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Studies on cytotoxicity induced by DES and SA in *Vicia faba* var. *major*

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Abstract: The effect of diethyl sulphate (DES) and sodium azide (SA) was studied on the meiotic behaviour of *Vicia faba* L. var. *major* in the M_1 generation. Five concentrations of SA (0.01%, 0.02%, 0.03%, 0.04%, and 0.05%) were combined with DES (0.02%) to study the synergistic and antagonistic effects of these 2 mutagens. A dose-dependent increase in chromosomal aberrations was recorded in populations treated with both the mutagens individually and their combination doses. The main chromosomal aberrations were stickiness, precocious separations, univalents, multivalents, laggards, unequal separations of chromosomes, micronuclei, disturbed polarity, multinucleate condition, and cytomixis. The stickiness and stray bivalents at metaphase I/II, bridges with or without fragments at anaphase I/II, disturbed polarity, and cytomixis at telophase I/II were the dominant anomalies. The univalents, rod bivalents, and multivalents showed dose-dependent increases, whereas the ring bivalents and chiasma frequency per cell showed dose-dependent decreases both in single as well as in combined mutagenic treatments as compared to the control.

Key words: *Vicia faba*, chromosomal aberrations, DES, SA, cytotoxicity, chiasma frequency

Introduction

Although induced mutations have great relevance for raising superior plant types in different crop plants, most of the mutations are lethal or semi-lethal and do not have any practical value possibly due to doses monitored or due to the mutagens employed. Thus to administer successful mutagenesis, selection of efficient mutagens and their treatment doses are pre-requisite as mutagens are potential tools for direct improvement or bringing about certain qualitative and quantitative changes in crop plants. Cytological analysis with respect to either mitotic or meiotic behaviour is considered one of the most dependable indexes to estimate the potency of mutagens. Therefore, investigations on meiotic aberrations and their genetic consequences form an integral part of most mutation studies. They also provide a considerable clue to assess the sensitivity of plants for different mutagens.

Many researchers (Zeerak, 1991; Dhamyanthi & Reddy, 2000; Singh et al., 2003) have assessed the mutagenic efficiencies of different mutagens on different crops and their results seem to be entirely specific for particular species or even for the varieties.

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Vicia faba L. (2n = 12), of the family Fabaceae, is commonly known as broad bean. It is an important pulse crop and is used as a vegetable, silage, forage, and stock feed. It is a common breakfast food in the Middle East, the Mediterranean region, China, and Ethiopia. The genotype of *Vicia faba* is homozygous because of self pollination; therefore, there is a need for its further improvement, which can be done by creating additional genetic variabilities in its genotype through mutagenesis. Chemical mutagens provide a good scope for selection as a tool for inducing alterations in the genotype to enhance the variability of characters. Hence, the present investigation was undertaken to study the DES and SA induced cytotoxicity and also to assess their roles for creating additional genetic variability for crop improvement.

Materials and methods

Abbreviations

DES: Diethyl sulphate, SA: Sodium azide, PMCs: Pollen mother cells, MI: Metaphase first, MII: Metaphase second, AI: Anaphase first, AII: Anaphase second, TI: Telophase first, TII: Telophase second.

Healthy and dry seeds (10%-12% moisture) of *Vicia faba* var. *major* were obtained from Cytogenetics and Mutation Breeding Laboratory, Department of Botany, Aligarh Muslim University, Aligarh. The seeds were subjected to 6 h treatment with 5 different concentrations (0.01%, 0.02%, 0.03%, 0.04%, 0.05%) of DES and SA after pre-soaking for 14 h in water. All 5 doses of SA were combined with a 0.02% concentration of DES. One percent stock solution of DES and SA were prepared and from their stock solution different concentrations of DES and SA were prepared by using the formula $S_1V_1 = S_2V_2$, where

S_1 = Strength of the stock solution.

