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Research Article

GENOTOXIC EFFECT OF ETHYLMETHANE SULPHONATE AND SODIUM AZIDE IN *LINUM USITATISSIMUM* L.

Alka*, M.Y.K. Ansari, Towseef Mohsin Bhat, Sana Choudhary and Rumana Aslam
Cytogenetics Lab, Department of Botany,
Aligarh Muslim University, Aligarh-202002, U.P. (INDIA)
Email: alka.aditya@gmail.com

ABSTRACT: The present investigation provides a comparative account of cytological and developmental effects on EMS (Ethylmethane Sulphonate) and SA (Sodium Azide) on meiotic features in *Linum usitatissimum*. Seeds of *Linum usitatissimum* were treated with five concentrations (0.02, 0.04, 0.06, 0.08 and 0.10%) of EMS and SA. Different types of meiotic abnormalities as laggards, bridges, chromosome Stickiness, stray chromosomes, precocious movement of chromosomes and multi-nucleate conditions were recorded. Chromosomal aberrations and chiasma frequency per bivalent were found to be correlated with the concentration of chemical mutagens. In general, the meiotic abnormalities increased along with the increase in concentration in mutagens. The maximum frequency of abnormalities both structural and behavioural was induced by EMS in *Linum usitatissimum*.

Key Words: *Linum usitatissimum*, EMS, SA, Meiotic aberrations.

INTRODUCTION

Mutations can be beneficially utilized for tailoring better varieties of crop plants. But in general, chemical mutagens like EMS affects a wide range of chromosomal aberrations resulting into abnormal behavior. Cytological analysis with respect to meiotic behavior is considered to be one of the most dependable index to estimate the potency of mutagens. Many researchers have mutagens on different crops. While many researchers like [27, 21, 9 ,4] found chemical mutagens to be more effective than physical ones, others like [39, 41] found the reverse case. Induced mutagenesis has rarely been used to increase genetic variability in flax breeding [11]. Chemical mutagens provide a good scope for selection, as a tool for alteration in the genotype to enhance the variability of characters. Cytological studies constitute an important component in breeding program involving the development of new original forms with aid of experimental mutagenesis [38]. Chromosome rearrangements are one of the most frequently produced classes of mutations that result from the action of both physical and chemical mutagenic agents [10] . Chemical mutagens are the one cause of mutations in living organism. It is known that various chemicals have positive or negative effects on living organisms. Many of these chemicals have clastogenic (chromosomal damaging) effects on plants through reactive oxygen-derived radicals [31]. The increased frequency of meiotic anomalies with increasing concentrations of mutagens was reported in several crops by many workers [16, 3, 9, 23, 15] . A number of workers have reported the role of chemical mutagens in enhancing genetic variability in higher plants [31]. Sodium Azide is a mutagen and has been one of the most powerful mutagens in crop plants and its mutagenic potential has been reported in several screening assays. The promutagen NaN_3 is highly used with seeds to create mutation, which must be metabolized by plant cells to the mutagenic agent, presumably azideoalanine [24]. To effect of chemical mutagens depends on the permeability of seed coat and nature of the mutagens. Chemical mutagens provide a good scope for selection, as a tool for alteration in the genotype to enhance the variability of characters. The present report deals with the cytological effects of EMS and SA at a range of concentratons in *Linum usitatissimum*.

MATERIALS AND METHODS

Healthy and dried seeds of *Linum usitatissimum* L. was obtained from Acharya Narendradev University of Agriculture and Technology, Kumarganj, Faizabad, U.P. were subjected to 24 hrs treatment by five different concentrations (0.02, 0.04, 0.06, 0.08 & 0.10%) of EMS and SA after presoaking of 12 hrs. 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 20-30 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 hrs, washed and preserved in 70% alcohol. Anthers were squashed in 2% aceto-carmine, dehydrated in NBA series (50% acetic acid+ 50% N-butyl alcohol then passed through 100% N-butyl alcohol), mounted in Canada balsom and dried at 45^0C. Microphotographs were taken from freshly prepared slides using X30 olympus research photomicroscope.

