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EMS AND SA INDUCED MEIOTIC ANOMALIES IN *PLANTAGO OVATA* FORSK.

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ABSTRACT: The anomalies in meiotic chromosomal behavior of *Plantago ovata* were studied on treating with EMS and SA. The various meiotic anomalies viz. stickiness, laggards, bridges, micronuclei, precocious separation, cytomixis were observed. The maximum percentage of meiotic aberrations was observed in 1.0% EMS (32.75%) and the minimum in 0.25% SA (6.5%). The frequency of meiotic abnormalities increased with increase in concentration of mutagen. The percentage of meiotic abnormalities was higher in EMS than SA treatments showing that EMS was more deleterious and induced more meiotic chromosomal aberrations might be leading to create more genetic variability.

Key words: *Plantago ovata,* EMS, SA, meiotic anomalies.

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INTRODUCTION

Plantago ovata, 'isabgol' is of known economic and medicinal significance [1]. It is cultivated for mucilaginous seeds and husk which is pharmaceutically used [6]. India dominates the world market in the production and export of isabgol but is not able to meet global demand on account of low productivity. Therefore, there is an immense need to increase the productivity by developing new superior varieties. The narrow genetic base on account of low chromosome number (2n=8) with small chromosome size are also major hindrance in its improvement through conventional breeding methods [14]. Hence, mutagenesis may be useful in increasing genetic variability [7, 8, 12, 16, 17]. The cytogenetic anomalies have been regarded as authentic parameter for estimating the mutagenic potential of a mutagen.

In view of this, an experiment was carried out to utilize the mutagenic effectiveness of ethyl methane sulphonate and sodium azide on the chromosomal behaviour of treated population to aim at identifying the suitable mutagenic treatments to generate maximum genetic variability.

MATERIALS AND METHODS

The fresh and viable seed material of *Plantago ovata* var. Niharika was procured from CSIR-Central Institute of Medicinal and Aromatic Plant, Lucknow. Thereafter, definite numbers of seeds were treated with freshly prepared concentrations of EMS ($CH_3SO_3C_2H_5$) and Sodium azide (NaN_3) viz. 0.25, 0.50, 0.75, 1.0 in phosphate buffer (pH 7.0) for 8 hours at room temperature. The control was also soaked in distilled water for same hours. Treated seeds were sown in three replicates to raise the M_1 generation in Randomized Block Design (RBD) in the experimental field of CIMAP, located at 26.5º N latitude and 80.50º E longitude and 120 m above mean sea level. The climate was semi-arid to sub tropical in nature.

Unopened flower buds of appropriate size from control as well as mutagen treated M_1 population were fixed in freshly prepared Carnoy's fixative (Absolute alcohol, Chloroform and Acetic acid in 6:3:1 ratio) for 24 hrs, washed and preserved in 70% alcohol, refrigerated at 4 ºC until use. The anthers were squashed in 2% aceto-carmine, gently heated to fasten the chromosome staining and pressed between the fold of blotting paper for chromosome separation. The slides were observed under light microscope (Nikon Labophot) and photographs of PMCs were made.

RESULTS AND DISCUSSION

The meiosis in control plants (2n=8), was fairly normal, showing 4 bivalents at metaphase I and 4:4 separation at anaphase I. A number of meiotic aberrations were observed in M1 generation plants, raised from seeds treated with different concentrations of EMS and SA (Fig 1: a-t and Table 1). The most frequent anomalies were chromosomal stickiness, precocious separation of chromosomes, un-orientation of bivalents, stray chromosomes and the multivalents were observed at metaphases. The chromatin bridges, laggards, unequal separation of chromosomes were common anomalies at anaphases. Disturbed polarity, bridges, laggards and micronuclei were seen at telophases. The highest percentage of chromosomal anomalies was found in plants raised out of EMS treatment. The overall percentage of anomalies ranged from 9.21 to 32.75 at 0.25 to 1.0%, EMS. The anomalies percentage from 6.5 to 27.05 ranged in SA treatments. A dose dependent increase in meiotic aberrations was found with increasing dose of the mutagens. Among all the anomalies, the most prominent aberrations were stickiness, found to be distributed in metaphase I/II to anaphase I/II; chromatin bridges, laggards and bridges were most frequently found at anaphase I/II to telophase I/II. The disturbed polarity and micronuclei were most frequently observed at telophase II. In addition to these, the non-disjunction of bivalents, univalents, tri-polarity, disorientation, non- synchronous division, ring formation were also observed in very low frequencies.