V_1 = Volume of the stock solution

S_2 = Strength of the desired solution

V_2 = Volume of the desired solution

The treated seeds were thoroughly washed in tap water for 30 min after mutagenic treatment to remove the mutagens stuck to the seed coat. One set of seeds was kept untreated to act as a control for comparison. All the sets of treated as well as control (50 seeds in each set) seeds were sown in earthen pots of 30 cm size (10 seeds in each pot) to raise the 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. Microphotographs were taken from freshly prepared slides using a X30 Olympus Research Photomicroscope.

Results

Meiosis was perfectly normal in the control plants showing 6 perfect bivalents at metaphase-1 (Figure 1a), which showed 6:6 segregation at anaphase-I (Figure 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 multivalents (Figure 1c, d, and e), laggards (Figure 1f), bridges (Figure 1g), early anaphase with some bivalent on the equator (Figure 1h), stickiness, stray bivalents, unequal separation, micronuclei, disturbed polarity multinucleate condition, and cytomixis. A dose-dependent increase in meiotic aberrations was observed both in single as well as in combined mutagenic treatments. The frequency of cytological aberrations was greater at higher doses of the mutagens and combined treatments showed more chromosomal aberrations than the single mutagenic treatments. Although most of the abnormalities were similar in all the treatments, stickiness and stray bivalents were frequently found at metaphases-I/II. Bridges and laggards were dominant among the abnormalities found at anaphase-I/II, whereas disturbed polarity and cytomixis were dominant at telophases. The chromosomal aberrations were more at the metaphase, followed by the anaphase and telophase stages (Table 1).

The chromosomal bridges with or without fragments were found at anaphase I. However, the frequency of bridges with fragments was found in the population treated with higher concentrations of mutagens. Mostly cells with single and double bridges were observed. However, the cells with single bridges were frequent in single mutagenic treatments and the

