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Comparative Analysis of Mitotic Aberrations Induced by Diethyl Sulphate (DES) and Sodium Azide (SA) in *Vicia faba* L. (Fabaceae)

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Abstract: The present investigation provides a comparative account of different concentrations (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06%) of diethylsulphate (DES) and Sodium Azide (SA) on mitotic aberrations, seed germination, seedling survival, plant height and mitotic index in *Vicia faba* L. variety major. The control plants were normal while as treated ones showed significant alterations. The mutagens caused dose dependent decrease in seed germination, seedling survival, plant height and mitotic index. All the parameters were found negatively affected and were positively correlated with mutagenic concentrations. The cytological study revealed various types of mitotic aberrations, among them the dominant were fragments, stickiness, precocious separation, c-metaphase, ring chromosomes, unequal separation, laggards, bridges, micronuclei, disturbed anaphase etc. Stickiness and fragments were more frequent as compared to other types.

Key words: DES, SA, *Vicia faba*, mitotic index, mitotic aberrations

INTRODUCTION

Cytological analysis with respect to either mitotic or meiotic behaviour is one of the most dependable index to estimate the potency of mutagens. Therefore, investigations on mitotic 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. The mitotic variations so induced in root tip cells of *V. faba* L. may be useful in studying the mode of action of the mutagens as well as in inducing the cytomorphological mutations at later stage.

Vicia faba L. (2n = 12, Fabaceae) commonly known as broad bean is an important pulse 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. It is consumed either green or dried, fresh or canned. It is a common breakfast food in the middle east, Mediterranean region, China and Eithopia (Bond *et al*., 1985).

The aim of the present study was to investigate the comparative cytotoxic effects of DES and SA on mitotic division in root tip cells of broad bean (*Vicia faba* L.) and to explore their possible genetic consequences on seed germination, plant height and seedling survival and their potential to induce genetic variability which may prove asset for cytogeneticists and plant breeders for the improvement of *Vicia faba* L.

MATERIALS AND METHODS

The healthy and uniform sized seeds of *Vicia faba* L. var. Major were procured from IARI, New Delhi. 12 sets of seed each containing 100 seeds, were treated with six different concentrations (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06%) of DES and SA for 6 h, after presoaking for 14 h. During treatment the beakers were shaken frequently to provide sufficient aeration to the seeds. These were thoroughly washed in running tap water for half an hour to remove the residual effect of mutagen sticking to the seed coat. One set of seeds presoaked in double distilled water was kept untreated to act as control. Each set containing 100 seeds was divided into two parts: Each part of 50 seeds in each concentration as well as control, kept in petriplates lined with moist cotton, was subjected to the BOD experiment at 25±2°C in cytogenetics research laboratory, Department of Botany, Aligarh Muslim University, Aligarh, till the emergence of radicals. The radicals (2 mm) were carefully cut and fixed in a mixture of Glacial Acetic Acid (GAA) and absolute alcohol in the ratio of 1:3 for 24 h and stored in 70% alcohol. The root tips were washed and hydrolysed in 1N Hcl and for 10 min at 60°C. After washing the tips were transferred to 4% Iron alum for 30 min. Finally the tips were transferred to 2% aceto-haematoxylin stain for 2 h. The squashing

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was done in 45% GAA. The slides showing uniform spreading were sealed with wax and screened for mitotic study. Mitotic index was calculated for all the concentrations of both the mutagens. Microphotographs were taken using Olumpus Research Photomicroscope. Another part of 50 seeds were sown in rabi session of 2005-06 in earthen pots and were kept in Net house of Botany Department, Aligarh Muslim University, Aligarh, to study the mutagenic effect on seed germination, seedling survival and plant height.

RESULTS AND DISCUSSION

Dose dependent reduction was observed in seed germination, seedling survival, plant height and mitotic index (Table 2). The mitotic aberrations were positively correlated with mutagenic concentrations (Table 1). The highest values of MI (43.47% in DES and 41.60% in SA) were recorded in 0.01% concentrations of both the mutagens and the lowest values (31.45% in DES and 28.57 in SA) were observed in 0.06% concentration of both the mutagens. In general, among the two mutagens DES was found to be more effective. The fall in mitotic index clearly indicate the prolongation of cell generation time.

