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Meiotic Studies in Two Varieties of *Vicia faba* L. (Fabaceae) after EMS Treatment

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Abstract: Seeds of 2 varieties viz., major and minor of *Vicia faba* L. were subjected to 4 different concentrations i.e., 0.1, 0.2, 0.3 and 0.4% of Ethyl Methane Sulphonate (EMS). Meiotic studies were carried out in the control as well as in the treated materials. Different types of meiotic abnormalities such as stickiness, univalents, multivalents, unorientation of chromosomes, precocious separation of chromosomes at metaphase, bridges, laggards and unequal separation of chromosomes at anaphase were recorded. In general, the meiotic abnormalities in both the varieties increased along with the increase in concentration of mutagen. However, *Vicia faba* L. variety minor showed more chromosomal abnormalities as compared to *Vicia faba* L. variety major at the same treatment.

Key words: *Vicia faba* L., ethyl methane sulphonate, meiotic abnormalities

INTRODUCTION

Vicia faba L. (2n = 12) of family Fabaceae, is an important pulse crop also used as vegetable, silage, forage and stock feed. It is consumed either green or dried, fresh or canned. It is a common breakfast food in the middle east, Medittarian region, China and Eithopia (Bond *et al*., 1985). According to Abdalla and Hussain (1977), the most popular dishes of faba bean are Medamis (Stewed beans), Falaful i.e., Deep fried cotyledons paste with some vegetables and spices, Bissara i.e., Cotyledon paste poured into plates and Nabet soup Boiled germinated bean (Jambunathan *et al*., 1994). Roasted seeds are eaten like peanuts in India (Duke, 1981).

Mutations can be beneficially utilized for tailoring better varieties of crop plants. But in general, chemical mutagens like Ethyl Methane Sulphonate (EMS) affects a wide range of chromosomal alterations resulting into abnormal behaviour during meiosis leading to varying degree of sterility. Cytological studies provide information regarding the response of various genotypes to a particular mutagen. The present study has been under taken to assess the effect of ethyl methane sulphonate in the 2 varieties of *Vicia faba* L. viz. *Vicia faba* L. var. minor and *Vicia faba* L. var. major.

MATERIALS AND METHODS

The seeds of 2 varieties of *Vicia faba* L. obtained from Indian Agricultural Research Institute (IARI), New Delhi, were treated with 4 different concentrations (0.1, 0.2, 0.3 and 0.4%) of Ethyl Methane Sulphonate (E. Merck India Ltd., grade-AR) for 6h after presoaking in distilled water for 8 h and sown along with the control after thorough washing in running tap water to remove the residual effects of mutagen sticking to the seed coat. These were grown under strictly similar conditions of growth. For meiotic studies flower buds were collected from 15-18 randomly selected plants, both in the control as well as treated plants and fixed in freshly prepared Carnoy's fixative (Alcohol: Chloroform: Acetic acid in 6:3:1 ratio) for 24 h, washed and preserved in 70% alcohol. Anthers were squashed in 2% acetocarmine, dehydrated in NBA series (50% acetic acid + 50% normal butyl alcohol and then passed through 100% normal butyl alcohol), mounted in canada balsom and dried at 45°C. Micro photographs were taken from freshly prepared slides using X30 Olympus research photomicroscope. Pollen fertility was determined by staining pollen grains in 1% acetocarmine and the stained pollen grains with regular margins were considered fertile.

RESULTS

Both the varieties showed almost similar response such as general reduction in seed germination, survival of plants and pollen fertility with increasing concentrations of EMS. The meiotic abnormalities were found dose dependent in both the varieties (Table 1). The above mentioned parameters were used for determining the comparative sensitivity of the 2 varieties under study.

Meiosis was perfectly normal in the control plants showing 6 bivalents at metaphase-1 and 6:6 disjunction at anaphase-I in both varieties (Fig. 1a and b). The data on

