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Meiotic changes in *Vicia faba* L. subsequent to treatments of hydrazine hydrate and maleic hydrazide

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ABSTRACT

Assessing the impact of mutagens for creating variations in crops like faba bean (*Vicia faba* L.) is an important criterion in the contemporary world where food insecurity and malnutrition is alarming at the doors of various nations. Impact of two chemical mutagens viz. hydrazine hydrate (HZ) and maleic hydrazide (MH) on the two varieties (NDF-1 and HB-405) of *Vicia faba* were analysed in terms of meiotic behavior and pollen sterility. Since there are not enough data about the effect of these mutagens on the chromosomal behaviors of *Vicia faba*, this study presents the role of hydrazine hydrate and maleic hydrazide as well as various types of chromosomal aberrations in crop improvement. The lower concentration of mutagens showed less pollen sterility compared to the higher concentrations. Manipulation of plant structural component to induce desirable alternations provides valuable material for the breeders and could be used favorably for increasing mutation rate and obtaining a desirable spectrum of mutation in faba beans based on preliminary studies of cell division.

Key words: food insecurity, *Vicia faba*, genetic diversity, mutagens, chromosome aberrations, yield

Introduction

Food insecurity is becoming major constraint for the development of national building programmes in various countries, including India. With an increase in human population the ghost of hunger are making its impact among millions of people all around. The conditions are worse at present due to growing undernourishment (FAO, 2009). The rescue lies in tailoring the better varieties of crop plants, high in nutrition and yield, and induced mutagenesis is one of those novel techniques, which impart variation in subject crops (Sharma & Kumar, 2004) through sustainable approach. Using chemical mutagens, wide range of chromosomal alterations leading to abnormal behavior during meiosis and consequently varying degree of sterility provide information regarding the role and effect of various genotypes to particular mutagen, especially of the crops which are partially cross-pollinated like *Vicia faba*.

Vicia faba, also known as bakla in India, originates from one of the forms of Greek verb mean "to eat" which highlights its use for food and feed by the ancient Greeks and Romans (Muratova, 1931; Cubero, 1974). *Vicia faba* has the advantage of having relatively large chromosomes that are excellent for assessing chromosomal aberrations. Pollen mother cells of faba bean are the most frequently used higher plant material for assessing chromosome damage. In spite of substantial production potential of faba bean, no attention has been paid to its improvement for increasing the production of local strains in different parts of the country. Mutation is the only method by which allelic differences between the genes can arise. Many researchers report that induced mutations are considered as the alternative to the naturally occurring variations, as the source of germplasm for plant improvement programmes and as alternative to hybridization and recombination in plant breeding (Brock, 1970). The application of mutation techniques, i.e. physical and chemical mutagens, has generated a vast amount of genetic variability and has played a significant role in plant breeding and genetic

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studies (Ranalli, 2012).

In the present study, a breeding programme for *Vicia faba* L. varieties HB-405 and NDF-1, using hydrazine hydrate (HZ) and maleic hydrazide (MH), has been undertaken to induce genetic variability in the crop. The objectives of the study were to study the effect of chemical mutagens (HZ and MH) on pollen fertility and meiotic behavior in M_1 generation.

Materials and Methods

Fresh and healthy uniform seeds of two varieties of *Vicia faba*, namely NDF-1 (local collection from Pithla village, Distt. Faizabad, U.P.) and HB-405 (line derived from the cross between cultivars EL-243835 × Vikrant and this hybrid is released as a variety for cultivation) were procured from Government Seed Store, Aligarh, U.P. India. The seeds were presoaked in distilled water for 12 hours and treated with 0.01%, 0.02%, 0.03%, 0.04% of HZ (base analogue, manufactured by Qualigen Fine Chemical, Mumbai, India) or 0.004%, 0.006%, 0.008%, 0.01% MH (synthesized from maleic anhydride and hydrazine, decomposing at 260°C, manufactured by Loba Chemie, Mumbai, India) prepared in phosphate buffer adjusted to pH 7 for 6 hours. Treated seeds were sown in the field during winter (rabi) season of 2009 at the Agricultural Farm, Aligarh Muslim University, Aligarh to raise M1 generation with three replications in a complete randomized block design (CRBD), with each replication consisting 100 seeds. The distance between the seeds in a row and between the rows was kept as 30 cm.

