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Comparative Analysis of Meiotic Aberrations Induced by Diethylsulphate and Sodium Azide in Broad Bean (*Vicia faba* L.)

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Abstract: The present investigation provides a comparative account of cytological and developmental effects of Diethylsulphate (DES) and Sodium Azide (SA) on meiotic features, pollen fertility, seed germination and seedling survival in *Vicia faba* L. Studies undertaken in M_1 generation on the variety major of this species, showed that both the mutagens DES and SA elicit various chromosomal aberrations in meiosis and reduction in seed germination, pollen fertility and seedling survival. Such effects were dose dependent and positively correlated with seed germination, pollen fertility and seedling survival. However, the induction of meiotic aberrations was observed to be higher in DES than SA treatments, suggesting that DES could be more effective in inducing additional variability than SA, in *Vicia faba* L. var. major.

Key words: Meiotic aberrations, seed germination, pollen fertility, seedling survival, M_1 generation, DES, SA, variability

INTRODUCTION

Cytological analysis with respect to either mitotic or meiotic behaviour is considered to be one of the most dependable index to estimate the potency of mutagen. Therefore, investigations on meiotic aberrations and their genetic consequences form an integral part of most of the mutation studies. It also provides a considerable clue to assess sensitivity of plants for different mutagens.

Many researchers have compared the mutagenic efficiencies of different mutagens on different crops. Their results seem to be entirely specific for particular species and even varieties. While many researchers like Rao and Rao (1983), Kumar and Dubey (1998c), Dhanayanth and Reddy (2000) and Bhat *et al.* (2005) found chemical mutagens to be more effective than physical ones, many others like Tarar and Dnyansager (1980), Zeerak (1991) and Singh (2003) found the reverse case.

Vicia faba L. ($2n = 12$) of family Fabaceae, commonly known as broad bean, is an important pulse crop used as vegetable, silage, forage and stock feed. Faba bean is used either green or dried, fresh or canned. It is a common breakfast food in the middle east, Mediterranean region, China and Ethiopia (Ismail *et al.*, 1976). According to Abdulla and Hussain (1977), the most popular dishes of faba bean are Medamis (Stewed beans), Falaful (Deep fried cotyledons paste with some vegetables and spices), Bissara (Cotyledon paste poured into plates) and Nabet soup (Boiled germinated bean). Roasted seeds are eaten like peanuts in India (Mark, 1983). Since, 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 by mutation breeding. Chemical mutagens provide a good scope for selection, as a tool for inducing alteration in the genotype to enhance the variability of characters. In the present investigation two potent mutagens that is DES and SA were used to treat *Vicia faba* seeds to assess their effect on the microsporogenesis.

MATERIALS AND METHODS

Healthy and dry (10-12% moisture) seeds of *Vicia faba* L. variety major, provided to me by the Cytogenetics and Mutation Breeding Laboratory, Department of Botany, Aligarh Muslim University, Aligarh, were subjected to 6 h treatment by six different concentrations (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06%) of DES and SA after presoaking of 14 h. The treated seeds were thoroughly washed in running tap water for half an hour to remove the residual effects of mutagen sticking to the seed coat. One set of seeds was kept untreated to act as control for comparison. All sets of seeds (including control), containing 50 seeds in each set, were sown in pots with 10 seeds in each pot to raise M_1 generation.

For meiotic studies young flower buds from 30-40 randomly selected M_1 plants were fixed in freshly prepared Carnoy's fixative (absolute alcohol, chloroform and acetic acid in 6:3:1 ratio) for 24 h, washed and preserved in 70% alcohol. Squashing was done in 2%

acetocarmine and slides were made permanent through butyl-alcohol series (Bhaduri and Ghosh, 1954). Microphotographs were taken from freshly prepared slides using X30 Olympus Research Photomicroscope. Pollen fertility was determined by staining pollen grains in 2% acetocarmine and stained pollen grains with regular margins were considered fertile.

