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EMS Induced Karyomorphological Variations in Maize (*Zea mays* L.) Inbreds

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Abstract: Chemical mutagen induced chromosomal variations were broadly observed from the point of view of understanding the mechanics of EMS induced anomalies and biological dosimetry in *Zea mays* L. Seeds of 6 inbred lines of maize, i.e. CM-135, CM-136, CM-137, CM-138, CM-142, and CM-213 inbreds, were first presoaked in distilled water and then these seeds were treated with 0.5% solution of EMS for 3 durations, i.e. 3, 5, and 7 h, and genetic segregations were carefully observed. During the present investigation, through EMS treatment, many chromosomal anomalies, namely precocious movements, stickiness, univalents, bridges, laggards, multivalents etc., were induced in all the inbred lines of maize. Higher frequencies of chromosomal anomalies were displayed at the maximum dose (7 h) of treatment in all the inbred lines of maize. Maximum chromosomal anomalies were observed in CM-213 (i.e. 22.92%) but inbred CM-138 displayed better responses in all the morphological parameters at the maximum dose (7 h) of EMS treatment. However, CM-142 was found to be the most suitable inbred line for induced mutagenesis since it registered minimum chromosomal damage with maximum variability.

Key Words: Maize inbreds, EMS, meiosis, chromosomal anomalies

EMS ile Uyarılmış Mısır Hatlarında Karyomorfolojik Değişiklikler

Özet: Mısırda EMS ile muamele ile edilerek elde edilerek kimyasal mutagenler ile oluşan kromozomal varyasyonlar çalışılmıştır. Mısırda altı hat CM-135, CM-136, CM-137, CM-138, CM-142 ve CM-213 hatları ilk olarak distile su içinde bekletilmiş ve daha sonra % 5 lik EMS çözeltisi içinde 3 saat, 5 saat ve 7 saat muamele edilerek genetik segregasyon incelenmiştir. Çalışma esnasında, EMS muamelesi sonucu birçok kromozomal anormallik, erken hareketlilik, yapışma, univalant köprüler, lagard ve multivalan gibi değişiklikler mısır hatlarında gözlenmiştir. Bütün mısır hatlarında kromozomal anormalliklerin yüksek sıklığına maksimum dozda (7 saat) da raslanmıştır. Maksimum kromozom anormalliği CM-213 de (% 22,92), gözlenmiş fakat, CM-138 de bütün morfolojik parametreler EMS maksimum dozunda (7 saat) daha iyi sonuçlar vermiştir. Fakat, CM-142 çok az kromozomal hasar göstermesi nedeniyle en uygun mısır hattı olduğuna karar verilmiştir.

Anahtar Sözcükler: Mısır hatları, EMS, mayoz, kromozom anormalliği

Introduction

Mutation breeding makes extensive use of deviations from the norms to improve the characteristics of important crops. However, an efficient genetic improvement of a cultivar depends on the knowledge of mode of gene action, genetic variability, and the interrelationship among important plant characters. Induced mutagenesis is a significant tool to break through the limitations of variability and to create variability in a short period of time (1,2). The degree of cytological aberrations in either mitosis or meiosis is regarded as one of the dependable criteria for estimating the effect of a mutagen.

Mutagen induced anomaly of the chromosome is the primary basis of genetic change; therefore, investigations on the mechanism of chromosome breakage, type of aberrations, and their genetic consequences form an integral part of most mutation studies (3).

Cytogenetical investigation is one of the best documented experimental proofs for the elucidation of the mode of speciation on different groups of plants (4). Ethyl methane sulfonate (EMS) has recently received much attention as the most effective mutagenic agent in higher plants known today. Studies reveal that EMS is an effective mutagen and has been used to induce genetic variability in a number of crop plants (5,6).

Innovative research on genetic amelioration of kharif maize at the University of Allahabad on maize inbred lines has been carried out to elucidate the mutagenic effectiveness of EMS and its effect on the chromosome biology of *Zea mays* L.

Materials and Methods

Seeds of 6 inbred lines of maize, namely CM-135, CM-136, CM-137, CM-138, CM-142, and CM-213, were obtained from the Division of Genetics, Indian Agricultural Research Institute (I.A.R.I.), New Delhi, India. The seeds were first presoaked in distilled water and then treated with a 0.5% solution of EMS for 3 durations (3, 5, and 7 h). The seeds were then washed thoroughly in running tap water for 12 h and excess moisture was blotted off. Three replicates were maintained for each dose of treatment and then they were sown under natural conditions to raise M_1 generation.

