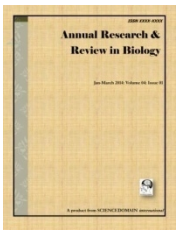

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Cytology of Morphological Mutants of *Vicia faba* L. var. vikrant

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Authors' contributions

This research work was carried out by two authors. Author S. Khursheed carried out the field experiment under the supervision of author S. Khan and drafted the entire manuscript. Author S. Khan finalized the manuscript with necessary corrections.

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ABSTRACT

Mutation breeding is an important tool in bringing genetic variability in plants. Different physical and chemical mutagens are used in mutation breeding to enhance variability. The present experiment was conducted to induce genetic variability in *Vicia faba* L. var. Vikrant, an autogamous crop, by gamma rays and ethyl methanesulphonate (EMS) used singly or in combinations. Some mutants were identified in M_2 generation which differed morphologically from the control plants. These mutants showed various chromosomal abnormalities. Higher combination treatment plants showed more frequency of meiotic abnormalities than individual doses/concentrations treated plants. Most of the desired mutants viz., bushy mutants, opposite leaves mutant and mutant with four flowers at a node were obtained in lower doses/concentrations of individual treatments whereas the higher combination treatments produced undesired mutants viz., unbranched mutant and one sided branched mutant.

Keywords: Vicia faba; gamma rays; EMS; chromosomal abnormalities.

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1. INTRODUCTION

India grows a large variety of pulses under different climatic conditions. The variability found in most of the pulse crops is considerably low due to the self-pollination. To cope this problem, mutation breeding is an important method to induce desirable variability in the plant. Mutagenesis brings novel changes in a plant in short duration of time than the conventional methods. The present experiment was conducted to study the cytology of morphological mutants isolated in M_2 population of *Vicia faba* L. var. Vikrant using single and combination treatments of ethyl methanesulphonate (EMS) and gamma rays. Morphological mutants are phenotypically visible, easily perceivable genetic alterations and include mutants isolated on the basis of altered form, phenotype or morphological architecture [1].

Vicia faba L., commonly known as faba bean or broad bean, is an important pulse crop. It belongs to family *Fabaceae* and has diploid chromosome number 12 (i.e., 2n=12). Faba Beans were a common food for many Mediterranean and Near East civilizations including the Egyptians, Greeks and Romans. The genotype of *Vicia faba* L. is homozygous due to the occurrence of self-pollination. Therefore, variation induced through mutation is an important method to reduce the homozygosity in the plant.

2. MATERIALS AND METHODS

Fresh and healthy seeds of *Vicia faba* L. var. Vikrant, obtained from IARI, New Delhi were used in this experiment. Seeds of faba bean were irradiated with four doses (100, 200, 300 and 400 Gy) of gamma rays from a Co^{60} source at N.B.R.I, Lucknow and 0.03 and 0.04 percent ethyl methanesulphonate for 6 hours at room temperature. Before chemical mutagenic treatment, seeds were pre-soaked in distilled water for 9 hours. EMS solution was prepared in phosphate buffer of pH 7. After chemical treatment, the seeds were thoroughly washed in running tap water to remove any residual effects of mutagen on seeds. Seeds were also treated with combined treatments of gamma rays and EMS (300 Gy + 0.03% EMS, 400 Gy + 0.04% EMS). 100- seeds were used for each treatment. The treated seeds were sown at the Agricultural Farm, Aligarh Muslim University, Aligarh during the rabi season of the year 2012 to raise the M_1 generation. The distance between seeds in a row and between the rows was kept 30×60 cms., respectively. M_1 plants were harvested individually and their seeds were sown in plant progeny rows to raise M_2 generation in the crop season of 2013. The M_2 generation was screened for different morphological mutants. Cytology was observed in these mutants. For cytological studies, flower buds of mutants and control were fixed in Carnoy's fluid (1 part Glacial acetic acid: 3 parts Chloroform: 6 parts Alcohol) for 24 hours. Traces of ferric chloride were added to improve the stainability of chromosomes. The flower buds were then washed with and preserved in 70% alcohol. Anthers were smeared in 1% acetocarmine solution. The pollen mother cells were examined at various stages of microsporogenesis. Photographs were taken from temporary preperations. The photographs were taken in 100X magnification of compound binocular microscope.

