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Original Research Paper

# A Comparison of the Effects of A Direct and an Indirect Mutagen on the Meiosis of Barley

Botany

**KEYWORDS :** Barley, chromosomal aberrations, Ethyl Methane Sulphonate, Sodium Azide.

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## ABSTRACT

*The success of any mutation breeding exercise lies in proper selection of mutagens for creation of mutations that can be selected for improvement programmes. Although numerous studies on Barley enlist a wide choice of mutagenic agents for mutagenesis but generally direct mutagens like EMS, MMS, dES etc have been conventionally tested. A comparison between an indirect mutagen like Sodium Azide and a conventional mutagen like EMS are very few. The present study compares these two mutagens on the basis of their ability to induce chromosomal aberrations. It is believed that more number of aberrations would lead to more number of heritable mutations. It would thus be easier to select the desired mutant for improvement programmes*

## INTRODUCTION

Genetic variations are continuously added to the population by means of mutations but their frequency of occurrence is extremely low. Mutations can be defined as sudden heritable changes in the characteristics of an organism. This definition requires that the change in the characteristics be heritable, but it does not state the genetic basis of heritable change. Clearly a mutation may be the result of a change in a gene, in a chromosome or a plasmagene. Mutations produced by changes in base sequences of genes are known as gene or point mutations. Some mutations may be produced by changes in chromosome structure, or even in chromosome number; they are termed as chromosomal mutations. Gross chromosomal mutations, eg, changes in chromosome number, translocations, inversions, large deletions and duplications are detectable cytologically under the microscope. But small deletions and duplications can rarely be detected, and would be considered as gene mutations.

Mutations occurring in natural populations are too low (1 in $10^6$) to be exploited at any massive level for crop improvement. We therefore need artificial induction of mutations by treatment with physical or chemical agents known as mutagens. The available evidences suggest that mutation induction rarely produces new alleles; it produces alleles that are already known to occur spontaneously but increase the frequency of their occurrence. However it would be reasonable to say that induced mutations are comparable to spontaneous mutations in their effects and in the variability they produce.

The usefulness of a mutagen and the type of treatment required to obtain a high efficiency are dependent upon specific properties of the mutagenic agent employed (its effectiveness, dose effect relationship and mode of application) as well as on specific characteristics of biological systems to be treated (the sensitivity of the treated tissues depending upon anatomical, physiological, biochemical and genetic peculiarities). In view of this, it is of utmost importance to first select a suitable mutagenic agent for the test crop. The present study seeks to compare the mutagenic efficacies of EMS and SA on Barley (*Hordeum vulgare* L.) through an evaluation of their induced cytological aberrations.

Ethyl methanesulfonate (EMS) is a mutagenic, teratogenic, and possibly carcinogenic organic compound with formula $C_3H_8SO_3$. It produces random mutations in genetic material by nucleotide substitution; particularly by guanine alkylation. This typically produces only point mutations. The ethyl group of EMS reacts with guanine in DNA, forming the abnormal base O-6-ethylguanine. During DNA replication, DNA polymerases that catalyze the process frequently place thymine, instead of cytosine, opposite O-6-ethylguanine. Following subsequent rounds of replication, the original G:C base pair can become an A:T pair (a transition mutation). EMS is always freshly prepared and never stored as they tend to be hydrolyzed producing non-mutagenic toxic products viz an alcohol and an acid.

Sodium azide ($NaN_3$) is a chemical mutagen and has been one of the most powerful mutagens in crop plants. The mutagenicity is mediated through the production of an organic metabolite of azide compound. This metabolite enters into the nucleus, interacts to DNA and creates point mutation in the genome. Sodium Azide (SA) is an indirect mutagen as it is not itself mutagenic; the mutagenic effects are produced by a metabolite generated from it inside the cell. It has been reported to be mutagenic in a number of crops but ineffective in others. This type of object specificity is typical for indirect mutagens. Azide is also known to be a respiratory poison, which accounts for some of its toxic properties.

