai, India, were prepared in phosphate buffer at pH 7.0. The pH of the solution was maintained using buffer tablets (MERCK manufactures, Mumbai, India). The fresh, healthy and uniform seeds were pre soaked in distilled water for 12 h and then treated with 5 different concentration (0.1, 0.25, 0.50, 0.75 and 1.0 %) of stock solution of Ethyl Methane Sulphonate (EMS) for 12 h with intermittent shaking at room temperature of 25±2°C. After treatment, the seeds were thoroughly washed in running tap water for 30 min to remove the excess of mutagen. Thoroughly washed 100 seeds were sown in four replicates of each treatment of the mutagen as well untreated seed in earthen pots filled with soil manure and kept in the Net House of the Department of Botany, Aligarh Muslim University during the Rabi season of the year 2013-2014 to raise M_1 generation. In M_1 generation, breeding behavior was observed and different agronomic traits viz., plant height, Number of branches per plant, pods per plant, seeds per pod, 100 seed weight and total yield/plant were evaluated. To determine pollen fertility (%), staining the pollen grain with acetocarmine and glycerin solution (1:1) and five slides per treatment were observed. The pollen grains stained as uniform deep red colors were counted as fertile and others as sterile. For meiotic studies, young flower buds from control as well as variant

plants were selected and also fixed in freshly prepared Carnoy's fluid (absolute alcohol: chloroform: acetic acid in 6:3:1 v/v ratio) for 24 h and preserved in 70% alcohol. Anthers from the collected flower buds were squashed in 1% acetocarmine for staining and made permanent slide through an NBA-GAA series, mounted in Canada balsam and dried at 45°C. Microphotographs were taken from freshly prepared slides using a X30 Olympus Research photomicroscope. For meiotic studies 275 pollen mother cells in control whereas 268, 272 and 260 pollen mother cells are observed in dwarf variant, tall variant with high yield and bushy variant with high yield respectively. Total 228 plants were screened in M_1 and selected the variants from it, which deviate from the control. The treated as well as control populations were carefully screened for morphological variants s throughout the growth period in M_1 generations. Some formulas[13] were used for calculating germination (%) in M_1 and frequency of meiotic abnormalities in different plants (variants) of M_1 generation[13]:

$$\text{Germination (\%)} = \frac{\text{No.of seed germinated}}{\text{No. of seed sown}} \times 100 \quad (1)$$

$$\text{Frequency of meiotic abnormality (\%)} = \frac{\text{Total no. of abnormal PMC's of particular treatment}}{\text{Total no. of PMC's observed in that particular treatment}} \times 100 \quad (2)$$

Statistical analysis: Statistical analysis, namely, mean ($\bar{x}$), Standard Deviation (SD), Coefficient of Variation (CV %) were done to assess the intra and inter-population (mutagen) variations in different quantitative traits. Data collected for quantitative traits in M1 generation were subjected to statistical analysis in order to assess the extent of induced variation as indicated below:

Mean ($\bar{x}$)**:** The mean was computed by taking the sum of a number of values (X1, X2, ····· Xn) and dividing by the total number of values (N) involved[13], thus:

$$\text{Mean } (\bar{X}) = \frac{X1 + X + X\ldots\ldots\ldots\ldots + X}{N} \quad (3)$$

where, X_1, X_2, X_3·····X_n are observations and N is total number of observations involved.

Standard deviation (SD): The standard deviation was calculated by the following formula for each parameter of study[13]:

$$SD = \sqrt{\frac{(X1-\bar{X})^2 + (X2-\bar{X})^2 + (X3-\bar{X})^2 + \ldots\ldots\ldots\ldots + (Xn-\bar{X})^2}{n}} \quad (4)$$

$$SD = \sqrt{\frac{\sum(X1-\bar{X})^2}{n-1}} \quad (5)$$

where:

SD = Standard deviation

ΣX = Sum of all individual aberration i.e., $X_1+X_2+X_3+$·····X_n

$\bar{x}$ = Mean of all observation

n = No. of all observation

Coefficient of variation: It measures the relative magnitude of variation present in the observations relative to their magnitude of arithmetic mean. The following formula was applied to compute Coefficient of Variability (CV)[13]:

$$CV = \frac{\text{Standard deviation}}{\text{Arithmatic mean}} \times 100 \quad (6)$$

$$CV = \frac{SD}{\bar{X}} \times 100 \quad (7)$$

RESULTS

In the present investigation three variants namely (i) Dwarf, (ii) Tall variant with high yield and (iii) Bushy variant with high yield were isolated at 0.75, 0.50 and 0.25% concentration of EMS followed by selection. These plants were differentiated on the basis of height (cm), flower morphology, leaf character and yield character (Table 1). The untreated plant showed normal growth and yield used as control plant (Fig. 1a). Morphological features of induced variants are shown in (Fig.1b-d). The Dwarf variant was isolated at 0.75% EMS with short internodes, reducing the height as compared to the control (Fig.1b). The tall variant with high yield was isolated at 0.50% EMS treatment; it had long internodes, enhance the height and yield as compared to control (Fig. 1c). The bushy variant with high yield was isolated at 0.25% EMS and had increase number of branches that makes it bushy appearance (Fig. 1d). The plant height, number of branches per plant, number of pod per plant, number of seeds per pod, 100 seed weight and total yield of variants were increased in tall variant with high yield and bushy variant with high yield as compare to control plant while it decreased in dwarf variants

Fig .1(a-d): Morphological variants of *Vicia faba* selected in M_1 generation (a) Control, (b) Dwarf variant, (c) Tall variant with high yield and (d) Bushy variant with high yield

over control (Table 2). Germination percentage and pollen fertility was also found to be moderately affected i.e., it decreased in all variants comparing with control (Table 2).

Meiosis was found to be normal in control (untreated) plants, which formed 6 bivalents at metaphase I and normal separation (6:6) in the anaphase I cells (Fig. 2a), while the meiosis of selected variants exhibited various chromosomal aberrations. The most common type of abnormalities were observed such as multivalents at metaphase I, two stray bivalents at metaphase I, stickiness at anaphase I, bridge with fragment at anaphase I, laggard at anaphase I and broken bridge at anaphase I (Fig. 2b-g). Such meiotic abnormalities were present at all the stages diakinesis through anaphase and telophase stages of meiosis. In the present investigation the highest percentage of abnormal pollen mother cells were found in dwarf variant, followed by tall and bushy variant with high yield (Table 3).

DISCUSSION

The present study proved beneficial in inducing morphological variants isolated on selection of M_1 generations, including, plant height (tall and dwarf), growth habits (bushy). In hybridization programmes, the isolated variants might be useful to plant breeders as a source of many beneficial genes. In the present study, germination percentage reduced due to treatment of chemical mutagen and this reduction may be due to demolition of the activity of gibberellic acid, following the radiation treatment and metabolic disruption during germination. The percentage of germination is inhibited due to interaction between mutagen and the seed cell system or it may also be due to toxicity of mutagens followed by mutational changes at genic or chromosomal level.