At the time of flowering, young floral buds were fixed in 1:3 acetic alcohol solutions for 24 h, after which they were transferred to 70% alcohol and stored at 4 °C. For cytological analysis, slides were prepared using the chromosomal squash technique with 2% acetocarmine stain.

Observations

Cytological Effects

Meiosis was normal in the control plants (2n = 20) (Figures 1-3) of the 6 inbred lines of maize tested with relatively negligible amounts of chromosomal anomalies. However, the plants under EMS treatment displayed varying degrees of chromosomal anomalies in all the inbred lines (Table 1). A dose-dependent increase in meiotic irregularities was observed along with the mutagenic treatments. Unoriented bivalents (Figure 4), precocious movement (Figure 5), multivalents (Figure 6), scattering (Figure 7) etc. were the pronounced effects at metaphase I after EMS treatment, while the first and second anaphases were characterized by stickiness (Figure 8), laggards (Figure 9), unequal separation, and multipolarity. The most prominent abnormality induced by EMS was stickiness of chromosomes at metaphase I and II as well as at anaphase I and II. During stickiness chromosomes formed a compact mass and the identity of individual chromosomes was lost. Varying numbers of univalents were also observed in all the inbred lines but their frequency was higher at the highest dose duration of EMS treatment. Non-synchronization in the divisional stage of PMCs and late separation of bivalents were observed in all the 6 inbreds tested. Multivalents at metaphase I were also noted in considerably frequency at the maximum duration of mutagenic treatment. Rarely

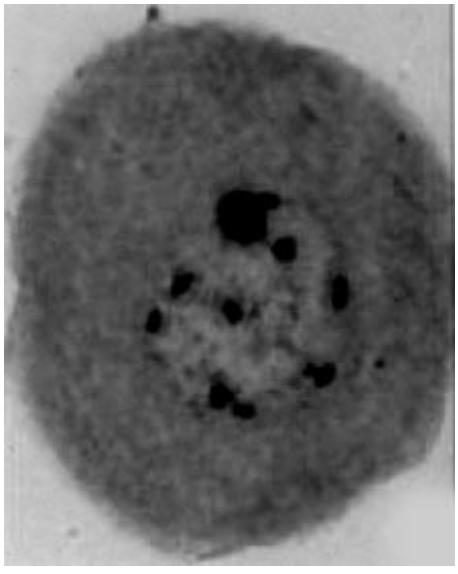

Figure 1. Normal diakinesis (n = 10).

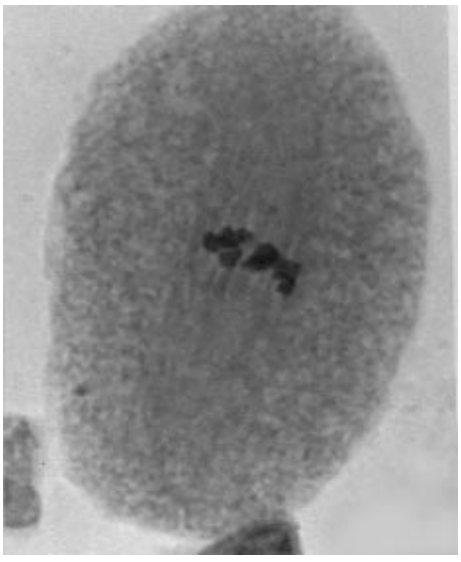

Figure 2. Normal metaphase I (n = 10).

Table 1. EMS induced chromosomal anomalies in 6 inbred lines of *Zea mays* L. as affected by 0.5%.