## MATERIAL AND METHOD

The seeds of Barley var K12 were soaked in distilled water for 6 h to initiate metabolic activities. The chemicals used for treatment were of AR grade and manufactured by LOBA Chemei, Mumbai. Ethyl Methane Sulphonate (EMS) is a partially photosensitive liquid while Sodium Azide is a white crystalline powder. For EMS treatment, 0.5% solution was prepared (v/v) by pipetting requisite volume of the mutagen in double distilled water and presoaked seeds were put into it. A calculated number of seeds were periodically removed from the solution after 1h, 3h, 5h, 7h, 9h and 11h. The treated seeds were washed and immediately planted in soil along with suitable controls. Same procedure was employed for SA with the difference that the chemical was weighed to prepare 0.5% solution (w/v) in double distilled water. The durations of treatment were similar.

Dose selection: Since the duration of treatment was to be varied, the concentration of solution was fixed at 0.5%. The durations of treatment were limited between the lowest effective duration and $LD_{50}$ for the mutagens used. In order that the durations of treatment of one mutagen may be compared with their counterparts in the other mutagen treatment, similar dose-durations were used for both crops. For ascertaining the lowest effective dose, the seeds were immediately put for germination following treatment. The minimum dose, at which the plants showed a significant variation from control in the germination frequency, was treated as the lowest effective dose. It came out to be 3h duration for EMS in both the cases. The highest duration of treatment ie., the $LD_{50}$, was found to lie somewhere

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between 7h and 9h for both the test crops, but since 9h duration showed a lethality greater than 50%, the highest duration was limited to 7h in both the plants, although it was somewhat less lethal than $LD_{50}$. The results were almost similar for SA and so, on the basis of equivalence, similar durations were used of SA treatment also. Thus, the durations of treatment for EMS and SA used for barley and pearl millet were 3h, 5h and 7h.

The treated seeds were planted in 5 replicates with suitable controls and at the time of maturity, young spikes were fixed in 1:3 Acetic Alcohol. These were then transferred in 70% alcohol after 24 h and stored at 4OC for cytological analysis. The slides were prepared using Acetocarmine squash technique.

**RESULTS**

Cytological investigations: About 1450-1500 PMCs were studied / treatment set, and the abnormalities encountered were grouped in 5 categories depending upon the meiotic phases at which they occurred.

EMS Treatment set: Table 1 presents a comparative account of cytological behaviour in controls and EMS treated plants of barley.

Meiosis was almost perfectly normal in control sets with regular formation of 7 bivalents at Metaphase I and 7:7 separation at Anaphase I. All the treated plants showed varying degrees of abnormalities. Stickiness of chromosomes formed the most dominant of all anomalies both at Anaphase as well as Metaphase. Its percentage was high even at the lowest duration set being 2.03% and further rose rapidly to reach a maximum value of 4.14% in 7h set. The case was almost similar at Anaphase I and II. Clumping of chromosomes was also frequent but not to the extent of stickiness.

Abnormalities related to spindle dysfunction were also common with highest frequency of late movement of bivalents to Metaphase plate (1.24% at 7h set). Disturbed orientation of bivalents was high at higher doses. Overall frequency of abnormalities related to spindle dysfunction was lower than that observed in case of irradiation treatments.

Multivalents and univalents were evident right from the lowest dose but in much smaller numbers. The percentage of multivalents was highest (0.83%) at 7h set. Few to many univalents could be in PMCs and their percentage increased with dose. Univalents showed consistency in both the generations. Fragmentation of chromosomes by heavy or moderate shredding was less frequent, observed only at the two highest doses being 0.27% and 0.55% in 5h and 7h sets respectively.

Secondary associations characterized by attachment between bivalents were quite common being 1.55% in 3h treatment duration but showed a reduction at higher doses.

Among Anaphasic anomalies, laggards, stickiness and bridges occupied the foremost position. Laggards reached the highest value of 2.69% at 7h set. Bridges also recorded a similar trend. Anaphase PMCs also exhibited high stickiness at Anaphase I and II. Non-synchronous disjunction of chromosomes at Anaphase II could be observed at 5h and 7h set only. Both, unequal separation of chromosomes and multipolarity at Anaphase I were of low occurrence.

The laggards and bridges observed at Telophase I and II, might have persisted from Anaphase. Laggards were common but bridges were seen only at the highest dose. Other common Telophase anomaly was the presence of micronuclei at the doses. Their percentage was high at 7h set and showed considerable increase with duration of treatment.

Cytokinesis revealed the limited presence of polyads and rare presence of triads alongwith normal tetrads at higher doses only. Other anomalies included cytomixis and transmigration of chromatin as well as shrinking of PMCs. Cytomixis was found in all doses but shrinking of PMCs was extremely rare. They were present only at highest doses and their numbers were few.