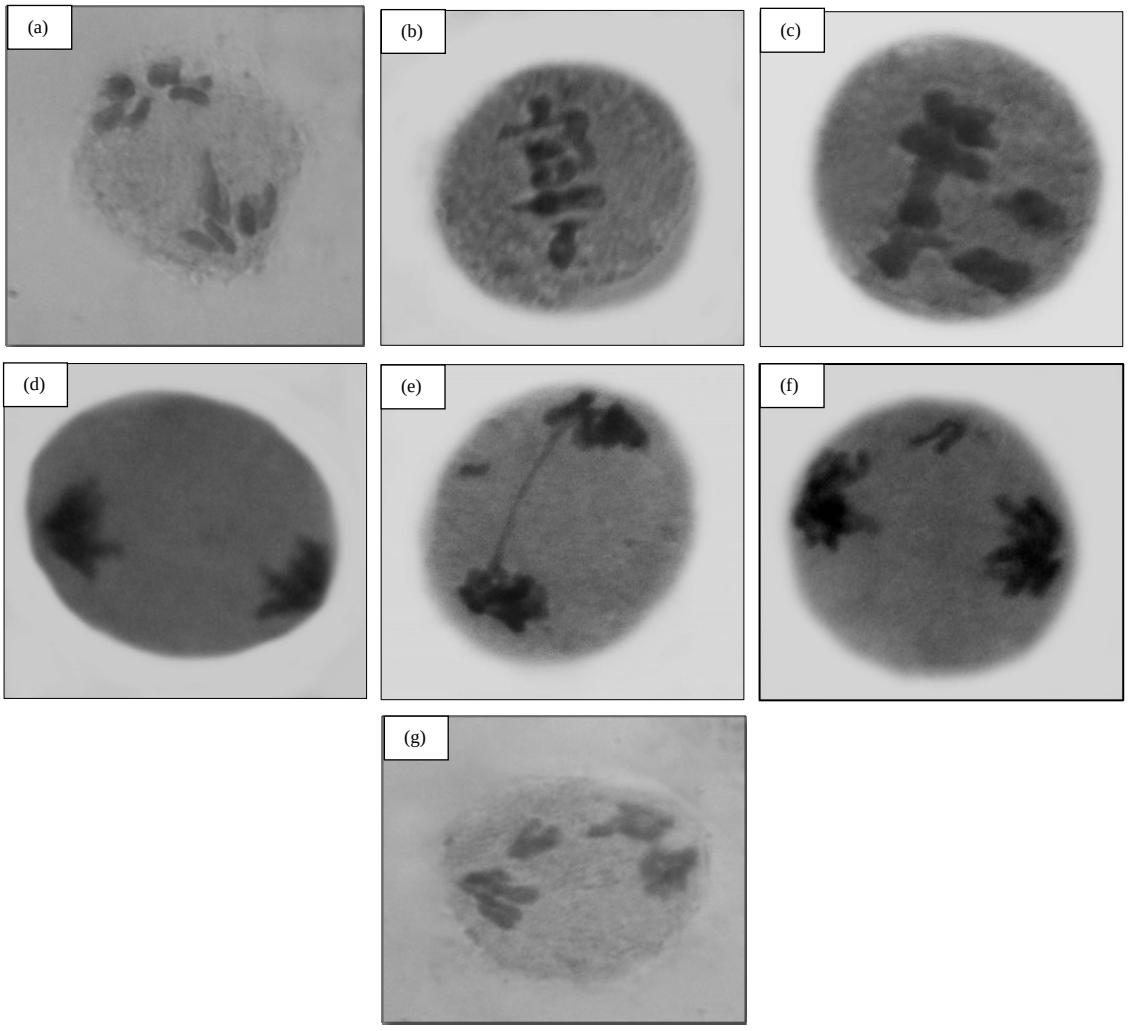

Fig. 2(a-g): Chromosomal aberration in *Vicia faba* induced by ethyl methane sulphonate treatment (a) PMC showing anaphase I (control), (b) PMC showing of multivalents at metaphase, (c) PMC showing two stray bivalents at metaphase I, (d) PMC showing sticky anaphase I, (e) PMC showing bridge with fragment at anaphase I, (f) PMC showing laggard at anaphase I and (g) PMC showing broken bridge at anaphase I

Table 1: Exo-morphological features of the selected variants of *Vicia faba*

Variant codes	Plant types	Mutagens	Salient features
A	Control	-	Normal plants bearing normal leaves and medium height and white flower with purplish blotches
B	Dwarf variant	0.75% EMS	Plant dwarf as compared to control with short internodes, normal leaf size and flower size
C	Tall variant with high	0.50% EMS	Tall plant with long internodes, normal leaf size and high yield
D	Bushy variant with high yield	0.25% EMS	Bushy plant with more branches, thick stem and high yield