Pollen fertility

Pollen fertility was estimated from fresh pollen samples. From mature anthers, some amount of pollen was dusted on a slide containing a drop of 1% acetocarmine solution. Pollen grains, which took stain and had a regular outline were considered as fertile, while empty and unstained ones as sterile.

The following formula was used to calculate the percentage inhibition or injury or reduction:

$$\%\ \text{inhibition (injury, reduction)} = \frac{\text{Control} - \text{Treated}}{\text{Control}} 100$$

Cytological studies

Meiotic studies were carried out to estimate the potency of chemical mutagens (HZ and MH) in relation to the induction of chromosomal abnormalities at various stages of cell division. Young flower buds from 20-25 randomly selected M_1 plants from each treatment in both the varieties were fixed in Carnoy's fluid (1:3:6, glacial acetic acid:chloroform:ethyl alcohol) for 30 minutes. The material was then transferred to propionic alcohol saturated with ferric acetate for 24 hours. The flower buds were washed with and preserved in 70% alcohol. Anthers were smeared in 1% acetocarmine solution and pollen mother cells were examined for their behavior at various stages of microsporogenesis. Photographs were taken from temporary preparations from Nikon 80i adjusted digital cam of triocular microscope.

Results and Discussion

Biological damages in terms of pollen fertility and meiotic behavior in M_1 generation are generally used to evaluate the mutagenic sensitivity of biological systems. Pollen fertility in the mutagen treated population forms a reliable index in assessing any internal change in the plant as well as in determining the efficiency of a mutagen. The pollen fertility was dose-dependent as evident from a proportionate decrease with increasing concentrations of HZ and MH in both varieties (Table 1).

Table 1. *Effect of mutagens on pollen fertility in the two varieties of Vicia faba.*

Treatment	Variety	Pollen fertility	
		Actual (%)	% reduction
0% (Control)	HB-405	99.50	-
	NDF-1	99.20	-
0.01% HZ	HB-405	93.99	-5.53
	NDF-1	93.80	-5.44
0.02% HZ	HB-405	93.90	-5.62
	NDF-1	93.25	-5.99
0.03% HZ	HB-405	92.58	-6.95
	NDF-1	92.09	-7.16
0.04% HZ	HB-405	92.25	-7.28
	NDF-1	92.00	-7.25
0.004% MH	HB-405	90.21	-9.33
	NDF-1	90.01	-9.26
0.006% MH	HB-405	90.10	-9.44
	NDF-1	90.00	-9.27
0.008% MH	HB-405	89.20	-10.35
	NDF-1	89.00	-10.28
0.01% MH	HB-405	-	-
	NDF-1	-	-

Percent pollen sterility (% age reduction) induced by MH treatments was higher as compared to HZ treatments in both varieties. In general, var. NDF-1 showed higher pollen

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sterility than the var. HB-405. Dose-dependent pollen sterility, as observed in the present study, was also reported by Bhat et al. (2006) in *Vicia faba* and by Khan et al. (2000) in *Vigna radiata*. The high sterility observed in the treated population may be attributed to vast array of meiotic aberrations that were induced by chemical mutagens leading to aberrant pollen grains. The reason of pollen sterility caused by these chemical mutagens may be attributed to a gene mutation or more probably invisible deficiencies. The lower concentration of mutagens showed less pollen sterility compared to the higher concentrations.

Various chromosomal aberrations were noticed in mutagen treated population of *Vicia faba*. In control plants, meiosis was normal. Chromosomal aberrations were found to correlate with the concentration of chemical mutagens (Tables 2 and 3). Cytological effects observed following treatments with HZ and MH are shown in Figures 1-12. The frequency of meiotic aberrations was comparatively more in the var. NDF-1 than the var. HB-405. Maximum aberrations were noticed in MH than HZ treatments in both varieties of *Vicia faba*. Different reasons had been attributed for the caused chromosomal aberrations.