Table-1. Frequency of meiotic abnormalities induced by EMS and SA in *Plantago ovata* var. Niharika Forsk.

Treatment	Total no	Total no of	Metaphasic abnormalities %						Anaphasic abnormalities %						Telophasic abnormalities %				Oth. Ab.%	Total Ab. %
	dividing cells	ab. Cells	St	Pr	Stray	Mult	Unori	Fr	St	Lg	Br	Uneq	Mulp	Cytomi	Br	Lg	Dp	Micr		
EMS																				
Control	525	4	0.76	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.76
0.25%	716	66	1.67	0.83	0.83	0.41	0.27	-	0.97	0.69	0.27	0.14	-	-	0.27	0.55	1.11	-	0.83	9.21
0.50%	830	139	2.4	1.08	0.96	0.84	1.08	0.36	1.56	1.32	0.72	0.36	0.24	0.24	0.72	0.96	1.92	0.24	1.81	16.74
0.75%	890	204	2.02	1.46	1.46	1.12	1.79	0.78	2.24	2.02	1.12	1.01	0.33	0.33	0.89	1.23	2.8	0.11	2.24	22.92
1.00%	925	303	2.7	1.94	2.16	1.29	2.48	1.29	3.02	2.59	1.51	1.62	0.75	0.54	1.4	2.05	4.1	0.43	2.81	32.75
SA																				
Control	420	3	0.47	0.23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.7
0.25%	723	47	2.07	0.55	0.69	-	0.13	-	0.55	0.27	0.13	0.27	-	-	0.13	0.41	0.69	-	0.55	6.5
0.50%	813	100	2.21	0.86	0.73	0.49	0.49	0.24	1.35	0.98	0.61	0.27	0.13	0.12	0.49	0.61	1.47	-	1.23	12.3
0.75%	838	157	2.5	1.07	0.95	0.71	1.19	0.47	2.14	1.43	0.83	1.07	0.23	0.35	0.71	1.07	2.26	0.11	1.55	18.73
1.00%	850	230	2.82	1.64	1.52	1.05	2.11	0.82	2.82	2.23	1.17	1.52	0.47	0.47	1.05	1.64	3.05	0.23	2.35	27.05

St- stickiness, Pr- Precocious movement, Multi- Multivalent, Unori- Unorientation, Fr- Fragmentation, Br- Bridges, Lg- Laggards, Uneq- Unequal separation, Mulp- Multipolarity, Cytomi- Cytoplasmic connections, Dp- Disturbed polarity, Micr- Micronuclei, Oth. Ab- Other abnormalities.