Mitosis was perfectly normal in control plants showing 12 chromosomes at metaphase (Fig. 1a) which segregated into 12:12 at anaphase and telophase was normal. However, a number of mitotic aberrations were recorded in root-tip cells treated with different concentrations of the mutagens. The most frequent aberrations were fragments, stickiness, precocious separation, c-metaphase, ring chromosomes, laggards, bridges, unequal separation, micronuclei etc. The maximum percentage of aberrations were found at higher dose of each mutagen. Stickiness (Fig. 1e and 1f), fragments (Fig. 1c), c-metaphase (Fig. 1d), were dominant aberrations recorded at metaphase. The observed decrease in the frequency of mitotic index in root meristems treated with DES and SA may be the result of accumulation of c-metaphase configuration. Inhibition of ATPase by the

Table 1: Frequency of chromosomal aberrations at different stages of mitosis induced by DES and SA in root tip cells of *Vicia faba* L.

Concentrations (%)	Total No. of RTCs observed	Total No. of dividing RTCs observed	Total No. of abnormal RTCs	Metaphase (%)				
				Fragments	Stickiness	Precocious separation	c-metaphase	Ring chromosomes
Control	1250	600	-	-	-	-	-	-
DES								
0.01	1035	450	33	1.11(5)	0.89(4)	-	-	0.44(2)
0.02	1084	455	66	1.54(7)	1.76(8)	0.88(4)	1.32(6)	0.88(4)
0.03	1132	463	98	2.16(10)	2.60(12)	1.30(6)	2.16(10)	1.10(5)
0.04	1158	460	133	2.61(12)	3.48(16)	1.96(9)	3.04(14)	1.52(7)
0.05	1215	465	161	3.44(16)	3.44(16)	3.01(14)	3.66(17)	1.51(7)
0.06	1488	468	199	4.27(20)	5.13(24)	3.85(18)	4.27(20)	1.92(9)
SA								
0.01	1120	466	21	0.86(4)	0.43(2)	-	-	0.21(1)
0.02	1195	460	50	1.30(6)	1.30(6)	0.43(2)	0.87(4)	0.65(3)
0.03	1227	465	75	1.94(9)	2.15(10)	0.86(4)	1.51(7)	0.36(4)
0.04	1342	462	111	2.60(12)	2.81(13)	1.73(8)	2.60(12)	1.30(6)
0.05	1472	472	143	3.39(16)	3.39(16)	2.33(11)	3.39(16)	1.27(6)
0.06	1645	470	185	4.47(21)	5.11(24)	3.40(16)	3.83(18)	1.70(8)

Table 1: Continued

Concentrations (%)	Anaphase (%)					Telophase (%)			
	Laggards	Bridges	Unequal separation	Fragments	Disturbed anaphase	Micronuclei	Bridges	Laggards	Abnormal RTCs (%)
Control	-	-	-	-	-	-	-	-	-
DES									
0.01	0.44(2)	1.33(6)	-	1.11(5)	0.89(4)	-	0.89(4)	0.67(3)	7.33
0.02	0.88(4)	1.54(7)	-	1.54(7)	1.76(8)	-	1.32(6)	1.10(5)	14.51
0.03	1.73(8)	1.73(8)	0.65(3)	1.98(9)	2.16(10)	0.86(4)	1.30(6)	1.54(7)	21.17
0.04	1.96(9)	3.04(14)	0.87(4)	2.17(10)	2.39(11)	1.52(7)	2.39(11)	1.96(9)	28.91
0.05	2.15(10)	3.01(14)	1.29(6)	2.58(12)	2.80(13)	2.37(11)	3.01(14)	2.37(11)	34.62
0.06	2.56(12)	3.85(18)	1.28(6)	2.99(14)	3.42(16)	2.56(12)	3.42(16)	2.99(14)	42.52
SA									
0.01	-	0.64(3)	-	0.86(4)	0.64(3)	-	0.43(2)	0.43(2)	4.51
0.02	0.43(2)	1.30(6)	-	1.30(6)	1.52(7)	-	0.87(4)	0.87(4)	10.86
0.03	1.29(6)	1.29(6)	-	1.72(8)	1.94(9)	3.08(2)	0.86(4)	1.29(6)	16.13
0.04	1.30(6)	2.38(11)	0.43(2)	1.95(9)	2.16(10)	1.30(6)	1.95(9)	1.52(7)	24.03
0.05	1.48(7)	2.54(12)	0.85(4)	2.33(11)	2.54(12)	2.12(10)	2.54(12)	2.12(10)	30.30
0.06	2.13(10)	3.40(16)	0.85(4)	2.77(13)	3.19(15)	2.55(12)	3.19(15)	2.77(13)	39.36

Within parenthesis actual number of RTCs observed. (–) = Chromosomal aberrations not found

Table 2: Comparative account of mitotic index, seed germination, seedling survival and plant height affected by DES and SA in root tip cells of *Vicia faba* L.

