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COMPARATIVE ANALYSIS OF MEIOTIC ABNORMALITIES INDUCED BY GAMMA RAYS, EMS AND MMS IN *VICIA FABA* (L.)

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The present investigation provides a relative account of cytological and developmental effects of gamma rays, Ethyl Methane Sulphonate (EMS) and Methyl Methane Sulphonate (MMS) on meiotic features and pollen fertility in *Vicia faba* L. Studies undertaken in M_1 generation on the variety minor of this species show that both the physical and chemical mutagens elicit various kinds of chromosomal abnormalities and reduction in pollen fertility. Such effects were dose dependent and positively correlated with dose concentration. However, the induction of meiotic abnormalities was observed to be higher under MMS treatment, followed by gamma rays and EMS, suggesting that MMS could be more effective in inducingvariability followed by gamma rays and EMS in this crop.

Key words: Meiotic abnormalities, *Vicia faba* L., gamma rays, MMS, EMS.

Cytological analysis with respect to either mitotic or meiotic behaviour is considered to be one of the dependable indices to estimate the potency of mutagen. Therefore, investigations on disturbances in meiotic behaviour indicating mutational genetic load form an integral part of most of the mutation studies. It also provides a considerable clue to assess sensitivity of plants for different mutagens, and to ascertain the most effective mutagen for a given crop to realize maximum results.

Vicia faba (family Fabaceae, 2n = 12) commonly known as broad bean, is an important crop used as vegetable, silage, forage and stock feed. Since genotype of *Vicia faba* L. is homozygous because of often self-pollination, therefore, there is a need to create variation to facilitate genetic improvement by mutation breeding. Physical and chemical mutagens provide a good scope for selection, as a tool for inducing alterations in the genotype to enhance the variability of characters. The present investigation has been taken to assess the effectiveness of physical and chemical mutagens on an important variety of *Vicia faba* L.

MATERIALS AND METHODS

Dry seeds of *Vicia faba* L. var. minor were irradiated with 4 doses of gamma rays viz. 10kR, 20kR.30kR and 40kR from the ^{60}Co source at IARI,°New Delhi. In another two sets the seeds soaked in water for 12 hours were surface dried with soaking paper, and then treated with different concentrations of freshly prepared EMS (0.1%, 0.2%, 0.3% and 0.4%) and MMS (0.01%, 0.02%, 0.03% and 0.04%) at pH 7.0 for six hours. After the treatment, all sets of seeds were sown in 3 replicates with 50 plants/replicate, to raise M_1 generation, along with control sets for comparison. For meiotic studies, young flower buds from 25-35 randomly selected plants were fixed in freshly prepared Carnoy's fixative. Squashes were made in 2% acetocarmine and slides were made permanent through butyl-alcohol series. Pollen fertility was assessed using 2% acetocarmine-glycerine staining technique where unstained and shrunken pollen grains were considered sterile.

OBSERVATIONS

Meiosis was perfectly normal in the control plants showing 6 bivalents at metaphase-1 and 6:6 anaphase-I disjunction. However, a number of meiotic abnormalities were recorded in plants raised from seeds treated with varying doses /concentrations

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of different mutagens. The most frequent aberrations were stickiness, laggards, bridges, disturbed polarity, non-synchronisation, cytomixis, precocious separation, univalents, trivalents and multivalents. Representative cytological features are shown in the Fig.1 (A-H). A dose dependent increase in meiotic aberrations was observed with all mutagenic treatments. The maximum aberrations were found at higher doses of each mutagen. Although most of the abnormalities were present in all the treatments, multivalents, stickiness and precocious separation were dominant at metaphase. Among the abnormalities at telophase, disturbed polarity was dominant, whereas anaphasic abnormalities were found almost in equal proportion in all the three mutagen treated populations. Univalents, bivalents, trivalents and cytomixis were found at metaphase-I/II.