RESULTS

Meiosis was perfectly normal in the control plants showing 6 bivalents at metaphase-I (Fig. 1) which

segregated into 6:6 at anaphase I (Fig. 1b). However a number of meiotic aberrations were recorded in plants raised from seeds treated with different concentrations of the mutagens. The most frequent aberrations were stickiness (Fig. 1c and g) stray bivalents (Fig. 1f) secondary association (Fig. 1d), multivalents and univalents, laggards, bridges, disturbed anaphase, cytotoxicity, micronuclei and disturbed polarity. A dose dependent increase in meiotic aberrations was observed with all mutagenic treatments in both the mutagens. The maximum aberrations were found at higher doses of the mutagens (Table 1). Although, most of the abnormalities

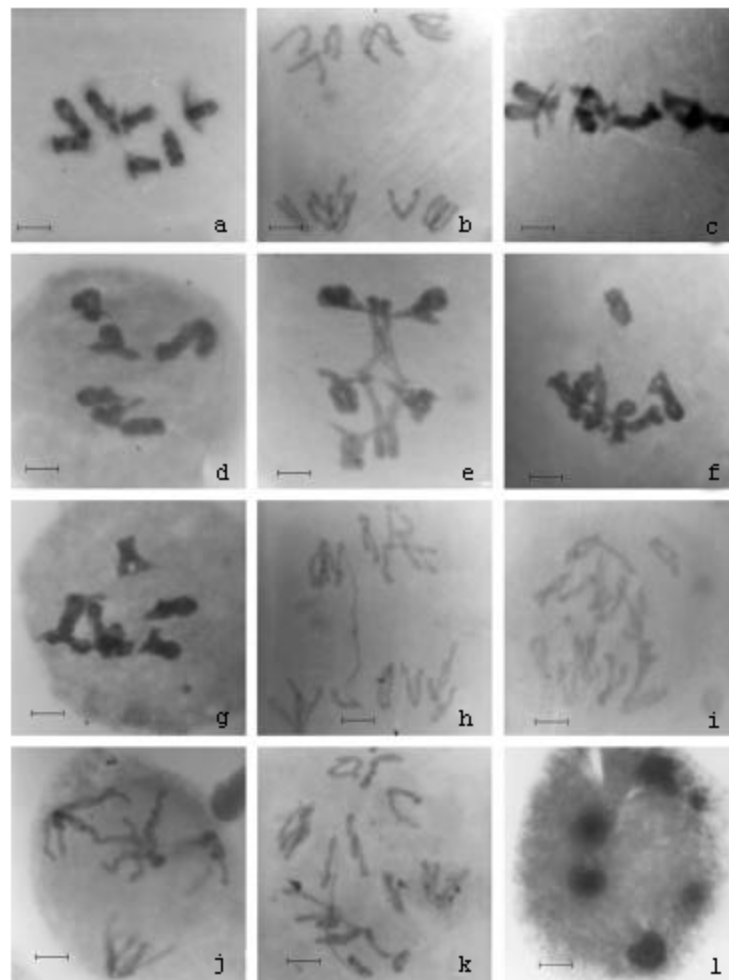


Fig. 1: Cytological features in *Vicia faba* L.; (a) PMC at metaphase-I showing 6 bivalents (Control), (b) PMC at anaphase-I showing 6:6 distribution of chromosomes (Control), (c) PMC at metaphase-I showing stickiness (0.03% DES), (d) PMC at metaphase-I showing the secondary association of three bivalents (0.05% DES), (e) PMC at metaphase-I showing the secondary association of six bivalents (0.04% SA), (f) PMC at metaphase-I showing a stray bivalent (0.06% DES), (g) PMC at metaphase-I showing stickiness of three bivalents (0.04% SA), (h) PMC at anaphase-I showing a bridge (0.06% DES), (i) PMC at anaphase-I showing multiple bridge (0.06% SA), (j) PMC showing disturbed anaphase-II (0.03% SA), (k) PMC at anaphase I showing many laggards (0.05% DES) and (l) PMC at telophase-II showing 5 macronuclei and one micronucleus (multinucleate condition) (0.05% DES)

Table 1: Percentage of meiotic abnormalities at different stages of meiosis induced by DES and SA in *Vicia faba* L.