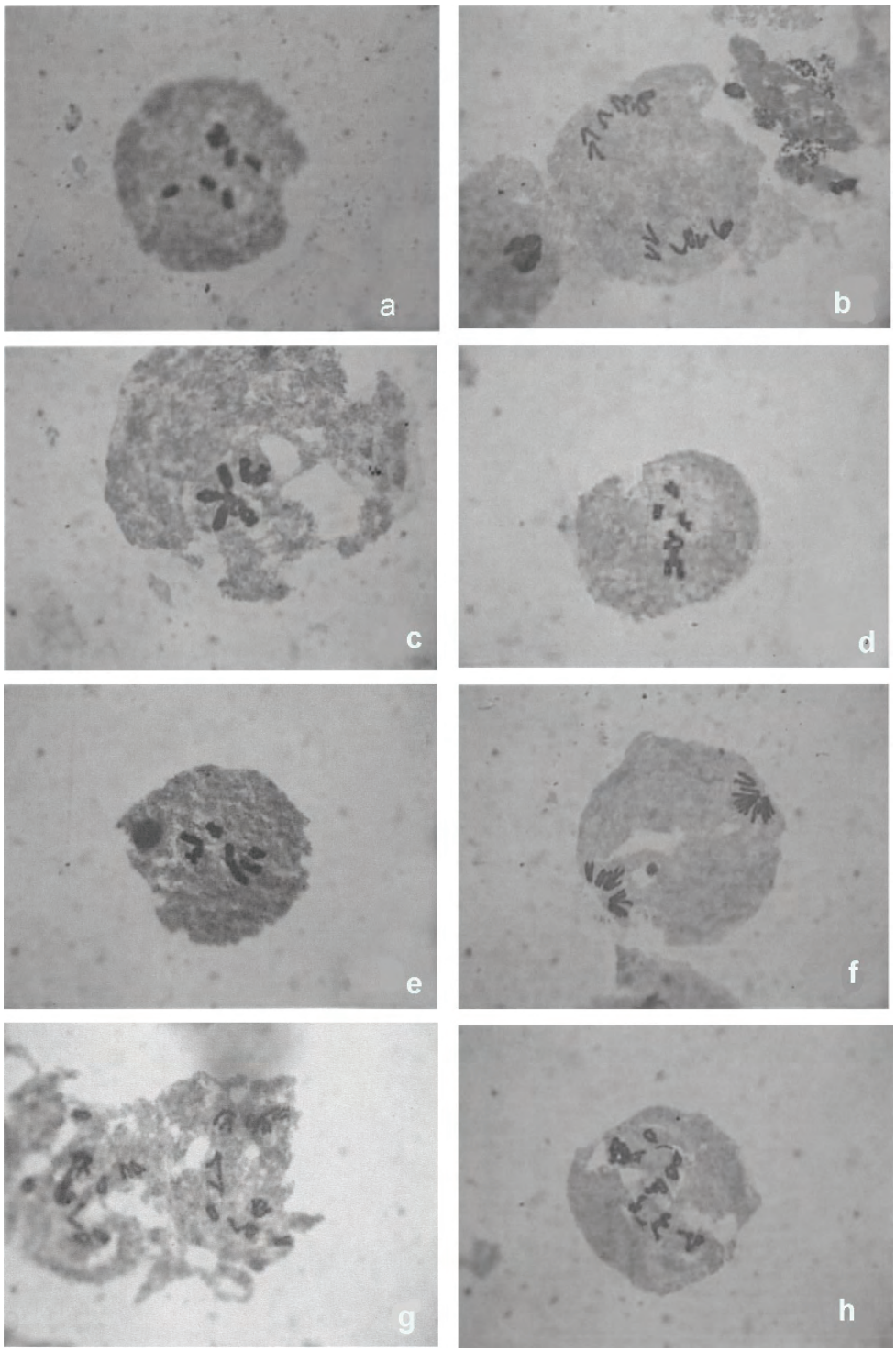

Figure 1. a. PMC showing 6 bivalent at metaphase-I in the M_1 generation of *Vicia faba* (Control); b. PMC showing 6:6 segregation at anaphase-I in the M_1 generation of *V. faba* (Control); c. PMC showing 1 octavalent + 2 bivalents at metaphase-I in the M_1 generation of *V. faba*; d. PMC showing 1 hexavalent + 3 bivalents at metaphase-I in the M_1 generation of *V. faba*; e. PMC showing 1 tetravalent + 4 bivalents at metaphase-I in the M_1 generation of *V. faba*; f. PMC showing laggard at anaphase-I in the M_1 generation of *V. faba*; g. PMC showing bridges at anaphase-II in the M_1 generation of *V. faba*; h. PMC showing early anaphase I (the other bivalent still on equator) in the M_1 generation of *V. faba*.

Table 1. Percentage of meiotic aberrations induced by DES, SA, and their combinations doses at different stage of meiosis in the M_1 generation of *Vicia faba* var. *major*.