Some formulas were used for germination in M_1 and phenotypically different plants (mutants) in M_2 as:

Percentage germination (%) =

$$\frac{\text{No. of seeds germinated} \times 100}{\text{Total no. of seeds sown}}$$

Mutant frequency (%) =

$$\frac{\text{No. of mutants isolated in particular treatment} \times 100}{\text{Total no. of } M_2 \text{ plants of that particular treatment}}$$

Frequency of meiotic abnormality (%) =

$$\frac{\text{Total no. of abnormal PMC's of particular treatment} \times 100}{\text{Total no. of PMC's observed in that particular treatment}}$$

3. RESULTS AND DISCUSSION

Data recorded on the morphology and cytology of the mutants of *Vicia faba* in M_2 generation are presented in Tables 2 and 3.

3.1 Germination

Percentage germination was calculated in M_1 generation Table 1. As is clear from the data, the seed germination decreased with increase in doses/concentrations of both individual and combination treatments. Ananthaswamy et al. [2], described that the decrease in seed germination may be due to inhibition in physiological and biological processes necessary for seed germination.

Table 1. Effect of different doses/concentrations on seed germination of *Vicia faba* L. var. Vikrant in M_1 generation

Treatment	Percentage germination (%)
Control	94
100Gy	92
200Gy	89
300Gy	84
400Gy	83
0.03% EMS	82
0.04% EMS	81
300Gy+0.03% EMS	80
400Gy+0.04% EMS	79

Table 2. Frequency of various morphological mutants of *Vicia faba* L. var. Vikrant in M_2 generation

Mutants	Dose/Conc. of mutagen (gamma rays /EMS)	Total No. of M_2 plants	No. of mutants isolated	Frequency (%)
Bushy	100 Gy	52	3	5.76
	0.03%	98	8	8.16
One sided branches	400 Gy + 0.04%	38	2	5.26
Unbranched	300 Gy + 0.03%	40	1	2.50
Narrow leaves	0.04%	44	2	4.54
Opposite leaves	200 Gy	50	4	8.00
Mutant(four flowers at a node)	0.03%	50	4	8.00

Table 3. Frequency of meiotic abnormalities in various mutagenic treatments in *Vicia faba* L. var. Vikrant in M_2 generation

Treatment	Total No. of PMCs observed	Frequency of abnormal PMCs at different stages of meiosis					Total No. of abnormal PMCs	Total frequency (%)
		Metaphase		Anaphase	Telophase I/II			
		Fg	S	L	Tn	Dt		
Control	242	-	-	-	-	-	-	-
100 Gy	240	-	-	0.41	-	0.83	3	1.25
200 Gy	238	-	0.42	0.84	-	0.42	4	1.68
300 Gy	243	0.82	-	0.41	0.41	1.2	7	2.88
400 Gy	236	-	0.84	-	0.84	0.42	5	2.11
.03% EMS	243	0.41	-	0.82	0.41	1.2	7	2.88
.04% EMS	244	0.40	0.81	0.40	1.22	-	7	2.86
300Gy+.03%EMS	248	1.20	-	0.80	0.40	0.80	8	3.22
400Gy+.04%EMS	249	0.80	0.40	1.20	0.80	1.60	12	4.81

Fg: Fragment formation at Metaphse-I, S: Stickiness, I: Laggard, Tn: Trinucleate condition at telophase-II, Dt: disturbed telophase-I

3.2 Morphological Observations

Different morphological mutants, isolated in M_2 generation, included bushy, one sided branched, unbranched, narrow leaves, opposite leaves mutants and mutants with four flowers at a single node Plate 1. As shown in Table 2, the highest frequency of mutants was observed at 0.03% EMS and 200 Gy of gamma rays treatments. The Bushy mutants were observed in 100Gy gamma ray treated plants. The Bushy were also observed in 0.03% EMS treated plants. Several workers have reported that the bushy mutants were monogenic recessive [3]. One sided branched and unbranched mutants were observed mostly in higher combined treatments of gamma rays and EMS. Shimizu-Sato and Mori [4] observed that both hormones and genes have a role in determining the branching pattern of plants. The one sided branched mutants could be due to imbalance or defective synthesis of hormones. Leaves of faba bean are alternate, pinnate and consist of 2-6 broad leaflets. Narrow and opposite leaves mutants were observed with 0.04% EMS and 200 Gy of gamma ray treatments. During the mutagenic studies of

Capsicum [5] presence of needle like narrow leaves was noticed. The altered metabolism as a result of cellular damage may be one of the reasons for leaf abnormalities. Although most of the morphological mutants were uneconomical, nevertheless, some mutants can be used as a source of many beneficial genes in cross breeding programmes Khan et al. [6]. Such mutants might be either a result of pliotropic effects of mutated genes or chromosomal aberrations [7] and Wani et al. [6].