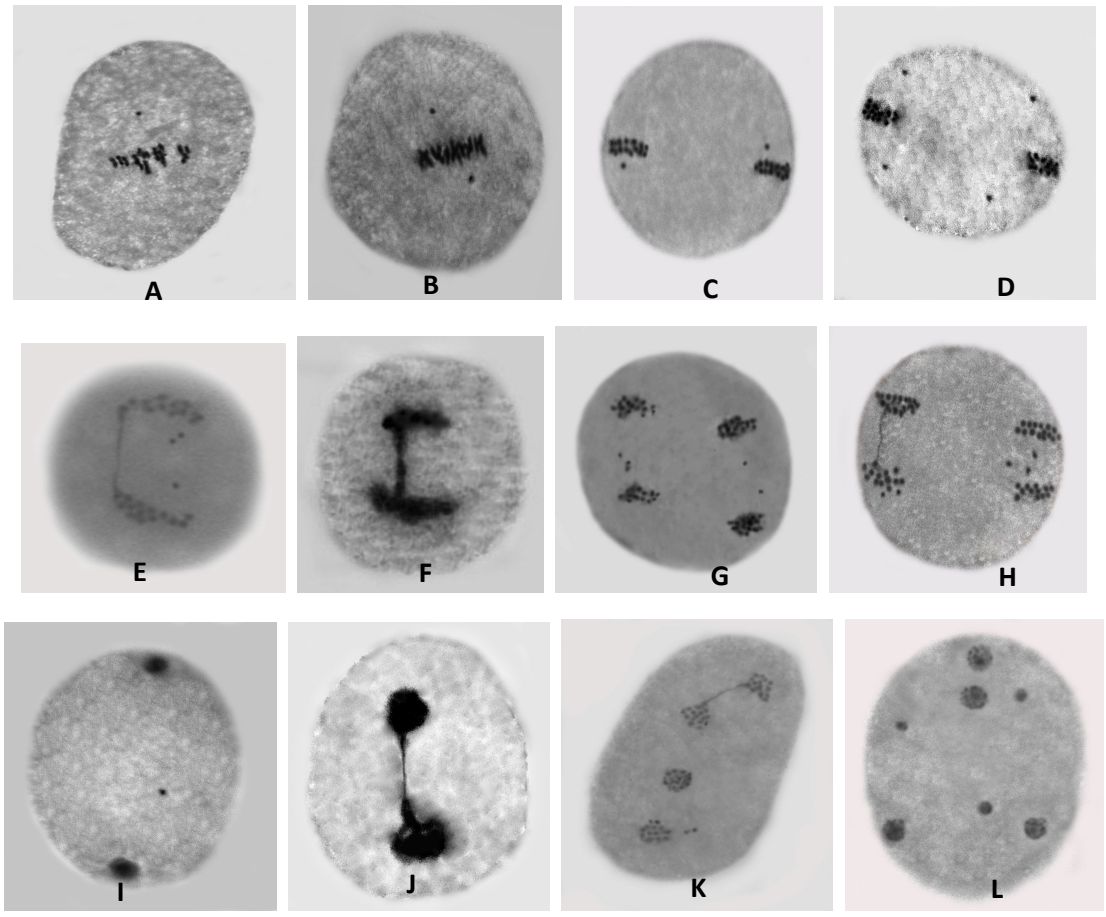

Fig.1 (A-L): Meiotic Aberrations Induced by EMS and SA in *Linum usitatissimum* L.

A: Metaphase-I: stray chromosomes (0.08% EMS). B: Metaphase-I: precocious separation of chromosomes (0.06% SA). C: Metaphase-II: stray chromosomes (0.02% SA). D: Metaphase-II: precocious separation of chromosomes (0.10% EMS). E: Anaphase-I: Chromatin bridge and 3 laggards (0.04% SA). F: Anaphase-I: Stickiness and chromatin bridge (0.06% EMS). G: Anaphase-II: five laggards (0.02% EMS). H: Anaphase-II: Chromatin bridge and 3 laggards (0.10% SA). I: Telophase-I: one laggard (0.08% EMS). J: Telophase-I: single chromatin bridge (0.08% SA). K: Telophase-II: Disturbed polarity, single chromatin bridge with two laggards (0.10% EMS). L: Telophase-II: Disturbed polarity and multinucleate condition (0.06% SA).