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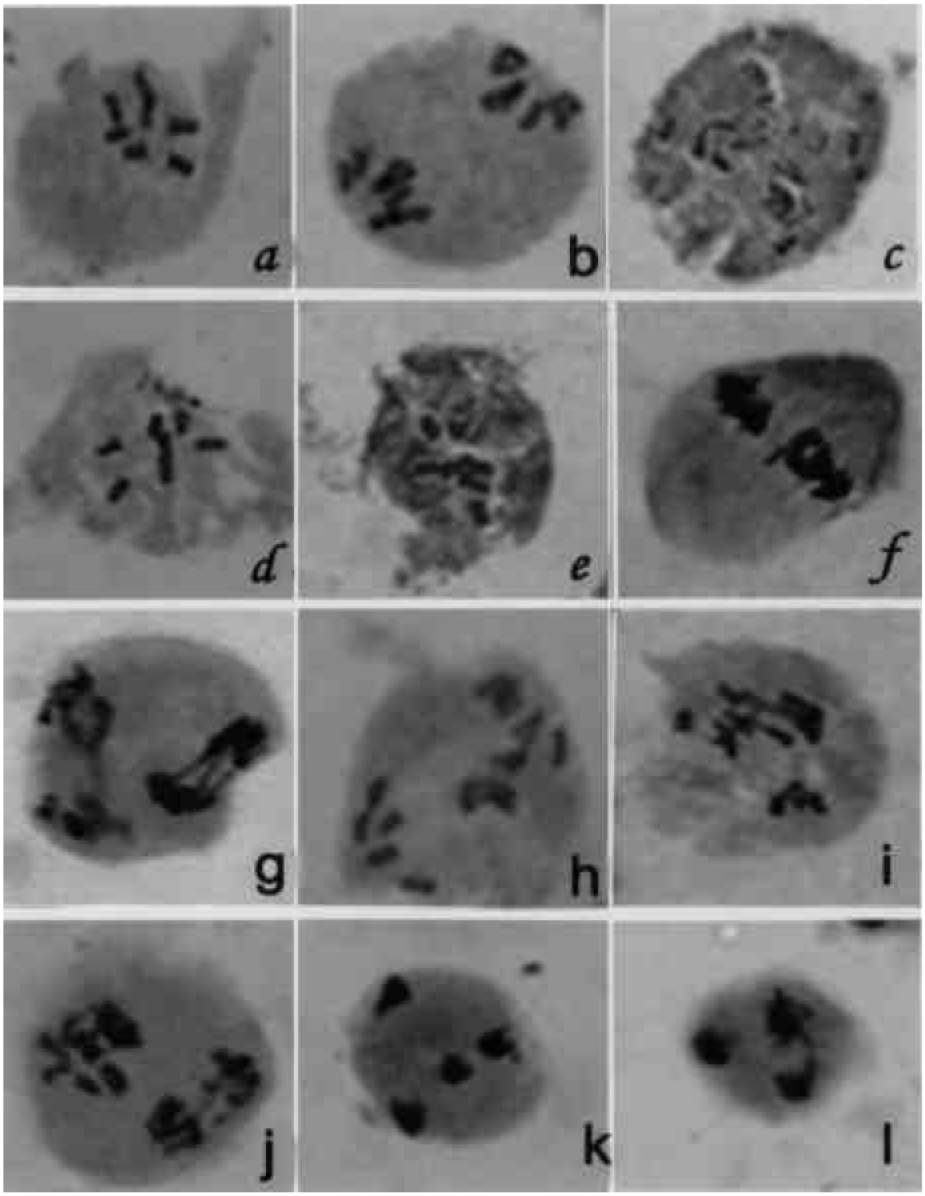

Fig. 1: a - l

a: PMC showing 6 bivalents at metaphase-1 of variety minor (Control)
b: PMC showing equal separation of chromosomes (6:6) at anaphase-1 of variety major (Control)
c: PMC showing 12 univalents and 2 fragments at metaphase-1 of variety minor (0.3% EMS)
d: PMC showing 4 bivalents and one tetravalent at metaphase-1 of variety major (0.4% EMS)
e: PMC showing 3 bivalents and one hexavalent at metaphase-1 of variety minor (0.3% EMS)
f: PMC showing stickiness at metaphase-II of variety major (0.2% EMS)
g: PMC showing multiple bridges at anaphase-II of variety minor (0.4% EMS)
h: PMC showing laggards at anaphase-I of variety major (0.3% EMS)
i: PMC showing unequal separation at anaphase-I of variety minor (0.1% EMS)
j: PMC showing bridge and unsychronisation at anaphase-II of variety major (0.4% EMS)
k: PMC showing disturbed polarity at telophase-II of variety minor (0.1% EMS)
l: PMC showing micro nuclei at telophase-I of variety major (0.2% EMS)

Table 1: Effects of EMS on seed germination, plant survival and meiotic abnormalities in two varieties of *Vicia faba* L.