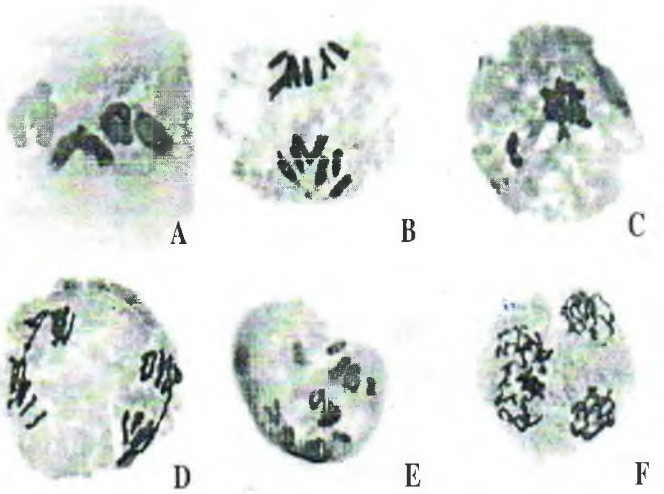

Fig.1 Representative meiotic features observed in the control and mutagen treated plants of *Vicia faba* L. : A, B. Metaphase/ Anaphase-I in control, C. Stickiness at metaphase-I, D. Bridges at anaphase-II, E. Laggards at anaphase-I, F. Non-synchronisation at anaphase-II / telophase-II.

Laggards were found both at anaphase and telophase-I/II. Bridges without fragments were observed more frequently at higher treatments both at anaphase and telophase-I/II.

Mostly the cells with single and double bridges were observed at anaphase and telophase. Disturbed polarity and non-synchronisation were also noted in all the treatments at anaphase and telophase-I/II respectively. The chromatin transmigration between PMCs with cytoplasmic channels was the common feature in treated plants. Table 1 shows a comparative account of various meiotic abnormalities induced by gamma rays, EMS and MMS. Varying frequencies of pollen sterility was observed in all the mutagenic treatments which increased with an increase in mutagenic concentration.

DISCUSSION

In the present investigation, all the mutagenic treatments exhibited similar types of meiotic abnormalities but the percentage of these abnormalities was different in different treatments. This shows that these mutagens have different mutagenic potential for *Vicia faba* L.

Bivalents were found clumped in single or different groups at metaphase/anaphase due to their stickiness. Similar results have been reported by Abbasi and Anis (2002) in *Trigonella foenum-graecum* L. Stickiness could be due to depolymerisation of nucleic acid caused by mutagenic treatment or due to partial dissociation of the nucleoproteins and alterations in their pattern of organisation (Evans 1962). Jayabalan and Rao (1987) suggested that stickiness might be due to disturbances in the cytochemically balanced reactions. 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. The occurrence of multivalent at metaphase have been reported in various plants like tomato (Gill *et al.*, 1980), lentil (Gupta *et al.*, 1999) and barley (Kumar and Singh 2003). Multivalent formation can be attributed to irregular pairing and breakage followed by translocations and inversions.

Precocious movement of chromosomes at metaphase-I/II seems to be caused by spindle disfunction. Roy *et al.* (1971) also observed precocious separation of one or more bivalents at metaphase-1 in *Cucumis sativus* raised from seeds irradiated with gamma rays.

Lagging chromosomes may be explained on the basis of abnormal spindle formation and chromosomal breakage. Bridges in gamma ray treatments seem more as a result of paracentric inversion (Swanson 1965) as they were found with fragments. Disturbed polarity may be due to improper spindle functioning. Besides these, the transmigration of chromatin material with cytomictic connections might have resulted in altered number of chromosomes in some pollen mother cells.

Pollen sterility was higher in MMS treatments followed by gamma rays and EMS and showed dose dependent increase (Table 1). Chary and Bhalla (1988) also reported an increase in pollen sterility with an increase in mutagenic treatment. Similar results by EMS were reported by Reddy and Annadurai (1992) in lentil, Dhamyanthi and Reddy (2000) in *Capsicum annum*. The high sterility observed in the treated populations may be attributed to vast array of meiotic aberrations that were induced by physical and

Table 1 A comparison of meiotic abnormalities induced by Gamma rays, EMS and MMS in *Vicia faba* L.