I.L.	Treatments	Metaphasic abnormalities (%)							Anaphasic abnormalities (%)						T. Ab. (%)	Total PMC's observed
		St. (%)	Pr. (%)	Fr. (%)	Uni. (%)	Multi. (%)	Sec. (%)	Ot* (%)	St. (%)	Lag. (%)	Br. (%)	Uneq. (%)	Mulp. (%)	Ot** (%)		
CM-135	Cont.	-	0.09	-	-	-	-	-	-	-	-	0.09	-	0.09	0.43	1154
	3 h	0.68	0.81	-	0.34	0.14	0.41	0.68	0.54	0.20	-	0.14	0.20	0.61	4.73	1481
	5 h	1.20	0.99	0.50	0.57	0.78	0.64	0.92	1.06	0.85	0.21	0.42	0.71	0.99	9.56	1412
	7 h	2.19	2.06	1.26	1.46	1.33	1.92	1.66	1.79	1.19	0.73	1.39	1.06	1.72	19.76	1508
CM-136	Cont.	-	0.14	-	-	-	-	-	-	0.07	-	0.07	-	-	0.28	1418
	3 h	0.65	0.58	0.14	0.22	-	0.43	0.29	0.94	0.72	0.07	0.65	0.36	0.58	5.63	1385
	5 h	1.49	1.09	0.31	0.39	0.63	0.86	0.86	1.25	0.86	0.55	0.94	0.70	0.39	10.32	1279
	7 h	2.01	2.46	1.34	1.19	1.12	1.94	1.57	2.54	2.31	1.12	1.34	1.49	1.57	22.00	1341
CM-137	Cont.	-	0.13	-	-	-	0.07	-	-	0.07	-	0.07	-	-	0.33	1503
	3 h	0.59	0.37	0.07	0.22	0.29	0.51	0.66	0.73	0.51	0.07	0.29	0.15	0.51	4.99	1364
	5 h	1.12	0.91	0.42	0.56	0.63	0.77	1.19	0.98	0.84	0.35	0.70	0.49	1.34	10.33	1423
	7 h	1.99	1.91	1.11	1.43	1.03	2.23	1.67	1.83	1.43	0.80	1.91	1.35	2.31	21.00	1257
CM-138	Cont.	0.14	-	-	-	-	0.07	-	0.14	0.07	-	-	-	0.07	0.41	1455
	3 h	0.71	0.42	0.14	0.28	0.21	0.35	0.28	0.85	0.78	0.07	0.28	0.21	0.35	4.89	1412
	5 h	1.38	1.25	0.21	0.69	0.42	0.76	0.83	1.31	1.04	0.28	0.97	0.62	0.69	10.45	1445
	7 h	2.15	2.08	0.65	0.93	1.43	2.01	1.22	2.30	1.87	0.86	1.51	1.65	1.29	19.94	1394
CM-142	Cont.	0.07	-	-	-	-	-	-	0.15	-	-	-	-	-	0.22	1350
	3 h	0.24	0.32	0.16	0.08	0.08	0.40	0.24	0.55	0.40	-	0.08	0.32	0.24	3.09	1263
	5 h	0.89	0.67	0.30	0.52	0.89	0.96	0.67	1.04	0.82	0.22	0.74	0.67	0.59	8.98	1348
	7 h	2.28	2.21	0.59	0.81	0.66	1.99	1.25	2.13	1.76	0.74	1.69	1.40	1.84	19.34	1360
CM-213	Cont.	-	0.06	-	-	-	0.06	-	0.13	0.13	-	-	-	0.06	0.44	1576
	3 h	0.86	0.78	-	0.29	0.21	0.50	0.43	0.71	0.36	0.14	0.50	0.29	0.5.	5.56	1402
	5 h	1.15	1.02	0.34	0.75	0.61	0.54	0.54	1.29	1.02	0.27	0.95	0.68	0.88	10.05	1473
	7 h	2.08	2.85	1.31	1.16	1.47	1.54	1.23	2.55	1.47	1.47	2.08	1.77	1.93	22.92	1296

I.L.- Inbred line, Cont.-Control, St.- Stickiness, Pr.- Precocious movements, Uni. - Univalents, Multi. - Multivalents, Fr.- Fragmentation, Sec- Secondary association, Mulp. - Multipolarity, Un. - Unorientation, Lag. - Laggards, Br.- Bridges, Uneq. - Unequal separation, Ot – other abnormalities, *- Cytoplasmic connections, scattering, **- Micronuclei, non-synchronous division.

