

## DIETHYLSULPHATE INDUCED MEIOTIC ABNORMALITIES IN *VICIA FABAL*.

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Seeds of two varieties of *Vicia faba* L., a self fertilized crop, namely EL117792 and EL354984 were subjected to different concentrations of diethylsulphate (dES). Various types of chromosomal abnormalities such as stickiness, bridges, nonsynchronization, misorientation and cytotoxicity were recorded in mutagen treated population. A dose dependent increase in meiotic abnormalities was observed in the two varieties. However, the induction of chromosomal abnormalities was higher in the var. EL117792 than the var. EL354984, indicating that it was more sensitive to chemical mutagenic treatments and the improvement in yield and yield related traits can easily be made.

**Keywords:** Chromosomal abnormalities; Diethylsulphate; *Vicia faba*.

### Introduction

Faba bean (*Vicia faba* L.), belongs to the family fabaceae, is a self fertilized crop. Seeds of faba bean are high in protein, vitamins and minerals. Faba beans are rich in L-dopa, a substance used medically in the treatment of parkinson disease. L-dopa is also an antiuretic agent, which might help in controlling hypertension. The pods of faba bean are reputed to be toxic when they are not cooked. The large chromosome size and small chromosome number ( $2n=12$ ) make the faba bean a suitable material for cytogenetic studies. Genotype of *Vicia faba* is homozygous because of self pollination; therefore there is need for its further improvement which can be done by creating additional variability in its genotype. The preliminary studies are necessary to find out the effectiveness of a mutagen to initiate mutation breeding programme in a crop. Among various parameters used to find out the effectiveness of a mutagen, meiotic chromosomal aberrations are important as they measure directly the genetic effects. The present paper deals with the effects of dES on various cytological parameters in two faba bean varieties. New genetic variability could be achieved by crossing land race with exotic material and/or through mutation breeding. Mutation can be beneficially utilized for tailoring better varieties of crop plants. Investigation on meiotic aberrations and their genetic consequences are the integral part of most of the mutation studies. Many researchers have compared the mutagenic efficiencies in different mutagens in different crops<sup>1,2</sup>.

### Material and Methods

Seeds of two varieties viz; EL117792 and EL354984 of

*Vicia faba* were presoaked in distilled water for 9 hours and then treated with different concentrations (0.02, 0.04, 0.06, 0.08 and 0.1%) of dES (diethylsulphate) for 6 hours. The solution was changed at every two hours interval. The mutagenic solution was prepared in phosphate buffer of pH-7. After the treatment, the seeds were washed thoroughly in running tap water and sown in the field to raise  $M_1$  plants. For meiotic studies, the floral buds were fixed in carnoy's fluid (absolute alcohol : chloroform : glacial acetic acid in 6:3:1 ratio). Meiotic studies, were conducted on 25 randomly selected plants from each treatment. Squashes were made in 2% acetocarmine.

Following abnormalities were recorded at different stages.

- (I) Chromatin bridges at anaphase- II
- (II) Micronuclei on telophase-II
- (III) Cells showing other chromosomal abnormalities.
- (IV) Pollen sterility.

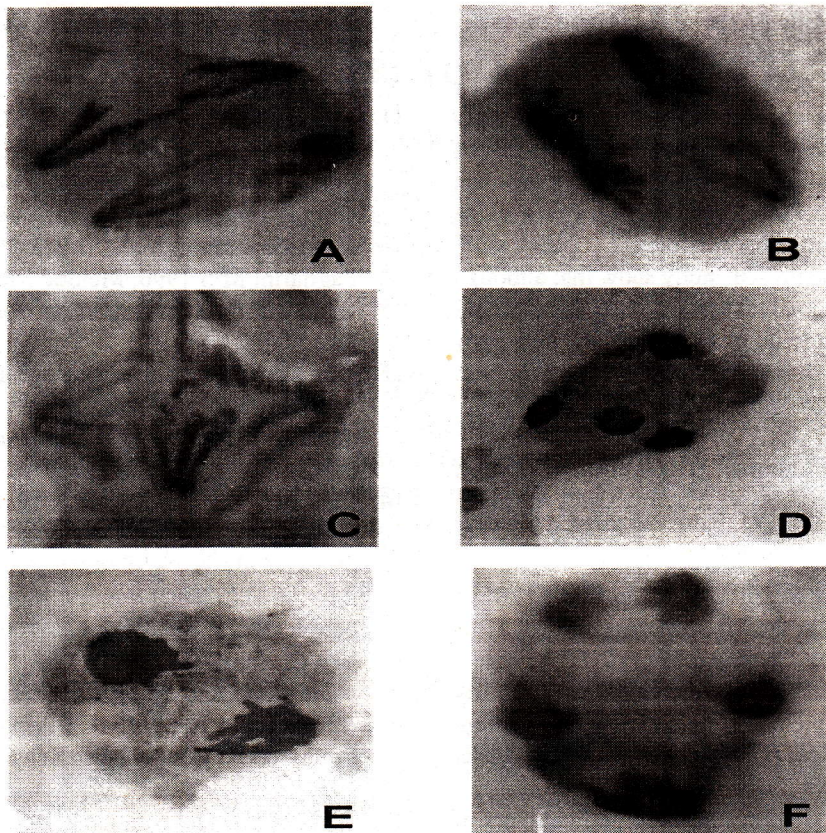
### Results and Discussion

Cytological studies of *Vicia faba* were carried out to estimate the potency of dES in relation to the induction of chromosomal aberrations at various stages of division. The floral buds from untreated plants revealed normal meiotic division and six bivalents were observed at metaphase-I. However, the treated plants showed chromosomal aberrations like chromatin bridges, nonsynchronization, misorientation, cytotoxicity, stickiness and micronuclei (Fig.1). Bridges were frequently observed at anaphase-II whereas at telophase-I and II stickiness and cytotoxicity found to be dominant. Statistical analysis revealed a positive correlation between aberration frequency and dose. Various concentrations of dES induced different

**Table 1.** Frequency of meiotic chromosomal abnormalities in *Vicia faba* L. after treating with different concentrations of dES.