Concentrations (%)	Total No. of observed PMCs	Total No. of abnormal PMCs	Metaphase I/II (%)			Anaphase I/II (%)			Telophase I/II (%)						
			Stickiness	Stray bivalents	Secondary association	Multivalents and univalents	Laggards	Bridges	Disturbed anaphase	Cytomixis	Micronuclei	Bridges	Disturbed polarity	Abnormal PMCs (%)	
Control	200	-	-	-	-	-	-	-	-	-	-	-	-	-	
DES															
0.01	210	10	0.95 (2)	-	-	0.48 (1)	1.43 (3)	-	-	-	-	-	0.95 (2)	0.95 (2)	4.76
0.02	215	21	1.86 (4)	0.93 (2)	1.40 (3)	0.93 (2)	1.86 (4)	-	-	-	-	-	1.40 (3)	1.40 (3)	9.76
0.03	208	37	2.88 (6)	1.44 (3)	2.40 (5)	0.96 (2)	1.92 (4)	0.48 (1)	0.96 (2)	0.97 (2)	0.96 (2)	0.96 (2)	1.44 (3)	1.44 (3)	17.79
0.04	206	56	3.88 (8)	2.43 (5)	3.40 (7)	1.94 (4)	3.40 (7)	0.97 (2)	1.94 (4)	0.97 (2)	1.50 (3)	1.94 (4)	2.91 (6)	2.91 (6)	27.18
0.05	200	72	4.00 (8)	3.50 (7)	4.50 (9)	3.50 (7)	4.00 (8)	1.50 (3)	3.00 (6)	1.50 (3)	1.36 (3)	3.00 (6)	3.50 (7)	3.50 (7)	36.00
0.06	220	83	5.45 (12)	4.09 (9)	4.55 (10)	3.64 (8)	4.09 (9)	1.36 (3)	2.73 (6)	1.36 (3)	1.36 (3)	2.73 (6)	3.64 (8)	3.64 (8)	37.73
SA															
0.01	220	5	0.45 (1)	-	-	-	0.91 (2)	-	-	-	-	-	0.45 (1)	0.45 (1)	2.27
0.02	208	14	1.44 (3)	0.48 (1)	0.96 (2)	0.48 (1)	1.44 (3)	-	-	-	-	-	0.96 (2)	0.96 (2)	6.73
0.03	215	26	2.33 (5)	0.93 (2)	1.86 (4)	0.47 (1)	1.40 (3)	-	0.47 (1)	0.47 (1)	0.47 (1)	0.47 (1)	0.93 (2)	0.93 (2)	12.09
0.04	206	45	3.40 (7)	1.94 (4)	2.91 (6)	1.46 (3)	2.91 (6)	0.49 (1)	1.46 (3)	0.49 (1)	0.49 (1)	1.46 (3)	2.43 (5)	2.43 (5)	21.84
0.05	210	60	2.86 (6)	2.86 (6)	3.81 (8)	2.86 (6)	3.33 (7)	0.95 (2)	1.90 (4)	0.95 (2)	0.95 (2)	2.38 (5)	2.86 (6)	2.86 (6)	28.57
0.06	228	72	5.26 (12)	3.51 (8)	3.95 (9)	3.07 (7)	3.51 (8)	1.32 (3)	2.19 (5)	1.32 (3)	1.32 (3)	2.63 (6)	3.07 (7)	3.07 (7)	31.58

Within parenthesis actual numbers of abnormal PMCs with abnormality

Table 2: Percentage of seed germination, seedling survival, pollen fertility in DES and SA treated *Vicia faba* L

Concentrations (%)	Seed germination (%)	Seedling survival (%)	Pollen fertility (%)	Abnormal PMCs (%)
Control	90.22	95.37	85.42	-
DES				
0.01	87.52	92.21	81.65	4.76
0.02	84.47	88.15	79.48	9.76
0.03	82.23	86.42	76.23	17.79
0.04	79.38	83.53	72.51	27.18
0.05	74.74	79.37	71.37	36.00
0.06	73.32	76.43	68.85	37.73
SA				
0.01	88.44	93.36	82.13	2.27
0.02	86.22	90.12	81.72	6.73
0.03	82.45	88.63	76.25	12.09
0.04	80.22	82.41	76.52	21.84
0.05	76.32	80.67	72.83	28.57
0.06	74.56	77.52	70.32	31.58

were present in all the treatments, stickiness and secondary association were dominant at metaphase I/II. Among the abnormalities at anaphase I/II, bridges (Fig. 1h) and laggards (Fig. 1k) were dominant, whereas dominant telophasic abnormalities were bridges, disturbed polarity and micronuclei (Fig. 1l).