The morphological changes that have been introduced in the variants through application of EMS are due to various changes in the structure of chromosomes as varying degree of chromosomal anomalies at different meiotic stages have been observed. The correlation of chromosomal aberrations with morphological changes and other characteristics of plants have also been reported by various researchers in different plants, using various chemical mutagens[14]. The dwarf variants with desynaptic behavior of chromosomes exhibited reduce plant height, seed yield and high pollen sterility. The reduction in plant height may be ascribed to reduction in number and length of internodes which could be the prominence of reduction in cell length and cell number, changes in gibberellic acids[15] and mitotic irregularities[16] are of the view

Table 2: Exo-morphological parameters of the selected variants of *Vicia faba* in M1 generation

Variants	Germination (%)	Plant height (cm)		No. of branches per plant		No. of pods per plant		No. of seeds per plant		100-seeds weight (g)	
		X±SD	CV	X±SD	CV	X±SD	CV	X±SD	CV	X±SD	CV
Control	100.00	36.44±3.11	8.530	2.00±0.707	35.35	4.00±0.75	18.75	3.25±0.66	20.30	41.84±1.17	2.74
Dwarf variant	66.66	35.41±5.00	14.12	2.08±0.39	18.75	3.66±0.91	24.86	2.83±0.73	25.79	39.64±1.75	4.41
Tall variant with high yield	80.00	38.66±5.13	13.99	2.25±0.33	14.66	4.10±0.90	21.95	3.00±0.71	23.66	42.28±1.63	4.40
Bushy variant with high yield	86.66	36.54±4.84	12.55	2.33±0.31	10.90	4.80±0.84	17.50	3.50±0.68	19.42	44.85±1.56	3.72

Table 3: Meiotic abnormalities as observed in the screened variants of *Vicia faba* L.

Control/variants	Total No. of M_1 plant	No. of variant plant	Frequency (%)	Total No. of pollen mother cells observed	Total No. of Abn. pollen mother cell observed	Diakinesis		Metaphase I/II			Anaphase I/II		Telophase I/II			Total % of Abn. PMCs Observed
						Unival Freq.	Multival	Precocious	Stray	Stickiness	Laggards	Bridge	Bridges	Disturbed polarity	Multinuc cond	
Control	65	0	0.00	275	0	0	0	0	0	0	0	0	0	0	0	0.00
Dwarf variant	49	2	4.08	268	21	0.74 (2)	0.37 (1)	1.11 (3)	0.74 (2)	0.74 (2)	0.37 (1)	0.74 (2)	0.74 (2)	0.00 (0)	0.74 (2)	7.78
Tall variant with high yield	61	5	8.19	272	20	0.36 (1)	0.36 (1)	0.73 (2)	0.36 (1)	0.73 (2)	0.73 (2)	0.36 (1)	0.73 (2)	0.73 (2)	1.10 (3)	7.29
Bushy variant with high yield	53	3	5.66	260	15	0.38 (1)	0.00 (0)	0.38 (1)	0.76 (2)	0.76 (2)	1.15 (3)	0.76 (2)	0.76 (2)	0.00 (0)	0.38 (1)	5.71

that a single dominant mutation controls the dominant dwarf mutation in rice. Dwarf mutant has also been reported by several workers in lentil[17], blackgram[18], chickpea[19], grasspea[20] and in *Vigna* spp[21]. In the existing study, bushy variant with high yield isolated, however became the advantage that sunlight reached to all plant parts which increases its sink potential at the time of flowering and reproductive phase. Excellence in yield performance and its component characteristics were also contributed by the plant, forming the optimum angle between leaflets and stems and contributing to higher photosynthetic efficiency[22]. Bushy mutant also have been observed by khan et al in chickpea[18]. The present investigation exhibits, tall variants in M_1 generations which were also observed in blackgram[23] and lentil[24] using different mutagens. Many authors have reported that many morphological mutants like tall, dwarf and bushy are monogenic recessive[25].