Chromosome stickiness may be caused by genetic or environmental factors. Genetically controlled stickiness has been described in other cultivated plants (Golubovaskaya, 1989; Caetano-Pereira et al., 1995). Bridges, as observed with HZ and MH treatments in the present study, may have been produced due to sub chromatic exchanges, unequal exchange of dicentric chromosomes. Ignacimuthu & Babu (1989) reported that chromosome breakage and reunion of broken ends could lead to the formation of bridges. The chromatin transmigration between PMCs with cytoplasmic channels was observed in treated plants. It is considered to be a source of production of aneuploid and polyploid gametes (Yen et al., 1993). The intensity of chromosome passage from one celled to the other during cytomixis depends upon the number and nature of cytoplasmic connections (Maria de Souza & Pagliarini, 1997).

The univalents and multivalents were found in treated population and their frequency was maximum at higher dose of mutagen. Mutagen-induced structural changes in chromosomes and gene mutations might be responsible for the failure of pairing among homologous chromosomes and hence the presence of univalents. Multivalents can be attributed to irregular pairing and breakage followed by translocation and inversions. According to Kodura & Rao (1981) the univalent, which are formed just before metaphase I would lie close to each other with their kinetochores directed towards the spindle axis and the arms towards the outside, and would also be near to the equator.

Kaul & Murthy (1985) refer to the genes that control, the normal spindle formation leading to proper separation of the bivalents and univalents. Mutation in these genes could lead to abnormal spindle formation and function leading to improper separation and uneven segregation of chromosomes to the opposite poles. Such gene multipolar telophase I/II that result in additional number of microspores or formation of micronuclei (Sjodin, 1970).

The laggards observed during the present study might be due to the delayed terminaliztaion, stickiness of chromosomal ends or because of failure of the chromosomal movements (Soheir et al., 1989). The occurrence of micronuclei at telophase II in the present case may results variation in number and size of pollen grains resulting from a mother cell. The disturbed polarity at anaphase and telophase stages could be due to the spindle disturbances. Cytomixis may occur between cells at different stages of division. The abnormal spindle observed in few cells has been reported also for other genera (Veilleux, 1985).

This study reaffirmed that meiosis is a complex that coordinate activity involving several genes and that mutation in any of these genes leads to irregularities.

Manipulation of plant structural component to induce desirable alternations in the yield components provides valuable material for the breeders and it may be concluded such mutagenic treatments could be used favorably for increasing mutation rate and obtaining a desirable spectrum of mutation in *Vicia faba* based on preliminary studies of cell division.

Conclusion

The possibilities offered by mutagenic agents to induced new genetic variation are of extreme interest. In many cases this might be the only answer to problems posed upon the practical breeder. An induced mutation is a very important event when it has a small effect for a specific character, because it change the balance established by natural selection in co-adapted blocks of genes. Therefore, it offers new situation for natural and artificial selection.

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Table 2. *Frequency and spectrum of chromosome abnormalities induced by HZ in two varieties of Vicia faba L.*