Bridges and laggards were found both at anaphase and telophase I/II. Bridges were found with or without fragments. However, the bridges with fragments were found in higher treatments. Mostly the cells with single and double bridges were observed at anaphase and telophase-I/II. Multiple bridges (Fig. 1) were also found in higher treatments but in low frequency. Disturbed polarity (Fig. 1j) and non-synchronisation were also noted in all the treatments at anaphase and telophase I/II, respectively. The chromatin transmigration between PMCs through cytoplasmic channels was the common feature in treated plants. The 0.01% of DES and SA showed 0.95 and 0.45% PMCs with stickiness respectively, while as 5.45 and 5.26% PMCs with stickiness were observed at 0.06% of both the mutagens. The stray bivalents and secondary association were not found at 0.01% of DES but 0.02% of DES showed 0.93 and 1.40% PMCs with stray bivalents and secondary association, respectively. While as the corresponding values for 0.02% of SA were 0.48 and 0.96%, respectively. The lower concentrations i.e., 0.01 and 0.02% of both the mutagens did not show univalents and multivalents, however, 0.03% of both the mutagens showed 0.96 and 0.47% of PMCs with multi-and univalents and their higher percentage was observed at 0.06% of both the mutagens.

In DES 0.01, 0.48 and 1.43% PMCs were observed with laggards and bridges, respectively at anaphase I/II while as 2.73 and 4.09% PMCs with laggards and bridges were found at 0.06% while as in SA no laggards were found at 0.01%. However 0.90% PMCs with bridges were found at the same concentration. The highest percentage of PMCs with laggards and bridges i.e., 2.91 and 3.51% were found at 0.06% of SA. In lower concentrations i.e.,

0.01 and 0.02% of DES, disturbed anaphase and cytomixis was not observed, but 0.48 and 0.96% PMCs with disturbed anaphase and cytomixis were observed at 0.03% of DES and the PMCs with the highest percentage of unequal separation and cytomixis were observed at 0.06% of DES. In SA, 0.01-0.03% concentrations did not showed disturbed anaphase and 0.01-0.02% of DES also did not showed cytomixis. However, in rest of the concentrations, both the aberrations were present and the highest percentage i.e., 1.32 and 0.88% of PMCs with these aberrations were observed at 0.06% of SA.

In telophasic abnormalities 0.01 and 0.02% of both the mutagens did not showed micronuclei, however, 0.96 and 0.47% PMCs with micronuclei were observed at 0.03% of DES and SA and the highest percentage of PMCs with micronuclei were observed at 0.06% of both the mutagens. The bridges and disturbed polarity were present in all the concentrations of both the mutagens at telophase-I/II. In DES and SA 0.95 and 0.45% PMCs with bridges were observed at 0.01% while as the highest percentage i.e., 3.64 and 3.07% of PMCs with bridges was observed at the highest concentration (0.06%) of both the mutagens. The telophase also showed 0.95 and 0.45% of PMCs with disturbed polarity at 0.01% of DES and SA, respectively while as the highest percentage i.e., 4.09%, 3.51% of PMCs with disturbed polarity was observed at 0.06% of both the mutagens, respectively.

Seed germination, pollen fertility and seedling survival showed dose dependent reduction in both the mutagens and were positively co-related with the meiotic aberrations observed (Table 2).

DISCUSSION

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

Bivalents were found clumped in single or different groups at metaphase I/II due to stickiness. Researchers Stickiness could be due to depolymerization of nucleic acid caused by mutagenic treatments or due to partial dissociation of nucleo-proteins and alterations in their pattern of organization (Evans, 1962). Jayabalan and Rao (1987) suggested stickiness might be due to disturbances in cytochemically balanced reactions. However, it seems most probable that some kind of a gene mutation leads to incorrect coding of some non-histone proteins involved in chromosome organization. When affected, these proteins lead to chromosome clumping. It may also be possible that the mutagen itself reacts with the histone proteins and brings about a change in the surface property of chromosomes due to improper folding of DNA, thereby causing them to clump or stick (Gaulden, 1987).