The induced variants were associated with cytological abnormalities such as univalent, multivalent, bridges laggard, stickiness, stray, disturbed polarity etc. Univalent formation was found in all variant but more frequent in dwarf isolated from the EMS-treated populations. The occurrence of univalent and multivalent observed in broad bean at metaphase I[26] has also been reported in other plants such as barley[27]. The prevalence of univalents may be due to the induction of structural changes at the chromosome and/or gene level and this may be responsible for the pairing disturbance among homologous chromosomes[28]. Multivalent formation can be attributed to partial irregular pairing between more than two chromosomes[29]. It was postulated to be consequence of defective functioning of one or two types of specific non histone proteins involved in chromosome organization which is needed for chromatid separation and segregation[28] or due to disturbance in cytochemical reactions[30]. Stickiness, observed in all variants, may be also due to damage of peripheral nuclear proteins, especially DNA topoisomerase II, which might interfere with chromosome segregation[31]. Stray chromosomes, at metaphase-I seem to be caused by spindle dysfunction and clumping of chromosomes[32]. The variants such as dwarf, tall and bushy also showed abnormality like laggard and bridges at anaphase I/II and telophase I/II. The laggards might be due to the disruption of spindle organization or due to delayed terminalization. Bridges with fragments were due to paracentric inversion, frequently observed in the present investigation and also have been reported in many other plants like fenugreek[33], in *Cichorium*[34] and in *Capsicum*[35]. The anaphasic bridges might be due to structural changes to

deficiency and translocation type, some of them surviving until the late telophase, indicative of their stability. Bridges observed seem to be due to non-separation of chiasma due to stickiness. Kumar and Gupta[36] reported that gene mutation or the direct action of a mutagen on the target protein responsible for chiasmata terminalization during diakinesis at meiosis-I, causes some structural defects in the protein which lead to their improper functioning, thus resulting in bridges. All these factors alone or together have resulted in the formation of non-viable gametes, which in turn considerably lower the pollen fertility but not lower enough to affect the yield[37,38].

This study revealed that the significant boost in correlation between yield components can be achieved through mutation breeding in faba bean. Overall, it was elucidated that the isolated variants were genetically of elite nature compared to the parents. Further, breeding for variant stability and trait expressivity of the elite phenotypes in the subsequent generation is recommended and required to establish novel farmer friendly cultivars.

CONCLUSION

The present investigation was undertaken to study the frequency and spectrum of morphological variants and cytological studies of these selected variants in M_1 generation of faba bean using treatments of EMS. Different morphological variants were isolated in M_1 population of faba bean. These variants involve traits affecting plant height (tall, dwarf), growth habit (bushy) and yield. Most of the useful doses of mutation were observed at 0.50, 0.75 and 0.25% EMS. The highest frequency of abnormal pollen mother cells was noted in the dwarf variants. Such variants are used for selecting diverse parents and monitoring the genotypic diversity periodically in the breeders working collection of *Vicia faba*.

SIGNIFICANCE STATEMENTS

This study discovers the high yielding variants plants through chemical mutagenesis and breeding program. This technique is widely used for improving and enhancing genetic variability of *Vicia faba* and the main advantage of this, to improve yield related traits without changing the rest of the genotype. This study will help the researcher to uncover the critical areas of mutation breeding program for crop improvement that many researchers were not able to explore. Thus by using micro and macro mutation, researchers need to broaden their narrowing genetic bases to boost the productivity and create a gene pool of numerous desirable traits of economically important crops.