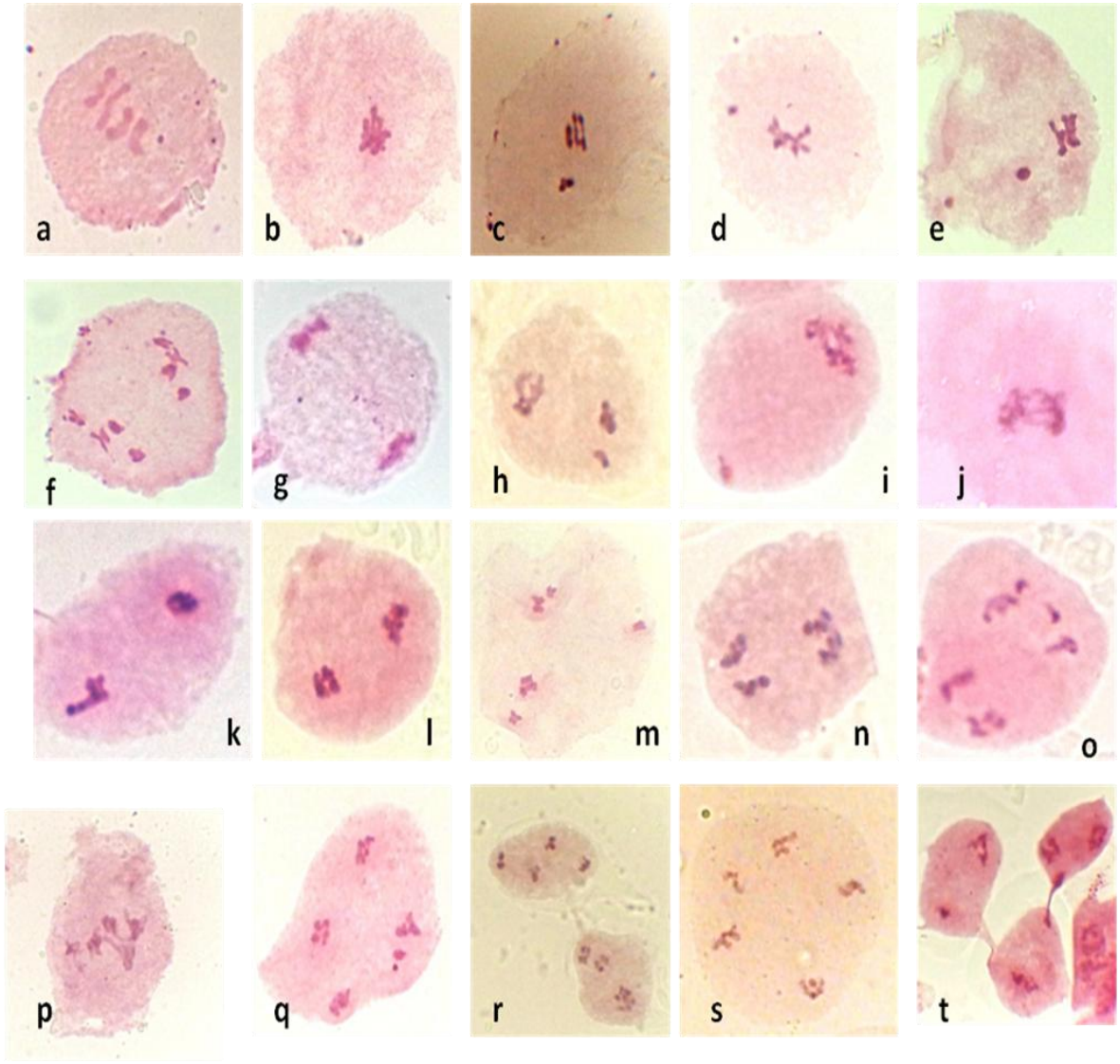

Fig 1. Meiotic anomalies induced by EMS and SA in *Plantago ovata* var. nihariak Forsk.

a- Metaphse I control, **b**- Meta I showing clumping of chromosomes, **c**- Meta I with stray chromosome, **d**- Meta I showing precocious separation of chromosome, **e**- Meta I with micronuclei, **f**- Control anaphase I, **g**- Ana I showing stickiness of chromosome, **h**- Anaphase I showing laggard, **i**- Ana I unequal separation of chromosome, **j**- Ana I bridges formation, **k**- Telo I with laggard, **l**- Meta II control, **m**- Meta II unorientation of chromosome, **n**- Ana II control, **o**- Ana II with laggards, **p**- Ana II multipolarity with chromatin bridge, **q**- Telophase I showing laggard, **r**- Telo II disturbed polarity, **s**- Telo II showing multipolarity, **t**- Cytomixix.

The stickiness, most prominent of the anomalies, observed at meta I/II to ana I/II, might be due to disturbances in cyto-chemically balanced reaction [5], while Gaulden [4] ascribed chemically induced stickiness is due to direct action of mutagen on histone proteins leading to improper folding of DNA. The major abnormalities at anaphase I was bridges which might be due to failure of chiasmata to terminalize and chromosome being stretched between the poles [13]. The laggards observed might be due to the delayed terminalization, stickiness of chromosomal ends or because of failure of the chromosomal movement [5, 15]. In metaphases the destruction or inhibition of spindle fibres causes scattering of chromosomes and un-orientation [10]. Disturbed polarity observed at anaphase and telophase might be due to spindle fibres. The micronuclei may be arisen from fragments of chromosome that failed to reach the pole and get integrated in daughter nuclei [11].

The various doses of EMS and SA were found to be considerably affecting the phenotypic traits in a number of plants. The chromosomal abnormalities observed in this study, have been reported in various plants on treatment with physical and chemical mutagens [3, 2]. Both of the mutagens exhibited similar types of meiotic aberrations in varying percentage showing that EMS and SA have different mutagenic potential. The meiotic abnormalities were increased alongwith increasing concentrations of mutagens. Mutagens may causes deviation in normal chromosome behavior which reflects (either positive or negative) in phenotypic traits of plant [9].

Though EMS and SA treatments exhibited similar types of meiotic abnormalities but the percentage of abnormalities was different, showing that both have different mutagenic potential. EMS was more deleterious than SA and induced more chromosomal aberrations and may lead to create more genetic variability in *Plantago ovata*, which could be exploited for improvement of this plant species.

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