Treatment	Total No. of PMCs	PMCs with Diakinesis to Metaphase I/II					PMCs with Anaphase I/II					PMCs with Telophase I/II				Total frequency (%)
		Univalents	Multi valents	Stickiness	Stray bivalents	Cytomixis	Misorientation	Bridges	Laggard	Non-synchronization	Unequal separation	Micro nuclei	Disturbed polarity	Multi nuclei	Cytomixis	
Var. NDF-1																
Control	255	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.01%HZ	250	0.40	0.81	0.30	0.45	0.52	1.20	1.25	0.59	0.52	-	1.50	1.22	1.08	1.19	11.03
0.02%HZ	241	0.80	0.85	0.60	0.55	0.59	1.25	1.26	0.62	0.58	0.98	1.55	1.23	1.20	1.20	13.26
0.03% HZ	245	0.90	-	2.10	1.45	0.79	1.26	1.28	0.65	0.59	1.19	-	1.50	1.60	1.25	14.56
0.04% HZ	252	1.20	0.80	2.11	1.50	0.99	1.27	1.30	1.20	0.60	1.20	1.56	-	1.65	1.28	16.66
	Total	**3.30**	**2.46**	**5.11**	**3.95**	**2.89**	**4.98**	**5.09**	**3.06**	**2.29**	**3.37**	**4.61**	**3.95**	**5.53**	**4.92**	**55.51**
Var. HB-405																
Control	255	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.01% HZ	253	0.30	-	1.00	0.43	0.50	1.10	1.23	0.53	0.50	0.91	1.45	1.20	1.01	1.18	11.34
0.02% HZ	245	0.75	0.80	-	0.59	0.55	1.17	1.25	0.59	0.58	-	1.52	1.21	1.19	1.19	11.39
0.03%HZ	248	0.80	0.81	1.50	1.25	-	1.20	1.27	0.59	0.59	0.99	1.53	1.28	1.20	1.21	14.22
0.04%HZ	254	1.19	0.83	2.08	1.48	1.10	1.21	1.29	-	0.60	1.19	1.50	-	1.64	1.20	15.31
	Total	**3.04**	**2.44**	**4.58**	**3.75**	**2.15**	**4.68**	**5.04**	**1.17**	**2.27**	**3.09**	**6.00**	**3.69**	**5.04**	**4.78**	**52.26**

Table 3. *Frequency and spectrum of chromosome abnormalities induced by MH in two varieties of Vicia faba L.*

Treatment	Total No. of PMCs	PMCs with Diakinesis to Metaphase I/II					PMCs with Anaphase I/II					PMCs with Telophase I/II				Total frequency (%)
		Univalents	Multi valent	Stickiness	Stray bivalent	Cytomixis	Misorientation	Bridges	Laggard	Non-synchronization	Unequal separation	Micro nuclei	Disturbed polarity	Multi nuclei	Cytomixis	
Var. NDF-1																
Control	225	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.004% MH	240	0.88	0.89	1.02	1.00	0.79	1.22	1.67	0.78	-	1.50	2.01	1.20	1.19	1.50	15.64
0.006% MH	241	1.10	0.99	2.15	1.50	0.98	1.29	1.68	0.79	2.20	1.88	-	1.50	2.20	1.70	19.96
0.008% MH	253	1.25	1.25	2.19	1.51	1.10	1.30	1.80	1.25	2.25	1.99	2.20	1.50	2.25	1.80	23.64
	Total	**3.23**	**3.13**	**5.36**	**4.01**	**2.87**	**3.81**	**5.15**	**2.82**	**4.45**	**5.37**	**4.21**	**4.20**	**5.64**	**5.00**	**59.24**
Var. HB-405																
Control	255	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.004% MH	245	0.86	0.87	1.00	1.00	0.69	1.10	-	0.60	2.19	1.50	2.00	1.19	1.18	1.40	15.58
0.006% MH	240	1.09	0.98	2.00	1.45	0.97	1.28	1.99	0.75	-	1.70	2.18	1.45	2.19	1.65	19.68
0.008% MH	250	1.20	1.23	2.18	1.48	1.09	1.29	2.00	1.19	2.20	1.89	-	1.45	2.20	1.78	21.18
	Total	**3.15**	**3.08**	**5.18**	**3.93**	**2.75**	**3.67**	**3.99**	**2.54**	**4.39**	**5.09**	**4.18**	**4.09**	**5.57**	**4.83**	**56.44**