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REFERENCES

1. Kharkwal, M.C., C. Cagirgan, T. Toker, M.M. Shah and H. Islam *et al*., 2010. Legume mutant varieties for food, feed and environmental benefits. Proceedings of the 5th International Food Legumes Research Conference and 7th European Conference on Grain Legumes, April 26-30, 2010, Antalya, Turkey, pp: 196.
2. Khursheed, S. and S. Khan, 2015. Cytology of morphological mutants of *Vicia faba* L. var. Vikrant. Annu. Res. Rev. Biol., 5: 366-371.
3. Jabeen, N. and B. Mirza, 2002. Ethyl methane sulfonate enhances genetic variability in *Capsicum annuum*. Asian J. Plant Sci., 1: 425-428.
4. Singh, G., P.K. Sareen, R.P. Saharan and A. Singh, 2001. Induced variability in mungbean (*Vigna radiata* (L.) Wilczek). Indian J. Genet., 61: 281-282.
5. Waghmare, V.N. and R.B. Mehra, 2001. Induced chlorophyll mutations, mutagenic effectiveness and efficiency in *Lathyrus sativus* L. Indian J. Genet. Plant Breed., 61: 53-56.
6. Sangsiri, C., W. Sorajjapinun and P. Srinivesc, 2005. Gamma radiation induced mutations in mungbean. Sci. Asia, 31: 251-255.
7. Lyakh, V.A. and V.A. Lagron, 2005. Induced mutation variability in *Linum grandiflorum* Desp. Mutat. Breed. Newslett. Rev., 1: 4-5.
8. Muthusamy, A., K. Vasanth and N. Jayabalan, 2005. Induced high yielding mutants in cotton (*Gossypium hirsuthum* L.). Mut. Breed. Newslett. Rev., 1: 6-8.
9. Zong, X., X. Liu, J. Guan, S. Wang, Q. Liu, J.G. Paull and R. Redden, 2009. Molecular variation among Chinese and global winter faba bean germplasm. Theoret. Applied Genet., 118: 971-978.
10. Azevedo, L., P.L.A. de Lima, J.C. Gomes, P.C. Stringheta, D.A. Ribeiro and D.M.F. Salvadori, 2007. Differential response related to genotoxicity between eggplant (*Solanum melanogena*) skin aqueous extract and its main purified anthocyanin (delphinidin) *in vivo*. Food Chem. Toxicol., 45: 852-858.