Varieties	Concentrations of EMS (%)	Germination (%)	Plant survival (%)	Pollen fertility (%)	Meiotic abnormalities (%)
Variety	Control	91.66	90.40	93.46	-
minor	0.1	83.33	86.30	89.40	10.46
	0.2	79.16	82.44	83.76	14.61
	0.3	66.66	78.24	78.52	18.77
	0.4	62.50	74.24	75.35	23.16
Variety	Control	93.75	91.60	93.46	-
major	0.1	87.50	88.20	89.40	7.71
	0.2	83.33	83.24	83.76	11.98
	0.3	75.00	80.44	78.52	16.66
	0.4	70.83	76.26	75.35	20.44

Table 2: Meiotic abnormalities in *Vicia faba* L. variety minor after EMS treatment

	Frequency of abnormal PMCs at metaphase I/II (%)							Frequency of abnormal PMCs at anaphase I/II (%)	
Treatments	Total No. of PMCs scored	Stickiness	Univalents	Multivalents	Precocious segregation	Un-orientation of chromosomes	Fragments	Laggards	Bridges
Control	432	-	-	-	-	-	-	-	-
EMS 0.1%	430	1.14 (5)	0.93 (4)	4.30 (6)	0.69 (3)	1.14 (5)	-	-	1.62 (7)
0.2%	438	1.35 (6)	1.14 (5)	1.59 (7)	0.91 (4)	1.59 (7)	-	0.91 (4)	1.82 (8)
0.3%	442	1.80 (8)	1.58 (7)	2.03 (9)	1.35 (6)	1.80 (8)	0.69 (3)	1.13 (5)	2.26 (10)
0.4%	436	2.29 (10)	2.06 (9)	2.52 (11)	1.83 (8)	2.06 (9)	0.89 (4)	1.60 (7)	2.75 (12)

	Frequency of abnormal PMCs at anaphase I/II (%)		Frequency of abnormal PMCs at telophase I/II (%)			
Treatments	Unequal segregation	Non-disjunction	Disturbed polarity	Micronuclei	Cytomixis	Total abnormalities
Control	-	-	-	-	-	-
EMS 0.1%	0.46 (2)	0.23 (1)	1.86 (8)	-	0.93 (4)	10.46 (45)
0.2%	0.86 (3)	0.45 (2)	2.05 (9)	0.68 (3)	1.35 (6)	14.61 (64)
0.3%	0.90 (4)	0.67 (3)	2.48 (11)	1.13 (5)	11.58 (7)	19.46 (86)
0.4%	1.14 (5)	0.91 (4)	2.75 (12)	1.37 (6)	1.83 (8)	24.05 (105)

Within parenthesis is number

Table 3: Meiotic abnormalities in *Vicia faba* L. variety major after EMS treatment

	Frequency of abnormal metaphase I/II (%)							Frequency of abnormal at PMCs at telophase I/II (%)	
Treatments	Total No. of PMCs scored	Stickiness	Univalents	Multivalents	Precocious segregation	Un-orientation of chromosomes	Fragments	Laggards	Bridges
Control	430	-	-	-	-	-			
EMS 0.1%	428	0.93 (4)	0.70 (3)	1.16 (5)	0.46 (2)	0.93 (4)			1.40 (6)
0.2%	434	1.15 (5)	0.92 (4)	1.38 (6)	0.69 (3)	1.15 (5)		0.69 (3)	1.61 (7)
0.3%	432	1.62 (7)	1.38 (6)	1.62 (7)	1.15 (5)	1.62 (7)	0.46 (2)	0.92 (4)	2.08 (9)
0.4%	445	2.02 (9)	1.79 (8)	2.24 (10)	1.57 (7)	1.79 (8)	0.67 (3)	1.34 (6)	2.47 (11)

	Frequency of abnormal at anaphase I/II (%)		Frequency of abnormal at PMCs at telophase I/II (%)			
Treatments	Unequal segregation	Non-disjunction	Disturbed polarity	Micronuclei	Cytomixis	Total abnormalities
Control	-	-	-	-		
EMS 0.1%			1.40 (6)		0.70 (3)	7.71 (33)
0.2%	0.46 (2)	0.46 (2)	1.84 (8)	0.46 (2)	1.15 (5)	11.98 (50)
0.3%	0.92 (4)	0.69 (3)	2.31 (10)	0.92 (4)	1.38 (6)	17.07 (74)
0.4%	1.12 (5)	0.89 (4)	2.47 (11)	1.12 (5)	1.57 (7)	21.11 (94)

Within parenthesis is number

various chromosomal aberrations such as stickiness, univalents, multivalents, non-orientation of chromosomes, laggards, bridges and micronuclei etc. are presented in Table 2 and 3.