There was a dose based decrease in the chiasma frequency over the controls. At 3h set, a mild depression was evident which intensified at 5h and 7h sets. Total abnormality percentage showed an exponential increase with dose. Lowest abnormality % was 10.73 at 3h treatment duration, which rose sharply to 27.44% at 7h.

SA Treatment Sets: Table 2 presents a comparative account of cytological behaviour in controls and SA treated plants of barley.

The usefulness of a mutagen and the type of treatment required to obtain a high efficiency are dependent upon specific properties of the mutagenic agent employed (its effectiveness, dose effect relationship and mode of application) as well as on specific characteristics of biological systems to be treated (the sensitivity of the treated tissues depending upon anatomical, physiological, biochemical and genetic peculiarities).

Stickiness of chromosomes being most frequent, dominated the scene at Metaphase I and II. It exhibited a rapid rise from 1.62% at 3h set to 3.57% 7h set. Secondary associations of bivalents maintained a common presence at all doses but did not show high frequency. Clumping was also present but not very high like in EMS treated sets.

Among spindle abnormalities, late movement of bivalents, precocious movement and disturbed orientation of chromosomes were common at all doses. Late movement of bivalents to equator recorded a moderate frequency right from 3h set (0.74%) to 1.30% in 7h set. Disturbed orientation showed an increase from 1.08% at 3h set to 1.79% at 7h set.

Univalents appeared in significant numbers at higher doses being 1.44% in 7h set. Multivalents were not so common at lower treatment durations but recorded a significant presence at 7h set (1.10%). Fragmentation was insignificant. Secondary associations were common at all sets and registered a linear increase with duration of treatment.

Laggards, bridges and stickiness were of common occurrence at Anaphase I and II. Laggards were extremely frequent even at 3h set but increased massively at higher duration sets. Bridges were substantial at all sets. Stickiness of chromosomes at Anaphase showed a more or less similar trend as in Metaphase.

Unequal separation and non-synchronous disjunction at Anaphase II appeared for the first time at 7h. Multipolarity was the rarest abnormality observed only in 7h set.

Laggards generally persisted up to Telophase and recorded a moderate presence in Telophase PMCs. Bridges were uncommon in Telophase I and II. Micronuclei, although present in insignificant numbers, revealed their presence at all doses.

Triads were absent altogether and polyads were found in extremely low frequencies, restricted to highest doses. Cytomixis was encountered at all 3 duration sets but in low numbers while shrinkage of PMCs only in one stray case.

The chiasma frequency/ bivalent ranged from 1.74 in controls to 1.65, 1.59 and 1.32 at 3h, 5h and 7h sets respectively. The total abnormality percentage was much lower at each dose as compared to other treatments being 7.89% at 3h set and rising upto 22.12% at 7h set.

**DISCUSSION**

The chemical mutagens have been reported to be one of the most potent mutation inducers, sometimes considered even better than the physical ones (Goud 1967, Sharma 1960 and Uhlik 1972). Although the present study does not support the last line but nonetheless, the efficiency of the two chemical mutagens in production of variations was high. However, the progress in the effective and efficient use of chemical mutagens is hindered by complex interplay of many physical and chemical factors that determine the ultimate yield of mutations (Konzak et al 1975).

Stickiness was the most common of all chromosomal abnormalities. Mitra and Bhowmik (1996) also obtained high stickiness of chromosomes following chemical treatments. Clumping could be the extreme culmination of stickiness where the chromosomes seem to lose identity and melt away (Gaul and Sato 1967). Both stickiness and clumping of chromosomes may be due to depolymerization of nucleic acids caused by a mutagenic treatment (Gaul et al 1966, Tarar and Dyansagar 1980).

Chemically induced fragmentation and breaks are attributed to coiling errors or duplication difficulties leading to chromosome breakage (Natarajan and Upadhyay 1961).

Presence of univalents in Metaphase I may be correlated to partial or complete lack of pairing between homologous chromosomes (Rao and Lakshmi 1980) or due to early terminalization of chiasmata. Inspite of high degree of sterility, the visible chromosomal rearrangements like translocations were rare.

Precocious movement of univalents, lagging chromosomes and disturbed or unorientation of bivalents may be because of discrepancies of spindle formation (Patil and Bora 1961, Khan 1996). Acentric fragments or laggards may result in the formation of micronuclei at Telophase I and II. Their random movement towards any pole may lead to unequal separation at Anaphase I (Mitra and Bhowmik 1996).