11. Gill, S.S., N.A. Khan and N. Tuteja, 2012. Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress (*Lepidium sativum* L.). Plant Sci., 182: 112-120.
12. Akhtar, A., M.Y.K. Ansari, A. Hisamuddin and M.I. Robab, 2012. Induced variations in quantitative traits by EMS and SA treatments in *Linum usitatissimum* L. Arch. Phytopathol. Plant Protect., 45: 667-671.
13. Khan, I.A. and A. Khanum, 2012. Fundamental of Biostatics. 3rd Rev. Edn., Ukaaz Publications, Hyderabad, Andhra Pradesh, India.
14. Kumar, G. and R. Tripathi, 2007. Anomalous nucleolar and chromosomal organization in induced phenodeviants of grasspea. Cytologia, 72: 345-350.
15. Hedden, P., 2003. The genes of the green revolution. Trends Genet., 19: 5-9.
16. Qin, R., Y. Qiu, Z. Cheng, X. Shan, X. Guo, H. Zhai and J. Wan, 2008. Genetic analysis of a novel dominant rice dwarf mutant 986083D. Euphytica, 160: 379-387.
17. Solanki, I.S. and B. Sharma, 2002. Induced polygenic variability in different groups of mutagenic damage in lentil (*Lens culinaris* Medik.). Indian J. Genet. Plant Breed., 62: 135-139.
18. Arulbalachandran, D. and L. Mullainathan, 2009. Chlorophyll and morphological mutants of black gram (*Vigna mungo* (L.) Hepper) derived by gamma rays and EMS. J. Phytol., 1: 236-241.
19. Khan, S. and K. Parveen and S. Goyal, 2011. Induced mutations in chickpea-morphological mutants. Front. Agric. China, 5: 35-39.
20. Talukdar, D. and A.K. Biswas, 2006. An Induced Internode Mutant in Grass Pea. In: Perspectives in Cytology and Genetics, Volume 12, Das, R.K., S. Chatterjee and G.C. Sadhukhan (Eds.). AICCG Publication, Kalyani, India, pp: 267-271.
21. Wani, M.R., S. Khan, M.I. Kozgar and S. Goyal, 2011. Induction of morphological mutants in mungbean (*Vigna radiata* (L.) Wilczek) through chemical mutagens. Nucleus, 48: 243-247.
22. Naik, B.S., B. Singh and C. Kole, 2002. A promising mungbean [*Vigna radiata* (L.) Wilczek.] genotype with high protein content and seed yield. Indian J. Genet., 62: 342-344.
23. Kumar, V., A.K. Sharma, V.P. Singh and M. Kumar, 2009. Characterization of Prebreeding Genetic Stocks of Urdbean (*Vigna mungo* L. Hepper) Induced through Mutagenesis. In: Induced Plant Mutations in the Genomics Era, Shu, Q.Y. (Ed.). Food and Agriculture Organization of the United Nation, Rome, Italy, ISBN-13: 9789251063248, pp: 391-394.
24. Solanki, I.S., D.S. Phogat and R.S. Waldia, 2004. Frequency and spectrum of morphological mutations and effectiveness and efficiency of chemical mutagens in Macrosperma lentil. Natl. J. Plant Improv., 6: 22-25.
25. Talukdar, D., 2009. Dwarf mutations in grass pea (*Lathyrus sativus* L.): Origin, morphology, inheritance and linkage studies. J. Genet., 88: 165-175.
26. Ishido, M. and M. Kunimoto, 2001. Regulation of cell fate by cadmium and zinc. J. Health Sci., 47: 9-13.
27. Kumar, G. and V. Singh, 2003. Comparative analysis of meiotic abnormalities induced by gamma rays and EMS in barley. J. Indian Bot. Soc., 82: 19-22.
28. Zeerak, N.A., 1991. Cytogenetical effects of gamma rays and ethyl methanesulphonate in brinjal (*Solanum melongena* L.). Cytologia, 56: 639-643.
29. Gaulden, M.E., 1987. Hypothesis: Some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenisis, 2: 357-365.
30. Jayabalan, N. and G.R. Rao, 1987. Gamma radiation induced cytological abnormalities in *Lycopersicon esculentum* Mill. var. pusa ruby. Cytologia, 52: 1-4.
31. Panda, B.B. and K.K. Panda, 2002. Genotoxicity and Mutagenicity of Metals in Plants. In: Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants, Prasad, M.N.V. and K. Strzalka (Eds.). Chapter 15, Springer, Netherlands, ISBN: 978-90-481-5952-9, pp: 395-414.
32. Bhat, T.A., S. Parveen and A.H. Khan, 2007. Meiotic studies in two varieties of *Vicia faba* L. (Fabaceae) after EMS treatment. Asian J. Plant Sci., 6: 51-55.
33. Srivastava, A. and K. Kapoor, 2008. Seed yield is not impaired by chromosome stickiness in sodium azide treated *Trigonella foenum-graecum*. Cytologia, 73: 115-121.
34. Jafri, I.F., A.H. Khan and M. Gulfishan, 2011. Genotoxic effects of 5-bromouracil on cytomorphological characters of *Cichorium intybus* L. Afr. J. Biotechnol., 10: 10595-10599.
35. Gulfishan, M., A.H. Khan, I. Haneef and T.A. Bhat, 2011. Genotoxic effect of diethyl sulphate in two varieties of *Capsicum annuum* L. Nucleus, Vol. 54.
36. Kumar, G. and P. Gupta, 2009. Induced karyomorphological variations in three phenodeviants of *Capsicum annuum* L. Turk. J. Biol., 33: 123-128.
37. Kakani, R.K., S.K. Singh, A. Pancholy, R.S. Meena, R. Pathak and A. Raturi, 2011. Assessment of genetic diversity in *Trigonella foenum-graecum* based on nuclear ribosomal DNA, internal transcribed spacer and RAPD analysis. Plant Mol. Biol. Rep., 29: 315-323.
38. Kumar, G. and P. Gupta, 2007. Mutagenic efficiency of lower doses of gamma rays in black cumin (*Nigella sativa* L.). Cytologia, 72: 435-440.