Treatment	Total no. of PMCs observed	Total no. of abnormal PMCs	PMCs with metaphase I/II abnormalities (%)						PMCs with Anaphase I/II abnormalities (%)			PMCs with Telophase I/II abnormalities (%)			% of abnormal PMCs	Pollen fertility (%)
			Univalents	Trivalents	Multivalents	Stickiness	Precocious separation	Cytomixis	Laggards	Bridges	Non-disjunction	Laggards	Bridges	Disturbed polarity		
Gamma																
30kR	316	13	–	–	1.58 (5)	1.89 (6)	0.63 (2)	–	–	–	–	–	–	–	4.11	93
50kR	315	22	0.63 (2)	–	2.22 (7)	3.17 (10)	0.63 (2)	–	–	–	–	–	–	1.26 (4)	7.93	83.22
90kR	320	53	1.87 (6)	1.25 (4)	3.12 (10)	5.0 (16)	1.25 (4)	0.62 (2)	0.31 (1)	0.93 (3)	0.62 (2)	0.93 (3)	0.62 (2)	0.93 (3)	17.49	69.44
130kR	325	84	2.46 (8)	1.84 (6)	4.30 (14)	5.53 (18)	2.15 (7)	1.84 (6)	0.92 (3)	1.53 (5)	0.92 (3)	1.23 (4)	1.53 (5)	1.53 (5)	25.83	64.22
EMS																
0.1%	340	11	–	–	0.88 (3)	1.17 (4)	0.58 (2)	–	–	–	–	–	–	0.58 (2)	3.23	91.22
0.2%	318	22	1.25 (4)	0.62 (2)	1.57 (5)	1.88 (6)	0.62 (2)	–	–	–	–	–	–	0.94 (3)	6.91	84.66
0.3%	316	36	1.26 (4)	0.63 (2)	2.22 (7)	2.53 (8)	1.58 (5)	0.31 (1)	0.31 (1)	0.31 (1)	–	0.63 (2)	0.31 (1)	1.26 (4)	11.39	70.00
0.4%	320	71	1.87 (6)	1.56 (5)	3.44 (11)	5.0 (16)	2.50 (8)	0.62 (2)	0.62 (2)	0.62 (2)	1.25 (4)	1.25 (4)	0.93 (3)	2.50 (8)	22.19	64.66
MMS																
0.05%	340	25	–	–	2.06 (7)	2.35 (8)	0.29 (1)	–	0.58 (2)	0.58 (2)	–	0.58 (2)	0.58 (2)	0.29 (1)	7.35	92.00
0.075%	318	56	1.25 (4)	1.25 (4)	3.15 (10)	3.77 (12)	1.25 (4)	0.62 (2)	0.94 (3)	1.25 (4)	0.94 (3)	0.62 (2)	1.25 (4)	1.88 (6)	17.61	85.22
0.10%	320	72	1.56 (5)	1.56 (5)	4.38 (14)	5.0 (16)	1.87 (6)	0.62 (2)	0.93 (3)	1.56 (5)	0.93 (3)	1.25 (4)	1.56 (5)	2.18 (7)	22.50	68.44
0.12%	340	94	2.05 (7)	2.05 (7)	5.58 (19)	5.29 (18)	2.05 (7)	0.88 (3)	0.88 (3)	1.76 (6)	1.76 (6)	1.76 (6)	1.76 (6)	2.65 (9)	27.55	60.00
Control	345	0	–	–	–	–	–	–	–	–	–	–	–	–	0	98

Within Parenthisis Number of Cells

chemical mutagens leading to aberrant pollen grains. The actual reason of sterility caused by these mutagens may be a gene mutation or more probably invisible deficiencies. The analysis of meiotic abnormalities at different stages of cell division clearly demonstrated MMS to be more effective followed by gamma rays and EMS in inducing the maximum frequency of aberrations.

The useful variability is a pre-requisite for crop improvement and therefore, the first step in any breeding programme would be the search for promising variability. In some crops such as maize, wheat and rice, a wide range of natural variability is known to occur in wild collections. However, in legumes, the available natural variability is not rich, therefore, mutagenesis has proved to be a handy tool to enhance the natural mutation rate, thereby enlarging the genetic variability and increasing the scope for desired selections. The production of cytological variations in *Vicia faba* L. may be considered as indicators to their corresponding mutagenic efficiency at the first instance, that may in turn lead to production of greater phenotypic variations, although not necessarily in terms of viable mutations.

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