The chromosome stickiness (Fig. 1) increased from 0.93 to 2.02 % in var. major and 1.14 to 2.29% in var. minor with the increasing concentrations of EMS i.e., from 0.1 to 0.4%, respectively (Table 2 and 3). The percentage of pollen mother cells with univalents (Fig. 1c) and multivalents (Fig. 1d and e) increased along with increasing concentrations of EMS in both the varieties. In the variety major, 1.79% univalents were observed at highest concentration (0.4%) of EMS, whereas, the variety minor showed about 2.06% univalents at the same concentration. At the highest concentrations (0.4%) variety major showed 2.24% pollen mother cells with multivalents as compared to 2.52% pollen mother cells with the same abnormality in variety minor at same concentration of EMS.

In the variety major, the pollen mother cells with non-orientation and precocious separation of chromosomes were less than in variety minor at all the concentrations of

EMS (Table 2 and 3). At anaphase I/II, the percentage of pollen mother cells showing laggards (Fig. 1g) unequal segregating bridges (Fig. 1h), non-disjunction of chromosomes also increased with increasing concentrations of EMS. The frequency of these abnormalities was maximum at 0.4% of EMS. However, laggards were not found in both the varieties treated with lowest concentration (0.1%) of EMS. The percentage of these abnormalities was more in variety minor than variety major. Unequal separation of chromosomes (Fig. 1i) and non-disjunction of chromosomes (Fig. 1j) were not found in lowest concentration of EMS in variety major but were found increasing from 0.2 to 0.4% of EMS. In variety minor these abnormalities were found in lowest concentration (0.1%) of EMS and showed dose dependent increase upto 0.4% of EMS. Disturbed polarity (Fig. 1k) and micronuclei (Fig. 1l) were observed in 2.47 and 1.37% of pollen mother cells respectively in variety major at highest concentration of EMS (i.e., 0.4%) but in variety minor these abnormalities were recorded in 2.75 and 1.37% of pollen mother cells respectively at 0.4% concentration of EMS. Cytomixis was commonly found in both the varieties in all the treatments at various frequencies. The increase in pollen sterility was consistent with the increase in meiotic irregularities in both the varieties.

DISCUSSION

Increase in meiotic abnormalities was found along with the increasing concentrations of ethyl methane sulphonate in both varieties. Different types of chromosomal abnormalities observed during the present investigation have also been reported by various workers in different plant materials after treatment with physical and chemical mutagens (Ahmad, 1993; Kumar and Dubey, 1998; Dhamayanthi and Reddy 2000; Bhat *et al*., 2005). Most of these workers have obtained a dose dependent increase in the meiotic aberrations. The varietal sensitivity to mutagenic treatment have also reported by some workers (Evans and Sparrow, 1961; Akbar *et al.* 1976; Ahmad and Godward, 1981; Bhat *et al*., 2006a).

Tarar and Dnyansagar (1980) have reported that stickiness at meiosis was due to depolymerisation of nucleic acid caused by mutagenic treatment. Gaulden (1987) attributed chemically induced stickiness to direct action of mutagen on the histone proteins leading to improper folding of DNA. 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. Anis and Wani (1997) have reported that precocious movement of chromosomes may be due to structural differentiation of homologous pair. Multivalent formation could be attributed to irregular pairing and breakage followed by translocations and inversions. Anaphasic bridges may be formed due to unequal exchange or dicentric chromosomes. Sax (1960) and Saylor and Smith (1966) suggested that the formation of bridges might be due to the failure of chiasmata in a bivalent to terminalise and chromosomes get stretched between the poles. In the present study the occurrence of bridges could be attributed to the general stickiness of chromosomes at metaphase stage or to the breakage and reunion of chromosomes.

The laggards observed during the present study might be due to the delayed terminalisation stickiness of chromosomal ends or because of failure of the chromosomal movement (Jayabalan and Rao, 1987; Soheir, *et al*., 1989). The occurrence of micronuclei at telophase-II in the present case may be due to presence of fragments or laggards and may result variation in number and size of pollen grains resulting from a mother cell (Bhattacharjee, 1953). The disturbed polarity at anaphase and telophase stages could be due to the spindle disturbances. Cytomixis refers to the migration of chromatin/chromosomes from one cell into the cytoplasm of another cell through cytoplasmic channels. The occurrence of cytomixis may be due to the influence of genes (Kaul and Nirmala, 1991), Pathological conditions (Bobak and Herich, 1978) and changes in biochemical processes (Koul, 1990) and abnormal genetic behavior due to chemical mutagen treatment (Bhat *et al*., 2006b). The cytomixis is considered to be a source of production of ane uploid and polyploid gametes (Yen *et al*., 1993; Bhat *et al*., 2006b).

Results obtained here indicate that although the types of abnormalities were more or less common in both the varieties but the frequency of aberrations were comparatively higher in variety minor indicating its greater sensitivity to the mutagen.

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