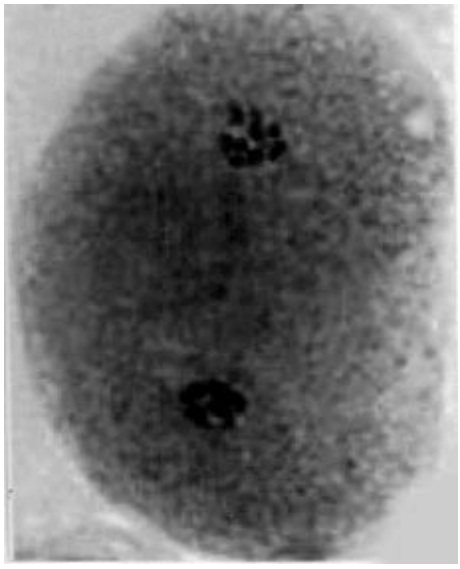

Figure 3. Normal anaphase I (10:10 separation).

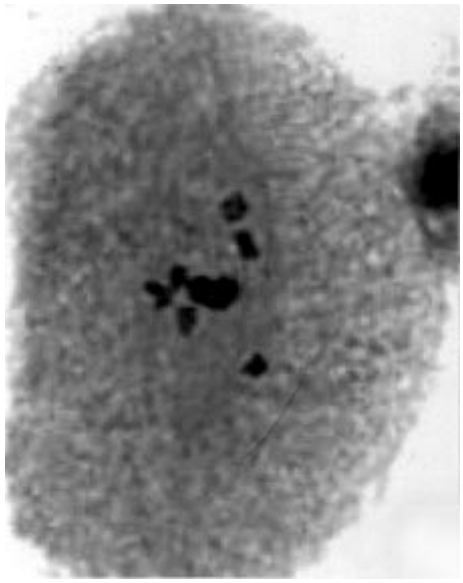

Figure 4. Unorientation at metaphase I.

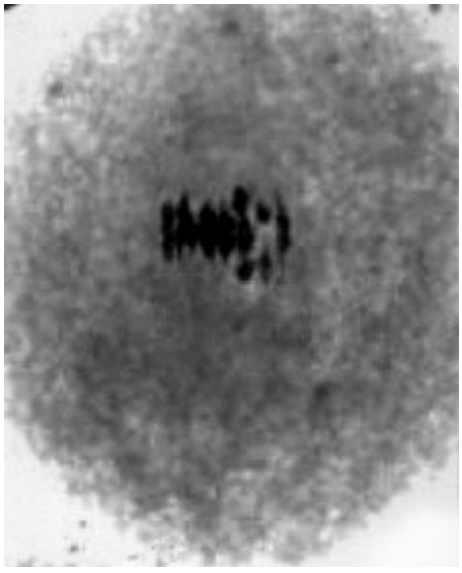

Figure 5. Precocious movement at metaphase I.

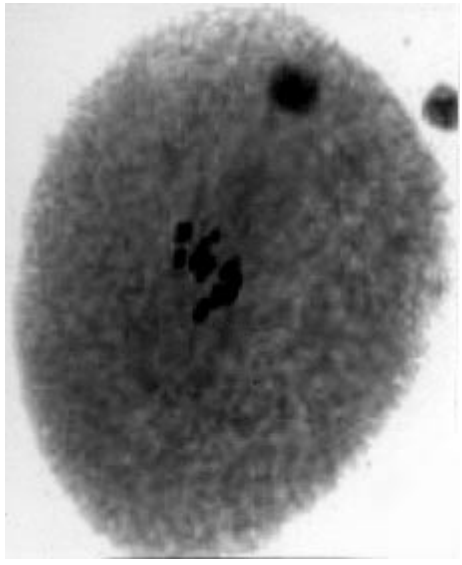

Figure 6. Multivalent formation at metaphase I.

bridges, fragments, and cytoplasmic connections were also registered at some of the treatment durations. Chromatin bridges were sometimes found accompanied by fragments, although their occurrence was mostly independent of each other. Non-disjunction of chromosomes was often observed at the first and second anaphase, resulting in aneuploid numbers of chromosomes in daughter cells. Some pollen mother cells had very few chromosomes probably representing examples of cells having undergone degeneration of chromatin material. The lagging chromosomes and fragments, which usually failed to be included in the daughter nuclei, formed micronuclei.