Anaphasic bridges may be formed due to stickiness of chromosomes or breakage and fusion (Kaur and Grover 1985b). They may also result from non-separation and stretching of chiasmata due to stickiness (Khan 1996).

The reduction in chiasma frequency following chemical treatment as observed in the present study might be a result of mutagen induced structural changes. The reduction observed here is common to most mutagenic treatments and has been demonstrated by workers like Singh and Mahapatra (1969) in *Zea,* Sinha and Roy (1976) in *Phaseolus* and Lal and Srinivasachar (1979) in *Pennisetum*.

There was a clear predominance of physiological abnormalities like stickiness and clumping as well as clastogenic ones like micronuclei. Such anomalies lead to high degree of gamete sterility and bring the plant into a growth disadvantage. As a result high degree of lethality is induced even at low doses.

Different types of meiotic anomalies observed in the present investigation have also been reported by different workers in different plants following chemical mutagen treatments (Reddy and Annadurai 1992, Anis and Wani 1997, Dhamyanthi and Reddy 2000).

**CONCLUSION**

Although it is reasonable to classify chemically induced variations into 3 types according to mechanism involved viz. chromosomal, genic and physiological, the boundary lines of these categories are not always clear. It is, for instance, almost impossible to discriminate a small chromosomal change from a gene mutation. Furthermore, it is probable that selective elimination of certain genotypes occurs during the haplophase under the unfavourable physiological condition caused by the mutagen. It is however, beyond doubt that both EMS and SA induce heritable changes in the genetic material, which controls the morphology as well as the fertility of the plant. However if the chromosomal aberrations are considered as the indicators of mutagenic potential Sodium Azide seems to be a better alternative as compared to the more common Ethyl Methane Sulphonate in this variety of Barley.

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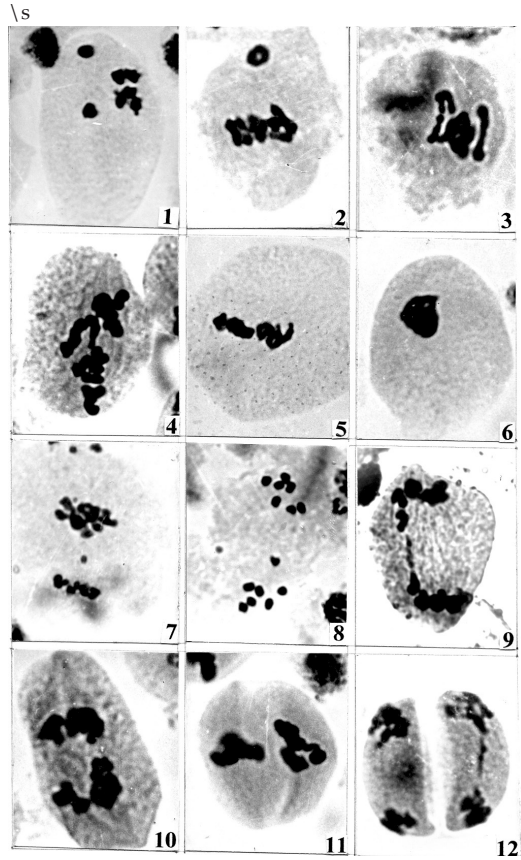


**Figures 1-12: Meiotic abnormalities in Barley Pollen Mother Cells due to the effects of EMS and SA treatments. 1. Disturbed orientation, 2. Precocious movement, 3,4. Multivalent associations, 5. Stickiness, 6. Clumping, 7. Laggard and unequal separation, 8. Laggard, 9. Sticky bridge, 10. Clumping and multipolarity, 11, 12. Lumping and bridge. (Bar: 1cm=4.2μm)**