Concentrations (%)	Total No. of cells observed	Total No. of dividing cells	Mitotic index (%)	Seed germination (%)	Seedling survival (%)	Plant height $\bar{X}$ ±SE
Control	1250	600	48.00	90.22	95.37	57.42±0.44
SA						
0.01	1120	466	41.60	88.44	93.36	58.23±0.64
0.02	1195	460	38.49	86.22	90.12	60.31±.53
0.03	1227	465	37.90	82.45	88.63	56.40±.83
0.04	1342	462	34.43	80.22	82.41	53.56±.51
0.05	1472	472	32.21	76.32	80.67	52.11±.72
0.06	1645	470	28.57	74.56	77.52	51.82±.38
DES						
0.01	1035	450	43.47	87.52	92.21	56.72±.58
0.02	1084	455	41.97	84.47	88.15	58.41±.72
0.03	1132	463	40.90	82.23	86.42	55.37±.43
0.04	1158	460	39.72	79.38	83.53	54.52±.64
0.05	1215	465	38.27	74.74	79.37	53.87±.31
0.06	1488	468	31.45	73.32	76.43	49.62±.29

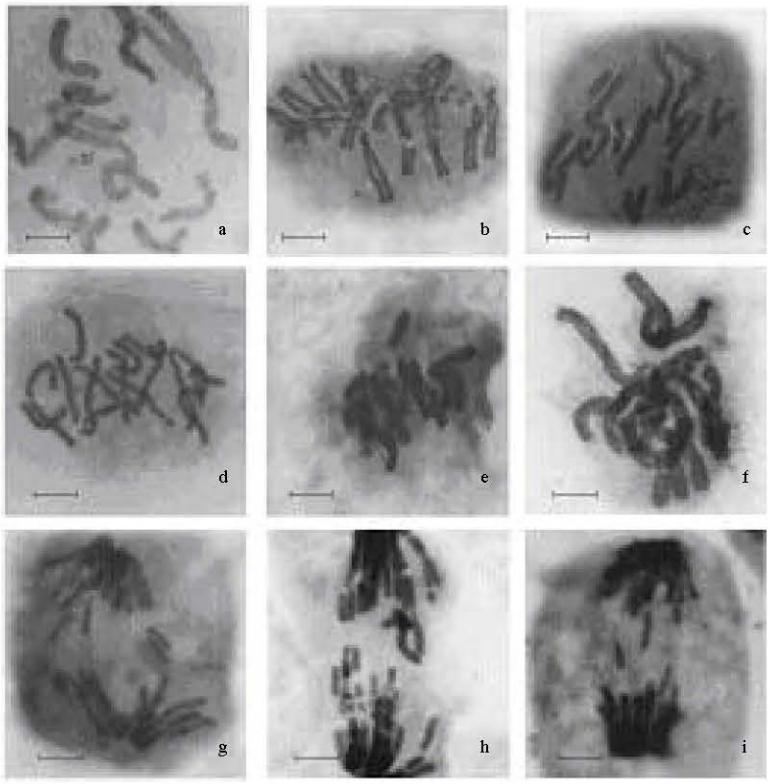

Fig. 1(a-I): Microphotographs showing different chromosomal aberrations in root tip cells of *Vicia faba* L.
a) Metaphase (Control), b) Metaphase chromosomes showing achromatic lesions, c) Fragmentation at metaphase, d) c-metaphase, e) Sticky metaphase with forward chromosome, f) Stickiness and ring chromosome at metaphase, g) 4 fragments at anaphase, h) Laggards and fragments at anaphase, I) 2 fragments at telophase, Bar = 10 μ

mutagens might be the cause of spindle disorganization (Reddy and Bashamohideen, 1991) leading to chromosome vagrancies as well as c-metaphases. The DES and SA showed 1.11 and 0.86% fragments in 0.01% concentration at metaphase respectively, while as 4.27 and 4.47% fragments were observed at 0.06% of DES and SA, respectively. In some cells broken achromatic lesions were also present (Fig. 1b). Stickiness, one of the

dominant abnormality at metaphase were 0.89 and 0.43% at 0.01% concentration and 5.13 and 5.11% at 0.06% concentration of DES and SA, respectively. Stickiness could be due to the depolymarization of nucleic acids caused by mutagenic treatments or due to partial dissociation of nucleo-proteins and alternations in their patterns of organization (Evans, 1962). Jayabalan and Rao, (1987) suggested stickiness might be due to disturbances in cytochemically balanced reactions. Gaulden (1987) attributed chemically induced stickiness to the action of mutagens on the histone proteins leading to improper folding of DNA. The induction of ring chromosomes suggest the possibility of two breaks occurring in the same chromosome. It is opined that if two breaks occur in the same chromosome, a ring chromosome may be formed by the process of rejoining.