On comparison of the 6 inbreds for their response against the mutagenic treatment, it was found that CM-142 was the most tolerant genotype, while CM-213 was

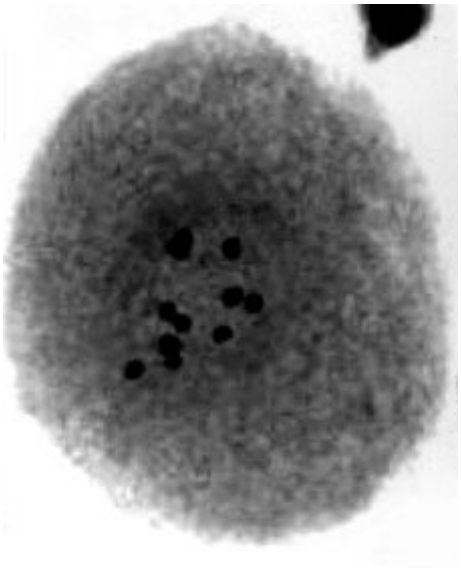

Figure 7. Scattering at metaphase I.

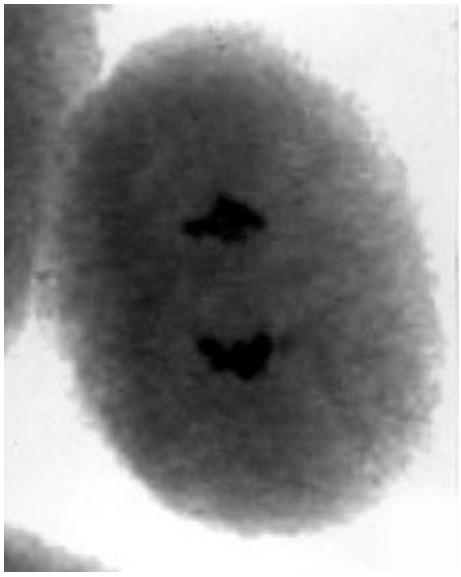

Figure 8. Stickiness at anaphase I.

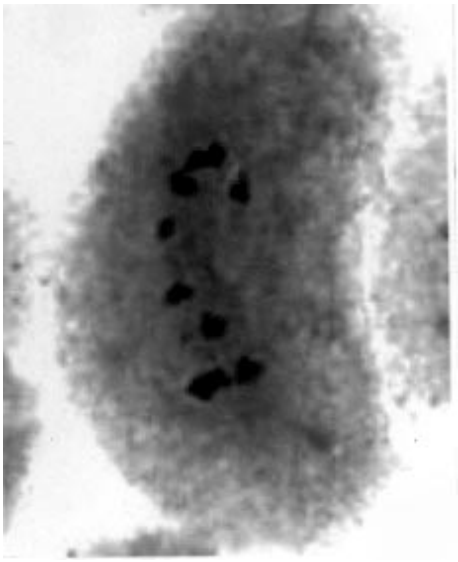

Figure 9. Laggards at anaphase I.

the least. Clastogenic abnormalities were registered in greater frequency in CM-136 (i.e. 22.00%) and CM-213 (i.e. 22.92%) at the maximum dose (7 h) of treatment (Table 1).

Morphological Effects

Mutagenic treatment also affected the morphological parameters of the treated sets. Germination percentage was found to be maximum (93.5%) in the control set of CM-138, while the minimum germination percentage (68.6%) was observed in the inbred line CM-135. It was observed that, along with increasing treatment duration of EMS, the germination percentage was reduced continuously. The maximum reduction in germination percentage was reduced continuously. The maximum reduction in germination percentage was registered in CM-136, where it decreased from 91.2% in the control set to 79.9% at the 7 h treatment duration. Plant height was also found to be significantly reduced at higher doses of mutagenic treatment but some of the plants at lower doses responded positively to the mutagen and recorded a slight increase in plant height. Similar trends in days to 50% silking, 100 seed weight, and pollen fertility were recorded after EMS treatment, while inbred CM-138 displayed better responses in all the morphological parameters at the maximum dose (7 h) of EMS treatment (Table 2).

Thus, in the present study, inbred CM-142 displayed the minimum chromosomal anomalies percentage among all the inbred lines, while the maximum chromosomal anomalies were configured in CM-213 (Table 1); however, inbred CM-138 displayed better responses among all the morphological parameters (Table 2).

Table 2. Effect of EMS on morphological parameters in 6 inbred lines of *Zea mays* L.