In the present case, mutagens used seems to be responsible for stickiness induced. Perhaps, the target proteins in this case are those responsible for chromosome condensation during active divisional stages. Their defective functioning, which may be due to gene mutation or direct action of the mutagen on the proteins, caused a disturbance in the chromosomes during the course of their condensation from prophase to metaphase-I. Probably this was the main reason that the stickiness was predominant from metaphase-I onwards. Stickiness in chromosomes interfered in normal arrangement at metaphase and further lead to their inability in separation and origin of thick sticky bridges. When the spindle fibers pulled the chromosomes towards the poles, these bridges broke into fragments, which either moved towards one pole or formed micronuclei. In either case, the daughter cells had an unequal distribution of chromatin material. Similar divisions at the second phases of division lead to abnormal pollen grains. Such pollen grains were usually non-viable and unable to fertilize the egg, leading to lower seed set.

Similar stickiness in mitotic cells might have hindered the normal growth of the plants and have lead to their weak morphology.

The occurrence of univalents and multivalents at metaphase have been reported in various plants like barley (Kumar and Singh, 2003) and broad bean (Bhat *et al.*, 2005b). Mutagen induced structural change in chromosomes and mutations might be responsible for the failure of pairing among homologous chromosomes and hence the presence of univalents. Multivalent formation can be attributed to irregular pairing and breakage followed by translocation and inversions. Stray bivalents at metaphase I/II seems to be caused by spindle disfunction.

The major abnormalities at anaphase I/II were bridges and laggards. Similar results were also observed earlier by different workers Bhat *et al.* (2006b) and Anis and Wani (1997). The laggards observed during the present study might be due to delayed terminalisation, stickiness of chromosomal ends or because of failure of chromosome movement (Permjit and Grover, 1985; Jayabalan and Rao, 1987; Soheir *et al.*, 1989). According to Bhattacharjee (1953) acentric fragments or laggards may result in the formation of micronuclei at telophase II and ultimately variation in number and size of pollen grains resulting from a mother cell. Anaphasic bridge formation was attributed to interlocking of bivalent chromosomes (Bhattacharjee, 1953), failure of chiasmata in a bivalent to terminalize (Saylor and Smith, 1966); unequal exchange or dicentric chromosomes etc. Besides these, the transmigration of chromatin material with cytotoxic connections might have resulted in altered number of chromosomes. In a few PMCs, deviation of chromosome number from the normal has been attributed to the cytotoxicity. Cytotoxicity between and among different stages of meiosis was also earlier reported Maria De Souza and Pagliarini (1997) and Bhat *et al.* (2005b). It is considered to be a source of production of aneuploid and polyploid gametes (Koul, 1990; Yen *et al.*, 1993; Kurobane *et al.*, 1979; Bhat *et al.*, 2006a).

Seed germination, pollen fertility and seedling survival were lower in DES than SA and showed dose dependent decrease in both the mutagens (Table 2).

Reduction in seed germination in mutagenic treatments has been explained due to delayed or inhibition in physiological and biological processes necessary for seed germination which include enzyme activity (Chrispeeds and Varner, 1976), hormonal imbalance (Ananthaswamy *et al.*, 1971) and inhibition of mitotic process (Sato and Gaul, 1967).

According to Sato and Gaul (1967) Natrajan and Shivshankar (1965) the reduction in seedling survival is attributed to cytogenetic damage and physiological disturbances. The greater sensitivity at higher mutagenic level has been attributed to various factors such as changes in the metabolic activity of the cells (Sree Ramula, 1972), inhibitory effects of the mutagens (Maherchandari, 1975) and to disturbance of balance between promoters and inhibitors of growth regulators (Krishna, 1984). The mutagens also cause disturbances in genetical and physiological activities leading to the death of the cells.

Pollen fertility is an index of meiotic behaviour. Greater the chromosomal abnormality, greater will be the pollen sterility. In the present investigation, pollen fertility decreased with the increasing concentrations of both the

mutagens. Similar decrease in fertility was reported earlier by Reddi (1977) and Bhat *et al.* (2007). According to Reddy and Rao (1982), the pollen sterility was the result of interchanges of segments between non-homologous chromosomes and considered that the presence of laggards, univalents, micronuclei and stickiness were closely associated with pollen sterility.

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