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**Table 1: A comparison of cytological abnormalities induced in 0.5% EMS treated barley.**

| 0.5%SA Treatment durations | | CF/biv ± SE | Metaphase I/II abnormalities (%) | | | | | | | | | Anaphase I/II abnormalities (%) | | | | | | Telophase I/II abn (%) | | Cytokinesis abn (%) | | | Other abn (%) | | T Ab (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mv | Uv | Fg | St | Cl | Sa | Lg | Br | Us | Ns | St | Mp | Lg | Br | Mn | Tr | Pa | Cy | Sh | | |
| Control | $M_1$ | 1.76±0.05 | 0.07 | 0.13 | | | | | | | | | | | | | | | | | | | | | 0.20 |
| 3h | $M_1$ | 1.58±0.06 | 0.61 | 0.67 | 0.33 | 0.27 | 0.20 | | 2.03 | 0.54 | 1.55 | 1.01 | 0.88 | | | 1.21 | | 0.40 | | 0.81 | | | 0.20 | | 10.73 |
| 5h | $M_1$ | 1.50±0.10 | 0.95 | 1.36 | 0.68 | 0.61 | 1.09 | 0.27 | 3.26 | 0.82 | 1.22 | 1.97 | 1.77 | 0.54 | 0.82 | 2.04 | 0.47 | 0.54 | | 1.02 | | 0.41 | 0.27 | | 20.06 |
| 7h | $M_1$ | 1.43±0.09 | 1.24 | 1.65 | 0.68 | 0.83 | 1.17 | 0.55 | 4.14 | 1.03 | 1.38 | 2.69 | 2.07 | 0.83 | 1.31 | 2.62 | 0.96 | 1.10 | 0.41 | 1.38 | 0.14 | 0.41 | 0.62 | 0.21 | 27.44 |

Lm=Late movement of bivalents; Do=Disturbed orientation of chromosomes; Pc=Precocious movement of chromosomes; Mv=Multivalent formation; Uv= Univalent formation; Fg=Fragmentation of chromosomes; St=Stickiness of chromosomes; Cl=Clumping of chromosomes: Sa=Secondary association of bivalents; Lg=Lagging chromosomes; Br=Bridge formation between poles; Us=Unequal separation of chromosomes at anaphase; Ns=Non synchronous disjunction; Mp=Multipolarity; Mn=Micronuclei; Tr=Triads; Pa=Polyads; Cy=Cytomixis; Sh=Shrinking of PMCs

**Table 2: A comparison of cytological abnormalities induced in 0.5% SA treated barley.**

| 0.5%SA Treatment durations | | CF/biv ± SE | Metaphase I/II abnormalities (%) | | | | | | | | | Anaphase I/II abnormalities (%) | | | | | | Telophase I/II abn (%) | | Cytokinesis abn (%) | | | Other abn (%) | | T Ab (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mv | Uv | Fg | St | Cl | Sa | Lg | Br | Us | Ns | St | Mp | Lg | Br | Mn | Tr | Pa | Cy | Sh | | |
| Control | $M_1$ | 1.79±0.09 | 0.13 | 0.13 | | | | | | | | | | | | | | | | | | | | | 0.26 |
| 3h | $M_1$ | 1.41±0.04 | 0.74 | 1.08 | 0.13 | | 0.20 | | 1.62 | 0.27 | 0.40 | 1.21 | 0.88 | | | 0.61 | | 0.54 | | 0.13 | | | 0.07 | | 7.89 |
| 5h | $M_1$ | 1.40±0.08 | 0.88 | 1.36 | 0.27 | 0.34 | 0.61 | 0.20 | 2.52 | 0.48 | 0.68 | 1.56 | 1.16 | | | 1.22 | | 0.54 | | 0.27 | | 0.14 | 0.41 | | 12.65 |
| 7h | $M_1$ | 1.43±0.10 | 1.30 | 1.79 | 0.61 | 1.10 | 1.44 | 0.48 | 3.57 | 0.96 | 1.03 | 2.68 | 2.06 | 0.62 | 0.76 | 1.24 | 0.27 | 0.82 | 0.21 | 0.34 | | 0.21 | 0.55 | 0.07 | 22.12 |

Lm=Late movement of bivalents; Do=Disturbed orientation of chromosomes; Pc=Precocious movement of chromosomes; Mv=Multivalent formation; Uv= Univalent formation; Fg=Fragmentation of chromosomes; St=Stickiness of chromosomes; Cl=Clumping of chromosomes: Sa=Secondary association of bivalents; Lg=Lagging chromosomes; Br=Bridge formation between poles; Us=Unequal separation of chromosomes at anaphase; Ns=Non synchronous disjunction; Mp=Multipolarity; Mn=Micronuclei; Tr=Triads; Pa=Polyads; Cy=Cytomixis; Sh=Shrinking of PMCs

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