Plate 1. Morphological mutants

A: Control; B: Unbranched mutant (100 Gy gamma rays + 0.03% EMS); C: One sided branches mutant (200 Gy gamma rays + 0.04% EMS); D,E: Bushy mutants (100 Gy gamma rays + 0.03% EMS); F: Mutant with narrow leaves (0.04% EMS); G,H: Opposite leaves mutant (200 Gy gamma rays); I: Leaves of control plant; J: Mutant with four flowers at a node (0.01%EMS)

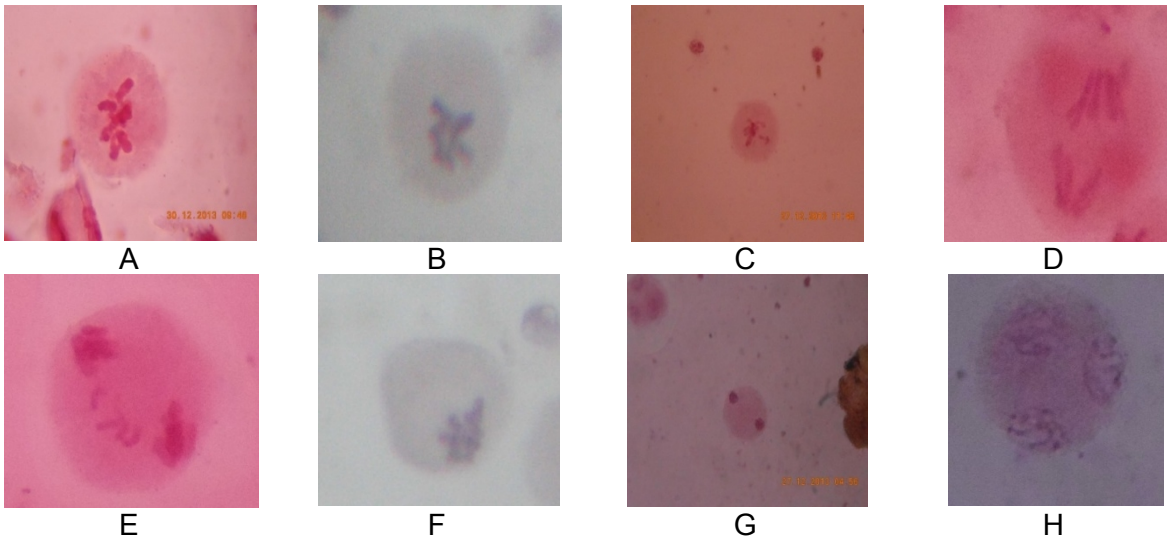

Plate 2. Cytology of mutants

A: Metaphase-1 (Control); B: Stickiness at metaphase-1; C: Fragment formation at metaphase-1; D: Anaphase-1 (Control); E: Laggards at anaphase-1; F: Disturbed telophase-1; G: Telophase-1 (Control); H: Trinucleate condition at telophase-II

3.3 Cytological Observations

Cytology was performed of the morphological mutants isolated on the screening of M_2 population of *Vicia faba.* Chromosomal abnormalities such as laggards, stickiness, fragments, trinucleate condition at Telophase-II and disturbed Telophase were noticed in the mutants Plate 2 above. In control plants, meiosis was normal. Laggard formation occurs due to delayed terminalisation, stickiness of chromosome ends or failure of chromosome movement [8,9,10]. Several agents have been reported to cause chromosome stickiness, including gamma rays Al Achkar et al. [11], temperature [12] and some chemicals present in soil Caetano-Pereira et al. [13]. Gaulden [14] postulated that sticky chromosomes may result from defective functioning of one or two types of specific non-histone proteins involved in chromosome organisation, which are needed for chromatid separation and segregation. The altered functioning of these proteins leading to stickiness is caused by mutations in the structural genes coding for them (hereditary) or by the action of mutagens on the proteins (induced stickiness). The fragment noticed in the mutants might be due to the failure of the broken chromosomes to recombine. Disturbed polarity occurs due to the alterations in genes controlling the biochemical pathways of the substance that determine the position of spindle poles. Disturbed polarity has also been reported by Fatma and Khan [15] in *Vicia faba.*

4. CONCLUSION

As is clear from the experiment that lower individual doses/concentrations produced less frequency of meiotic abnormalities Table 3, that can be tolerated by the plant. The lower individual doses/concentrations also produced desirable mutants such as bushy mutants, opposite leaves mutant and mutant with four flowers at a node Table 2 where as the higher combination treatments (300Gy+0.03% EMS and 400Gy+0.04% EMS) produced more frequency of meiotic abnormalities Table 3 and also undesirable mutants such as unbranched mutant and one sided branched mutants Table 2. So, it is better to use lower doses/concentrations of individual treatments to produce the desirable variability in plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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