Concentrations (%)	No. of Total PMCs	No. of abnormal Cells	Anaphase-II			Telephase-I/II			Total Frequency (%)	Pollen Sterility (%)
			Bridges	Nonsynchronization	Misorientation	Cytomixis	Stickiness	Micronuclei		
			Var-EL354984							
0.02	215	20	2.79 (6)	1.39 (3)	0.93 (2)	1.86 (4)	0.93 (2)	1.39 (3)	9.30	4.39
0.04	229	30	3.49 (8)	2.18 (5)	1.74 (4)	2.62 (6)	1.31 (3)	1.74 (4)	13.10	6.93
0.06	235	44	2.79 (7)	3.82 (9)	3.40 (8)	2.55 (6)	3.40 (8)	2.55 (6)	18.72	8.69
0.08	228	63	5.70 (13)	5.26 (12)	5.26 (12)	4.82 (11)	3.50 (8)	3.07 (7)	27.63	11.04
0.1	228	81	6.57 (15)	9.21 (21)	5.70 (13)	5.26 (12)	4.82 (11)	3.94 (9)	35.52	14.50
Total Frequency %	-	-	20.58	21.10	16.38	16.38	13.44	12.18	-	-
Var-EL117792										
0.02	210	21	2.38 (5)	1.42 (3)	2.85 (6)	1.90 (4)	0.95 (2)	0.47 (1)	10.00	3.96
0.04	229	38	3.49 (8)	2.18 (5)	3.93 (9)	3.05 (7)	2.18 (5)	1.74 (4)	16.59	6.35
0.06	223	58	5.38 (12)	6.72 (15)	3.13 (7)	7.17 (16)	2.24 (5)	1.34 (3)	26.00	8.06
0.08	235	79	5.95 (14)	8.08 (19)	5.10 (12)	5.53 (13)	3.82 (9)	5.10 (12)	33.61	10.47
0.1	224	84	6.69 (15)	8.92 (20)	6.25 (14)	4.91 (11)	5.35 (12)	5.35 (12)	37.50	16.29
Total Frequency%	-	-	19.28	22.14	17.14	18.21	11.78	11.42	-	-





**Fig.1. A :** Anaphase-II showing chromatin bridge; **B.** Non-synchronization at Anaphase-II; **C.** Disturbed Anaphase-II showing misorientation of chromosomes; **D.** Cytomixis and disturbed polarity at Telophase-II; **E.** Stickiness of chromosome at Telophase-I; **F.** Micronuclei at Telophase-II.

kinds of chromosomal aberrations in the two varieties of faba bean. Nonsynchronization at anaphase-II was the most prominent abnormality, it was 21.10 and 22.14 percent in the varieties EL354984 and EL117792, respectively (Table 1). A gradual reduction in pollen fertility was also noticed in the two varieties.

Various types of chromosomal aberrations observed during the present study have also been reported earlier<sup>3-5</sup>. Saylor and Smith<sup>6</sup> reported that the formation of bridges might be due to the failure of terminalisation in interspecific hybridization in *Pisum*. In the present study, the occurrence of bridges could be attributed to stickiness of chromosomes at metaphase stage or to the breakage and fusion of chromosomes and chromatids. Sticky chromosomes were first reported in maize<sup>7</sup> and are seen as intense chromatin clustering in the pachytene stage. Several factors<sup>8-10</sup> have been reported to induce chromosome stickiness<sup>11</sup>. Gaulden<sup>11</sup> postulated that sticky chromosomes may result from the defective functioning of one or two types of specific nonhistone proteins

involved in chromosome organization, which are needed for chromatid separation and segregation. The altered functioning of these proteins leading to stickiness is caused by mutation in the structural gene coding for them (hereditary stickiness) or by the action of mutagens on the proteins (induced stickiness). Laggards were not observed in the present study. However, the percentage of cells with micronuclei was higher at higher concentrations of mutagen in telophase II. Precocious migration of univalents to the poles is a very common abnormality among plants<sup>12,13</sup>. Koduru and Rao<sup>14</sup> suggested that laggards chromosomes are characteristic in that they generally lead to micronuclei formation. The disturbed polarity at telophase stage could be due to the spindle disturbances.

Cytomixis refers to the transfer of nuclear material or nutrients from one cell in to the cytoplasm of another cell through the cytoplasmic channels<sup>15</sup>. Cytomixis has been detected at a higher frequency in genetically imbalance species such as hybrids<sup>16</sup>. The occurrence of



cytomixis may be due to the influence of gene<sup>17</sup>, pathological conditions<sup>18</sup> and herbicides and temperature<sup>19</sup>. Cytomixis may have serious genetic consequences by causing deviation in chromosome number and may represent an additional mechanism for the origin of aneuploidy and polyploidy<sup>20</sup>. A gradual reduction in pollen fertility was noticed. Pollen fertility showed a close relationship with meiotic abnormalities<sup>21-23</sup>.

Results of the present study show that although the types of chromosomal aberration were more or less common in the two varieties of *Vicia faba*, the frequency of abnormalities was comparatively higher in the var. EL117792, indicating its greater sensitivity to dES.

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