I.L.	Treatments	Germination (%) (Mean ± S.E.)	Plant height (In cm) (Mean ± S.E.)	Days to 50% silking (In days) (Mean ± S.E.)	100 Seed weight (In gm) (Mean ± S.E.)	Pollen fertility (%) (Mean ± S.E.)
CM-135	Cont.	79 ± 0.48	153.8 ± 0.51	52 ± 0.27	119.0 ± 0.38	97.6 ± 0.11
	3 h	74.5 ± 0.78	152.1 ± 0.53	57 ± 0.43	112.43 ± 0.57	95.1 ± 0.17
	5 h	69.9 ± 0.97	141.9 ± 0.68	59 ± 0.67	109.11 ± 0.77	90.0 ± 0.43
	7 h	68.6 ± 0.85	128.4 ± 0.86	63 ± 0.91	100.13 ± 0.94	83.4 ± 0.66
CM-136	Cont.	91.2 ± 0.25	161.5 ± 0.43	54 ± 0.15	117.61 ± 0.25	98.9 ± 0.08
	3 h	87.11 ± 0.41	158.3 ± 0.65	59 ± 0.22	112.36 ± 0.49	96.2 ± 0.25
	5 h	81.12 ± 0.72	143.6 ± 0.82	63 ± 0.45	103.06 ± 0.87	88.7 ± 0.57
	7 h	79.9 ± 0.67	123.8 ± 1.27	67 ± 0.74	99.09 ± 1.20	79.6 ± 0.63
CM-137	Cont.	89.91 ± 0.33	156.1 ± 0.65	53 ± 0.31	109.41 ± 0.31	97.2 ± 0.23
	3 h	87.15 ± 0.56	148.5 ± 0.59	57 ± 0.49	101.11 ± 0.56	96.8 ± 0.15
	5 h	82.11 ± 0.29	133.7 ± 0.90	61 ± 0.68	97.49 ± 0.46	90.1 ± 0.37
	7 h	79.96 ± 0.78	124.2 ± 0.94	63 ± 0.88	93.56 ± 0.78	81.5 ± 0.48
CM-138	Cont.	93.5 ± 0.08	162.1 ± 0.47	50 ± 0.25	120.41 ± 0.33	96.1 ± 0.28
	3 h	86.9 ± 0.31	159.4 ± 0.51	56 ± 0.41	114.45 ± 0.57	94.4 ± 0.29
	5 h	83.1 ± 0.55	150.7 ± 0.69	59 ± 0.70	107.59 ± 0.85	90.2 ± 0.41
	7 h	80.0 ± 0.96	138.2 ± 0.88	61 ± 0.97	100.48 ± 1.06	85.5 ± 0.59
CM-142	Cont.	89.11 ± 0.15	150.1 ± 0.34	55 ± 0.39	108.45 ± 0.09	96.7 ± 0.18
	3 h	85.41 ± 0.23	149.7 ± 0.62	57 ± 0.55	101.11 ± 0.29	94.3 ± 0.32
	5 h	81.01 ± 0.21	144.2 ± 0.97	61 ± 0.89	99.82 ± 0.71	87.5 ± 0.49
	7 h	78.11 ± 0.52	127.6 ± 1.10	64 ± 1.09	96.81 ± 0.93	80.2 ± 0.51
CM-213	Cont.	90.14 ± 0.23	159.8 ± 0.45	51 ± 0.13	113.11 ± 0.26	97.3 ± 0.20
	3 h	87.44 ± 0.39	153.3 ± 0.64	58 ± 0.30	109.76 ± 0.59	92.8 ± 0.38
	5 h	82.10 ± 0.32	140.6 ± 0.79	62 ± 0.58	103.49 ± 0.70	85.9 ± 0.31
	7 h	77.61 ± 0.68	127.5 ± 0.82	64 ± 0.75	99.59 ± 0.86	73.7 ± 0.52

Discussion

The frequency and spectrum of aberrations observed during the present investigation clearly displayed that EMS is a very potent mutagen for *Zea mays* L. The results also showed a co-linearity between the duration of treatment and the percentage of chromosomal anomalies.