RESULTS

Meiosis was perfectly normal in the control plants showing 15 bivalents at metaphase-I which segregated into 15:15 at anaphse-I. However a number of meiotic aberrations were recorded in plants raised from seeds treated with different concentrations of the mutagens. Lower concentrations of mutagens revealed more or less normal pairing like that of control. However, a consistent increase in the frequency of various types of chromosomal abnormalities was observed with the increasing concentrations of mutagens . The maximum aberrations were found at higher doses of the mutagens (Table. 1). Although, most of the abnormalities were present in all the treatments, stray and precocious movements of chromosomes were dominant at metaphase I/II (Fig. 1: A, B, C & D). Among the abnormalities at anaphase I/II, laggards (Fig.1: E, G & H), bridges (Fig.1: E, F & H) and stickiness (Fig. 1: F) were dominant, whereas dominant telophasic abnormalities were bridges (Fig. 1: J & K), laggards (Fig. 1: I & K) and multinucleate condition (Fig.1: L). As a result percentage of abnormal PMCs ranged from 5.11-19.53% in EMS and 3.59-16.03% in SA (Table. 1). The maximum Frequency of abnormalities both structural and behavioural was induced by EMS in *Linum usitatissimum*. A dose dependent increase in the meiotic aberrations was observed in both the varieties . The maximum frequency of aberrations was recorded in the plants obtained by treating the seeds with the highest concentration of EMS. It is clear from the observations that EMS caused more chromosomal damage in *Linum usitatissimum*. A dose dependent decrease in chiasma frequency per cell and per bivalents was recorded in treated plants (Table 2).

Table. 1: Frequency of Meiotic Aberrations Induced by EMS and SA in *Linum usitatissimum* L.

Conc. of Mutagens	Total no. of PMCs observed	Metaphase-I/II			Anaphase-I/II				Telophase-I/II					Total No. of Abnormal PMCs	Percentage of total abnormal PMCs in all stages
		Precocious movement	Stray chromosomes	Percentage of abnormal cells	Laggards	Bridges	Stickiness	Percentage of abnormal cells	Laggards	Bridges	Multinucleate	Disturbed Polarity	Percentage of abnormal cells		
Control	133	-	-	-	-	-	-	-	-	-	-		-	-	-
EMS															
0.02%	137	2	1	2.19	1	1	-	1.46	1	1	-	-	1.46	7	5.11
0.04%	124	3	1	3.22	2	1	-	2.49	2	1	1	-	3.22	11	8.87
0.06%	130	3	2	3.84	2	1	1	3.07	2	2	1	1	4.61	15	11.54
0.08%	132	5	2	5.30	3	2	1	4.54	3	2	1	1	5.30	20	15.15
0.10%	128	6	3	7.03	3	2	2	5.47	4	2	2	1	7.03	25	19.53
SA															
0.02%	139	1	1	1.44	1	1	-	1.44	1	-	-	-	0.72	5	3.59
0.04%	130	2	1	2.31	1	1	1	2.31	1	1	-	-	1.54	8	6.15
0.06%	135	3	2	3.70	2	1	1	2.96	1	1	1	1	2.96	13	9.63
0.08%	137	4	2	4.38	3	1	1	3.65	2	1	1	1	3.65	16	11.68
0.10%	131	5	3	6.10	3	2	1	4.58	3	2	1	2	6.10	21	16.03

Table. 2: Effect of EMS and SA on chiasma frequency at Metaphase-I in *Linum usitatissimum* L.