			Metaphase-I/II				Anaphase-I/II			Telophase-I/II				
Concentration	**Total no. of PMCs observed**	**Total abnormal PMCs observed**	Stickiness	Precocious separations	Stray bivalents	Fragments	Laggards	Bridges	Unequal separation	Micronuclei	Multinucleate conditions	Disturbed polarity	Cytomixis	**Abnormal PMCs (%)**
Control	200	-	-	-	-	-	-	-	-	-	-	-	-	-
DES 0.01% (L)	210	15	(3)1.42	(2) 0.95	(4) 1.90	(3) 1.42	-	-	-	(1) 0.47	-	-	(2) 0.95	7.14
0.02% (L)	205	36	(4) 1.95	(3) 1.46	(5) 2.44	(3) 1.46	(2) 0.97	(5) 2.44	(3) 1.46	(2) 0.97	(2) 0.97	(3) 1.46	(4) 1.95	17.56
0.03% (I)	208	46	(6) 2.88	(3) 1.44	(6) 2.88	(4) 1.92	(3) 1.44	(5) 2.40	(3) 1.44	(3) 1.44	(3)1.44	(4) 1.92	(6) 2.88	22.11
0.04% (I)	215	57	(8) 3.87	(4) 1.86	(6) 2.79	(5) 2.32	(4) 1.86	(5) 1.86	(4) 1.86	(4) 1.86	(4) 1.86	(4) 1.86	(6) 2.79	26.51
0.05% (H)	206	63	(10) 4.85	(5) 2.42	(7) 3.39	(5) 2.42)	(6) 2.91	(4) 1.94	(4) 1.94	(4) 1.94	(4) 1.94	(5) 2.42	(7) 3.39	30.58
Pooled Mean			**(6.2) 2.96**	**(3.4) 1.62**	**(5.6) 2.68**	**(4) 2.50**	**(3.75) 1.79**	**(4.75) 2.27**	**(3.5) 1.67**	**(2.8) 1.33**	**(3.27) 1.55**	**(4) 1.91**	**(5) 2.39**	**20.78**
SA 0.01% (L)	207	10	(2) 0.96	(1) 4.83	(3) 1.44	(2) 0.96	-	-	-	(1) 0.48	-	-	(1) 0.48	4.83
0.02% (L)	211	26	(3) 1.42	(2) 0.94	(4) 1.89	(2) 0.94	(1) 0.47	(2) 0.94	(1) 0.47	(2) 0.94		(3) 1.42	12.32	12.32
0.3% (I)	217	37	(5) 2.30	(2) 0.92	(5) 2.30	(3) 1.38	(2) 0.92	(4) 1.84	(3) 1.38	(3) 1.38	(2) 0.92	(3) 1.38	(5) 2.30	17.05
0.04% (I)	219	43	(7) 3.19	(3) 1.36	(5) 2.28	(4) 1.82	(4) 1.82	(4) 1.82	(5) 2.28	(3) 1.36	(2) 0.91	(3) 1.36	(5) 2.28	19.60
0.05% (H)	220	54	(9) 4,09	(4) 1.81	(6) 2.27	(4) 1.81	(5) 2.27	(4) 1.81	(3) 1.36	(4) 1.81	(3) 1.36	(3) 1.36	(4) 1.81	(6) 2.72
Pooled Mean			**(5.2) 2.39**	**(2.4) 1.10**	**(4.6) 2.03**	**(3) 1.38**	**(3) 1.37**	**(4.25) 1.95**	**(3.5) 1.60**	**(2.4) 1.10**	**(2) 0.91**	**(3) 1.37**	**(4) 1.84**	**15.66**
DES+SA (L)														
0.02% DES +	215	18	(4) 1.86	(2) 0.93	(5) 2.32	(2) 0.93	(1) 0.47	-	-	(2) 0.93	-	-	(2) 0.93	8.37
0.01% SA (L)														
0.02%							+							0.03%
SA	209	28	(5) 2.39	(3) 1.43	(5) 2.39	(2) 0.95	(1) 0.48	(1) 0.48	(1) 0.48	(2) 0.95	(3) 1.34	(3) 1.43	(2) 0.95	13.39
0.02%							+							0.03%
SA (I)	205	37	(7) 3.41	(3) 1.46	(5) 2.43	(3) 1.46	(2) 0.97	(2) 0.97	(1) 0.49	(2) 0.97	(4) 1.95	(5) 2.44	(3) 1.46	18.04
0.02%							+							0.04%
SA (I)	208	46	(8) 3.84	(4) 1.92	(6) 2.88	(4) 1.92	(3) 1.44	(3) 1.44	(2) 0.96	(2) 0.96	(4) 1.92	(5) 2.40	(5) 2.40	22.11
0.01%							+							0.05
+ **SA** (H)	212	5	(13) 6.13	(5) 2.35	(6) 2.83	(6) 2.83)	(4) 1.88	(3) 1.41	(2) 0.94	(3) 1.41	(5) 2.35	(6) 2.83	(5) 2.35	27.35
Pooled Mean			**(7.4) 3.52**	**(3.4) 1.61**	**(5.4) 2.57**	**(3.4) 1.61**	**(2.2) 1.04**	**(2.75) 1.07**	**(1.5) 0.71**	**(2.2) 1.04**	**(4) 1.91**	**(4.75) 2.27**	**(3.4) 1.61**	**17.85**

Within parentheses in no. of cells, - = not observeds

cells with double bridges were observed in combined mutagenic treatments. The chromatin transmigration between PMCs through cytoplasmic channels was the common feature in treated plants.