At anaphase, bridges, laggards and fragments (Fig. 1g and 1h) were dominant. The laggards were 0.44 and 0.43% in 0.01% of DES and SA respectively. While as the percentage of bridges were 1.33 and 0.64 in 0.01% of both the mutagens. The lower concentrations of both the mutagens i.e., 0.01-0.03% of SA, 0.01 and 0.02% of DES did not showed unequal separation. However, 0.04 and 0.03% of SA and DES showed 0.43 and 0.65% of RTCs with unequal separation respectively and the highest percentage of RTCs with unequal separation were observed at 0.06% of both the mutagens. The DES showed 1.11 and 2.99% fragments at 0.01 and 0.06%, respectively. While as SA showed 0.86 and 2.77% fragments at 0.01 and 0.06%, respectively.

The dominant telophasic abnormalities were laggards (Fig. 1i), bridges and micronuclei in both the mutagens. Micronuclei observed may be attributed either to clastogenic events of the cells concerned or the result of single or a group of chromosomes forming individual nuclei. Based on the size of the micronuclei observed in the present study, most of the micronuclei observed might be the product of the later phenomenon (Degrassi and Rizzoni, 1982; Sparrow and Singlaton, 1953). The DES showed 0.89 and 0.67% bridges and laggards respectively at 0.01%. While as the corresponding value for bridges and laggards were 3.42 and 2.99% in 0.06% of DES. The SA showed 0.43% bridges and laggards at 0.01% while as 3.19 and 2.77% bridges and laggards were observed at 0.06% of SA. Bridges and laggards with or without fragments were found both at anaphase and telophase, bridges without fragments were found in lower concentrations while as bridges with fragments were found at higher concentrations of the mutagens, both single and double bridges were found but the multiple bridges were not also rare. Multiple bridges were mostly found at anaphase and the single bridges at telophase. Occurrence of chromosome bridge may be due to stickiness or formation of dicentric chromosomes caused by breakage and reunion (Dempong and Maxwell, 1973). Chromosomal bridges may also be due to the chromosomal stickiness and subsequent failure of anaphasic separation or may also be attributed to unequal translocation or in origin of chromosomal fragments. Lagging chromosomes may be explained on the basis of abnormal spindle formation and failure of chromosome movement. Mutagens may have caused chromosomal breakage by binding to DNA at GC rich regions and making the DNA unstable and hence the formation of fragments and laggards. Disturbed anaphase was indicated by irregular chromosome spreading and moving unsynchrosingly towards both poles. This may be due to the defective formation of the spindle apparatus (Badr, 1986, Abrham and Rajlakshmy, 199). The present effect of DES and SA is probably due to direct effect of mutagens on viscosity of protoplasm and DNA proteins of the chromosomes as stated by Savage (1975) and Abderrhman (1997).

Types of abnormalities as multipolarity, disturbed anaphase, c-metaphase, bridges, laggards as observed in the present investigation showing the direct effect of mutagen on spindle apparatus. These aberrations mostly cause somatic instability. The non-disjunction and micronuclei were also observed as a penalty of lagging and unoriented types of aberrations.

In the present investigation, there is strong negative relationship between MI and percentage of abnormalities as previously recorded for many chemical treatments. (Banerjee, 1992; Anis and Wani, 1997). Although the mechanism of mitotic disturbance is unknown, the possibility of genetic instability is ensured by using these chemical mutagens and we may conclude that genetic instability is associated with mutagenic treatments. The comparison of MI and aberrations brought by the two chemicals indicates that *Vicia faba* is more sensitive to DES than SA. Although both the chemicals showed inhibitory effect on seed germination, plant height and seedling survival but both the mutagens have enough potential to induce genetic variability for quantitative and qualitative variations which can be favourably exploited by cytogeneticists and plant breeders in improving the genotype of *Vicia faba* L.

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