Treatments	EMS		SA	
	No. of chiasma/cell	No. of chiasma/bivalent	No. of chiasma/cell	No. of chiasma/bivalent
Control	29.50	1.96	29.50	1.96
0.02%	27.70	1.84	28.60	1.90
0.04%	27.20	1.81	28.10	1.87
0.06%	25.90	1.72	27.40	1.82
0.08%	25.30	1.68	26.90	1.79
0.10%	24.80	1.65	25.80	1.72

DISCUSSION

In the present investigation the mutagenic treatments exhibited similar types of meiotic abnormalities but the percent abnormalities was different in different treatments. This showed that these mutagens have different mutagenic potential for *Linum usitatissimum*. Meiotic abnormalities increased along with the increasing concentrations of EMS and SA. Precocious movement of chromosomes was observed at metaphase I and II stages. Similar results were also observed earlier by different workers such as [25, 17]. Precocious movement of chromosomes might have occurred due to disturbed homology for chromosome pairing, disturbed spindle mechanism or inactivation of spindle mechanism [1] . It may also be ascribed to the presence of chromosome breakage. Some of the univalents disjuncted early and presumably this happened due to genic differences. Such chromosomal divergences in the form of precocious movement is pointed towards structural differentiation of homologus pair. Stray chromosomes at metaphase I & II stages occurred in all concentrations of EMS and SA. According to [20], along with the precocious separation of univalents, the bivalents were also observed to move ahead and seemed as stray chromosome, this may move to one pole resulting into unequal distribution of chromosome or loss of a complete bivalent at later stage. Laggards were present in almost all treatments and occurrence of laggards in the present study has also been reported previously by many workers such as [35, 13, 19] etc. Delayed terminalisation and/or failure of chromosomal movement, following spindle fibre discrepancies have lead to lagging chromosomes. The fragments which appeared on the breakage of bridges, as a result of spindle fibres functioning to pull the chromosome towards poles, formed laggards [22]. Asynaptic condition which results in abnormal meosis in later stages may also lead to laggard formation [37]. Stickiness among the chromosomes occurred at metaphase and anaphase I and II stages. Stickiness was a common meiotic abnormality reported by various workers such as [15, 18, 7, 26] hold the view that stickiness could be due to disturbances of cytochemically balanced reactions by the effects of alkylating agents. According to [7] stickiness appears as a result of disturbances in the nucleic acid metabolism in the cell. It may also cause movement of whole bivalent towards one pole at anaphase due to the non-disjunction of homologus chromosomes. Bridges occurred both at anaphase I & II and telophase I & II stages. The frequency was generally higher at anaphase than telophase. It may be due to sister chromatid exchange followed by delayed or failure of their separation at later stages. Bridge formation was also observed by [2, 40, 6]. Bridges might have occurred as a result of delayed terminalisation, stickiness of chromosome ends, failure of chromosome movement [8], late terminalisation [5], unequal separation of chromosomes [13]. Multinucleate condition (more than four nuclei) at telophase II stages were found in low frequency only at higher concentrations of mutagen. Occurrence of micronuclei was also reported by [28, 36, 40] etc. The laggards and non-oriented chromosomes when fail to reach the poles in time to be included in the main telophase nucleus, form micronuclei leading to multinucleate condition [29, 40].

A considerable decrease in chiasma frequency showed the prominent effect of EMS on chromosomes. Crossing over and chiasma formation are under genetic control [30]. It is reported in wheat, Sorghum and in Capsicum [32] that the reduction in chiasma frequency is the consequence of the mutagen induced structural changes. Disturbed polarity has been observed by [34, 38, 33] etc. The disturbed polarity at anaphase and telophase stages could be due to spindle disturbances. In the present investigation reduction in chaisma frequency may be attributed to the nature and potency of mutagen and to the underlying factors such as complex structural changes or to the nature of the genes responsible for chiasma formation and the varying responses of these two mutagens (EMS and SA) to *Linum usitatissimum*. Chromosomal abnormalities and reduction in chiasma frequency were higher in EMS than SA in *Linum usitatissimum*.

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