At diakinesis 6 bivalents were found regularly in the control and chiasma frequency per cell was 21.00 (Table 2). A dose-dependent increase in univalents, rod bivalents, and multivalents, and a dose-dependent decrease in mean number of ring bivalents and number of chiasma per cell were recorded in both single as well as combined mutagenic treatments. The higher concentrations of mutagens were more effective than intermediate or lower concentrations in reducing the chiasma frequency per cell (Table 2).

Discussion

In the present investigation similar types of meiotic abnormalities were found in all the mutagenic treatments but the frequency of abnormalities was different in different treatments, indicating the different mutagenic potentials of mutagens against *Vicia faba* L.

Bivalents were found clumped in single or different groups at metaphase I due to stickiness. The stickiness could be due to depolymerisation of nucleic acids caused by mutagens or due to partial dissociation of nucleo-proteins and alterations in their pattern of organisation (Evans, 1962). Jayabalan and Rao (1987) suggested that stickiness of chromosomes

Table 2. Chromosomal associations and chiasma frequency at diakinesis in the M_1 generation of *Vicia faba* var. *major*.

Concentrations	Chromosomal association per cell				Chiasma frequency per cell
	Univalents $\bar{X} \pm SE$	Multivalents $\bar{X} \pm SE$	Rod bivalents $\bar{X} \pm SE$	Ring bivalents $\bar{X} \pm SE$	
Control	**0.00**	**0.00**	**1.00 ± .0.00**	**5.00 ± 0.00**	**21.00 ± 0.10**
DES 0.01%	0.40 ± 0.22	050 ± 0.26	1.30 ± 0.57	3.90 ± 0.54	19.20 ± 0.30
0.02%	0.40 ± 0.22	0.60 ± 0.26	1.40 ± 0.55	3.80 ± 0.44	19.00 ± 0.33
0.03%	0.50 ± 0.28	0.60 ± 0.30	1.00 ± 0.64	3.20 ± 0.38	18.60 ± 0.42
0.04%	0.60 ± 0.32	0.70 ± 0.36	1.50 ± 0.65	3.10 ± 0.32	18.20 ± 0.27
0.05%	0.70 ± 0.32	0.80 ± 0.38	15.0 ± 0.70	3.10 ± 0.36	18.00 ± 0.29
Pooled Mean ± SE	**0.52 ± 0.27**	**0.64 ± 0.31**	**1.34 ± 0.62**	**3.42 ± 0.40**	**18.6 ± 0.32**
SA 0.01%	0.50 ± 0.32	0.80 ± 0.32	1.40 ± 0.15	3.80 ± 0.64	18.60 ± 0.36
0.02%	0.50 ± 0.38	0.70 ± 0.36	1.50 ± 0.25	3.70 ± 0.54	18.20 ± 0.41
0.03%	0.60 ± 0.36	0.70 ± 0.036	1.20 ± 0.60	3.60 ± 0.60	18.00 ± 0.28
0.04%	0.60 ± 0.32	0.80 ± 0.40	1.70 ± 0.65	3.00 ± 0.62	17.80 ± 0.46
0.5%	0.80 ± 0.36	0.90 ± 0.38	1.70 ± 0.70	3.00 ± 0.44	17.60 ± 0.40
Pooled Mean ± SE	**0.60 ± 0.34**	**0.78 ± 0.36**	**1.50 ± 0.44**	**3.42 ± 0.56**	**18.04 ± 0.38**
DES+SA					
0.2% DES+0.01SA	**0.80 ± 0.94**	**1.10 ± 0.37**	**1.10 ± 0.22**	**3.00 ± 0.22**	**18.00 ± 0.35**
0.2%+0.02% SA	1.00 ± 0.32	1.20 ± 0.41	1.20 ± 0.27	3.00 ± 0.32	17.70 ± 0.51
0.2%+0.03%SA	1.00 ± 0.28	1.30 ± 0.44	1.30 ± 0.32	2.80 ± 0.34	17.50 ± 0.53
0.02% + 0.04% SA	1.20 ± 0.32	1.40 ± 0.40	1.50 ± 0.65	2.80 ± 0.64	17.20 ± 0.49
0.02%+0.05% SA	1.20 ± 0.36	1.50 ± 0.22	1.60 ± 0.75	2.50 ± 0.68	16.80 ± 0.51
Pooled Mean ± SE	**1.04 ± 0.44**	**1.30 ± 0.36**	**1.34 ± 0.42**	**2.82 ± 0.44**	**17.4 ± 0.47**