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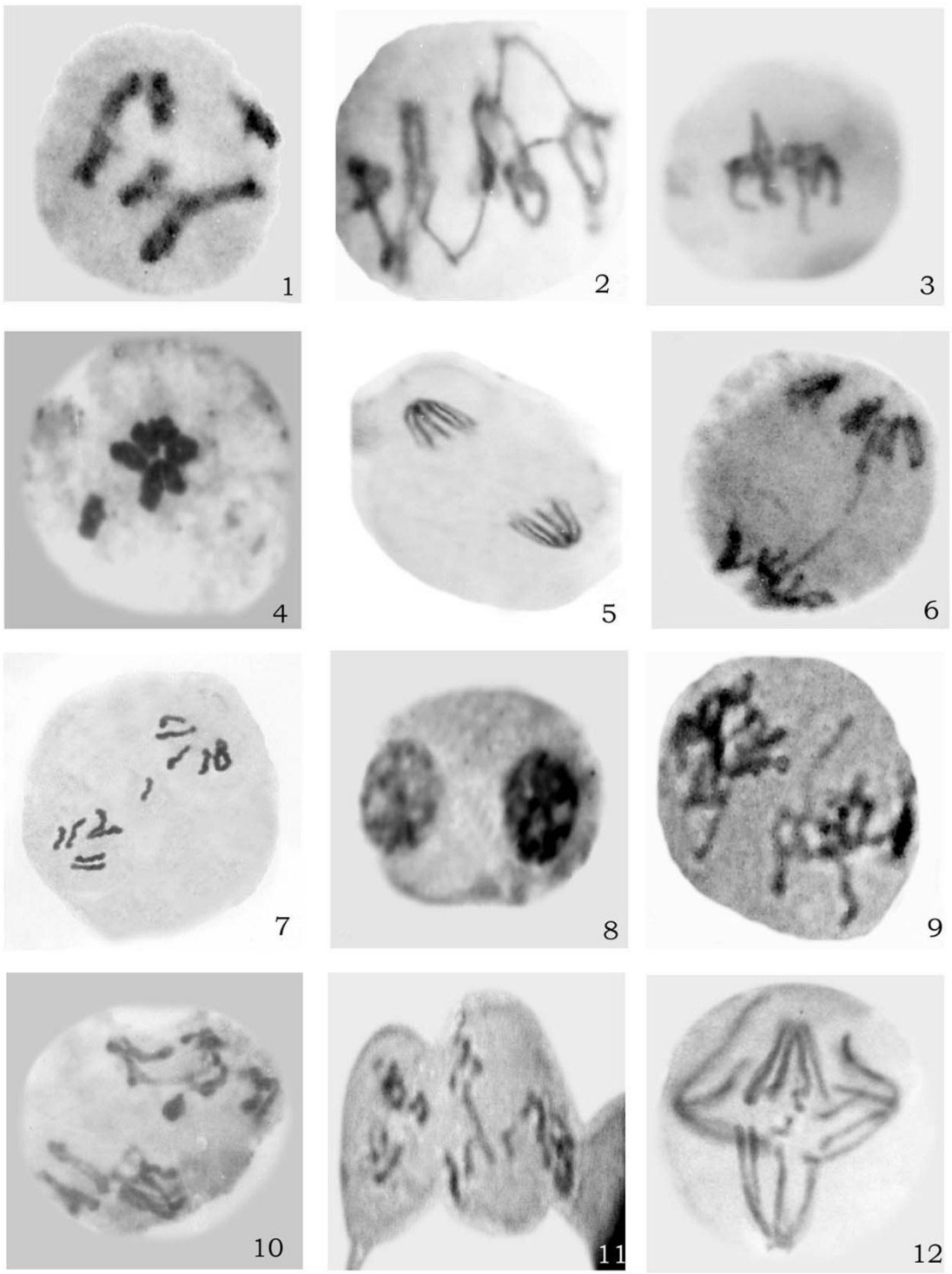

Figures 1-12. Plates. ***1****-Diakinesis (control);* ***2****- Metaphase-I (Control);* ***3****-Stickiness at Metaphase-I;* ***4****-Stray bivalent with sticky chromosomes Metephase –I;* ***5****-Anaphase-I (Control);* ***6****-Bridge at Anaphase-I;* ***7****- Laggard at Anaphase-I;* ***8****-Telophase-I (Control);* ***9****-Metaphase-II (Control);* ***10****- Stray chromosomes at Metaphase-II;* ***11****- Cytomixis at Metapaphase-II;* ***12****- Laggard at Anaphase-II.*

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