Chemical mutagen induced chromosomal variations have been widely investigated from the point of view of understanding the mechanics of EMS induced damage and biological dosimetry in *Zea mays* L. Enhancement in the frequency of meiotic chromosomal anomalies is wide and it included a high proportion of stickiness and secondary association and moderate frequency of laggards and multivalents. EMS induced chromosomal stickiness has also been reported by Kumar and Singh (7) in *Hordeum vulgare* L. It implies that the chemical mutagen may have brought some alterations in the pattern of organization of chromosomes. Similar results were also found by Kumar and Rai (5), and Sharma and Kumar (8).

The phenotypic manifestation of stickiness may vary from mild, when only a few chromosomes of the genome are involved, to intense, with the formation of pycnotic nuclei that may involve the entire genome, culminating in chromatin degeneration (9).

Chromosome stickiness has been documented to be due to genetic or environmental factors. Genetically induced stickiness in maize has been reported by Golubovskaya (10), Caetano-Pereira et al. (11), and Kumar and Rai (12), while stickiness has also been reported in other crops like *Glycine max* L. (7,13), *Hordeum vulgare* L. (7), pearl millet (14), and wheat (15). Gaulden (16) postulated that sticky chromosomes may result from the defective functioning of 1 or 2 types of specific non-histone proteins involved in chromosome organization, which are needed for chromatid separation and segregation.

The observed precocious chromosome migration to the poles may have resulted from univalent chromosomes at the end of prophase I or precocious chiasma terminalization at diakinesis or metaphase I. Univalents may originate from an absence of crossing over at pachytene or from synaptic mutants. Chiasmata are responsible for the maintenance of bivalents, which permit normal chromosome segregation. Precocious migration of univalents to the poles is a very common abnormality among plants (17-21), which was also evident in our case.

Unorientation and scattering of chromosomes may be due to either the inhibition of spindle formation or the destruction of spindle fibers formed. The behavior of these and of the laggard chromosome is characteristic in that they generally lead to micronucleus formation (22,23). Laggards and disturbed polarity might have appeared due to improper spindle functioning.

Bridges seem to be a result of non-separation of chiasma due to stickiness. Division of nucleolar material recorded here in some cases was in line with the observations reported by Shaikh and Godward (24) in irradiated populations of *Vicia ervilia* and *Lathyrus sativus*.

In many studies, chromosome cluster, fragments, laggard, chromatin bridges, and micronuclei were observed as the effects of physical and chemical mutagens (25-32).

Chromosomal damages may be the prominent causes of reduced seed germination and decreased yield as compared to controls. The reduction in germination percentage might have been due to the effect of mutagen on meristematic tissues of the seed. The mutagenic treatments also delayed the germination process. Kleinhofs et al. (33) reported a delay in the initiation of metabolism following germination, resulting in uniform delay in mitotic activity, seedling growth, and ATP and DNA synthesis. Although all doses of mutagen elicited a reducing effect on plant height, some of the plants at 3 h treatment duration displayed an increase in plant height, which may be due to the mutation in major or minor genes. Fertility depends on the efficiency of the meiotic process. Studies on different plant species have shown that the decline in seed production is correlated with meiotic irregularities (19,34). Reduction in pollen fertility also supports a decrease in seed production due to the meiotic anomalies. Similar results were also obtained by Kumar and Rai (7).

As a result of these studies, genetic segregations should be carefully observed. Efficient mutagenesis is defined as the production of desirable changes (mutations) free from the usually associated undesirable changes such as chromosomal aberrations, sterility, lethality, etc. (35). Ehrenberg (36) and Kawai (37) stated that the highest mutation rates also induce a high degree of lethality, sterility, and other undesirable effects. From the practical breeding point of view, the mutagenic treatments that induce high mutation rates with the least accompanying deleterious effects are desirable. During the present investigation, through EMS, many chromosomal anomalies were induced. The genetic structure of our material was highly affected, favoring new genetic changes in the following generations.

From the present study, it is quite evident that EMS is very efficient mutagen for creating genetic variability in the natural gene pool of *Zea mays* L. It has also been concluded from the present investigation that maximum chromosomal anomalies were noted in CM-213, suggesting that this inbred is most susceptible to EMS and hence the least tolerant, while inbred CM-138 displayed better responses in morphological parameters at the maximum dose of EMS treatment. CM-142 was, however, found to be the most suitable inbred line for induced mutagenesis through EMS since it registered minimum chromosomal damage with maximum variability.

Acknowledgments

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