might be due to disturbances in cytochemically balanced reactions in the nucleic acids. However, it seems most probable that some kinds of gene mutations lead to incorrect coding of some non-histone proteins involved in chromosome organisation. 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 together (Gaulden, 1987).

In the present case, the mutagens used seem to be responsible for stickiness. 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-I to metaphase-I. Probably this was the main reason that the stickiness was predominant from metaphase-I onwards. Stickiness in chromosomes interfered with the normal arrangement of chromosomes at metaphase and further led to their inability to separate and the origin of thick sticky bridges. When the spindle fibres pull the chromosomes towards the poles, these bridges may break at any point. Similar divisions at the second part of meiosis lead to formation of abnormal pollen grains, which usually are non-viable, leading to a lower seed set.

The occurrence of univalents and multivalents at metaphase I has been reported in various plants like barley (Kumar & Singh, 2003) and broad bean (Bhat et al., 2005a). Mutagen induced structural changes in chromosomes might be responsible for the failure of pairing among homologous chromosomes and hence the occurrence of univalents.

Occurrence of multivalent formation may be attributed to irregular pairing and breakage followed by translocation and inversions. Stray bivalents at metaphase I seem to be caused by spindle dysfunction and clumping of chromosomes (Bhat et al., 2007).

The major abnormalities at anaphase I/II were bridges and laggards. 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 & Grover, 1985; Jayabalan & Rao, 1987; Soheir et al., 1989; Bhat et al., 2006a). Acentric fragments or laggards may result in the formation of micronuclei at telophase II and ultimately variation in the number and size of pollen grains (Bhat et al., 2007). Bridge formation at the anaphase may be attributed to interlocking of bivalents (Bhattracharjce, 1953) and failure of chiasmata in a bivalent to terminalise (Saylor and Smith, 1996); besides these, the transmigration of chromatin material with cytomictic connections might have resulted in altered numbers of chromosomes. Variation in chromosome number in few pollen mother cells may be due to cytomixis, which is considered a source of production of aneuploid and polyploid gametes (Koul, 1990; Yen et al., 1993; Bhat et al., 2006b).

Chiasma frequency was variable in the populations treated with different mutagens. However, the reduction in chiasma frequency was dose dependent in different mutagenic treatments. The reduction in chiasma frequency was greater in combination treatments. The reduction in chiasma frequency may be attributed to the nature and potency of mutagens and to the underlying factors such as complex structural changes or to the nature of the genes responsible for chiasma formation. Many factors such as temperature, season, age, and amount and portion of heterochromatin in the chromosomes affect recombination and therefore changes in chiasma frequency.

Conclusion

In the present investigation dose dependent DES and SA induced chromosomal aberrations and chiasma frequency were reported. The DES proved more effective than SA and in combination doses antagonistic effects were reported. Therefore, it is concluded that lower and intermediate individual and combined doses of DES and SA are effective mutagenic concentrations for inducing genetic variability for the improvement of economic characters as assessed by cytotoxicity induced by these 2 mutagens in *Vicia faba* in the M_1 generation.

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