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# MUTAGENIC EFFECT OF ETHYLENE GLYCOL ON SOMATIC CELLS OF WHEAT (*TRITICUM AESTIVUM* L.)

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**Abstract**

Mutagenic effect of Ethylene Glycol on interphase chromosome volume (ICV) and structural chromosomal aberrations were studied using five different doses of mutagen along with a control. The ICV was found to be mutagen sensitive in somatic cells of wheat. It was found to increase with increasing the doses of mutagen in all the five varieties of wheat. The chemical mutagen, Ethylene Glycol was also found to induce high frequency of chromosomal changes like chromatid bridges, chromosome fragments, laggards etc. Their frequencies were also found to increase with increase of the doses. Regarding treatment durations the materials treated for 24 hours showed significantly higher values compared to that of 12 and 6 hours.

***Key words :*** Ethylene Glycol, Somatic cells, Wheat

mvi ms‡¶ct cvPwU wfb gvÎvi Bw_wjb MvBKj Gi cwie¨w³Ki cfv‡ei gva¨‡g Mg Mv‡Q AvštchwqK †µv‡gv‡Rv‡gi AvqZb I †µv‡gv‡Rv‡gi MvVwbK weP¨wZ ch‡e¶b Kiv n‡q‡Q| Mg Mv‡Qi ^`wnK †Kv‡li Avšt chwqK †µv‡gv‡Rv‡gi AvqZbI `e¨wUi cwZ †h ms‡e`bkxj Zv †ek ¯úó| Mg Mv‡Qi cvPwU wfb wfb Rv‡Zi †¶‡Î j¶bxq †h, cwie¨w³Ki `‡e¨i gvÎv ew×i mv‡_ mv‡_ D³ ms‡e`bkxjZv ew× cv‡"Q| †µv‡gv‡Rv‡gi wewfb MvVwbK weP¨wZ h_v, †µvgwUW †mZ, †µv‡gv‡Rvg LÛvsk, j¨vMvW †µv‡gv‡Rvg BZ¨w` mwó‡ZI Bw_wjb MvBKj Gi fwgKv i‡q‡Q| G‡¶‡Î j¶¨ Kiv †M‡Q †h, cwie¨w³Ki `‡e¨i gvÎvew×i mv‡_ mv‡_ †µv‡gv‡Rv‡gi MvVwbK weP¨wZI ew× cv‡"Q| ev‡iv I Qq N›Uv mg‡qi Zjbvq PweŸk N›Uv e¨wc c‡qvMKZ cwie¨w³Ki `‡e¨i D"PZi cfvelch‡ew¶Z n‡q‡Q|

## Introduction

A group of chemicals are reported to induce chromosomal aberration. These chemicals are commonly known as chemical mutagens having specific and limited action, and found to induce specific mutation or aberration in organisms. Chemical mutagens are being used in inducing variability in plant breeding programmes. Geneticists are using chemical mutagens as potential tools and it is reported that a number of chemicals influence the sensitivity as well as increase the frequency and spectrum of mutations.

The studies of a number of compounds and the development of chemical mutagenesis have been reported by different workers (Legator 1970; Liang and Liang 1972; Prasad 1972; Bose and Dutta 1973; Raghubansi et.al. 1978; Alam et. al. 1981; Rao and Rao 1983). In view of these research aspect, many mutant varieties have been developed through mutagenesis. Among them 94% were following the treatments of physical mutagen, 5% through chemical mutagen and the remaining 1% through a combined treatment of physical and chemical mutagens (Singh 1993). Considering these research attributes the present study was undertaken to investigate the mutagenic effect of a chemical mutagen *i.e.* Ethylene Glycol (EG) on somatic cells of wheat (*Triticum aestivum* L.).

## Materials and Methods

Five varieties of wheat *i.e.* Shourav ($V_1$), Gourab ($V_2$), Shatabdi ($V_3$), Protiva ($V_4$), Balaka ($V_5$) and a chemical mutagen *i.e.,* EG were used as experimental materials. The seeds of these varieties of wheat were procured from Regional Wheat Research Institute, Shaympur, Rajshahi. Five different doses of EG such as 1%, 2%, 3%, 4% & 5% designated as $D_1$, $D_2$, $D_3$,

$D_4$ and $D_5$, respectively along with a control ($D_0$) were used.

The dry and dormant seeds of the tested varieties of wheat treated with EG were placed on moist filter paper in Petri dishes for germination. When the roots grew up to 1.0-1.5 cm in length root tips of different varieties of wheat from each treatment were collected separately by a pair of fine forceps and fixed in 1:3 aceto alcohol solution for 48 h. Then, they were transferred to 70% ethyl alcohol and stored in the refrigerator till used. The root tip cells were stained by squash method using haematoxylin as stain following the schedule of Haque et al. (1976) with certain modifications.

In order to determine the interphase chromosome volume (ICV) from the cells of different varieties treated with different doses of EG, in the present study, the nuclear volumes (NV) were measured by oculometer and the values were calculated using the formula for a sphere $V = \frac{4}{3}\pi r^3$ (Nayer et al. 1971). The mean nuclear volume divided by the somatic chromosome number gave the interphase chromosome volume.

Different types of chromosomal abnormalities such as lagards, fragments, bridges etc. were detected from the desired preparations. They were detected from metaphase, anaphase and telophase stages of mitosis and data were recorded and analyzed statistically.

## Results

The ICV shown in Plate 1 was found to vary among the different doses of EG for 6 h treatment duration. Findings (Table 1) indicated that there was no significant variation among the different varieties, different doses and also in their interaction. Among the five varieties, the highest interphase chromosome volume was found in $V_3$ and lowest in $V_4$ (Fig. 1).

For 12 h treatment duration, ICV were found to vary among the different doses of EG. The ICV ($\mu^3$) due to $D_1$, $D_2$, $D_3$ and $D_4$ in $V_1$, $V_2$, $V_5$; $D_1$, $D_2$, $D_3$ and $D_5$ in $V_3$; $D_1$, $D_2$, $D_4$ and $D_5$ in $V_4$ showed non-significant variation. The analysis of variance indicate that there is no significant variation among different varieties, different doses and also in their interaction. Among the five varieties the highest interphase chromosome volume were found in $V_3$ and lowest in $V_4$ (Fig. 2).

For 24 h treatment duration, ICV were also found to vary among the different doses of EG. The ICV ($\mu^3$) due to $D_1$, $D_2$, $D_3$ and $D_4$ in $V_1$, $V_3$, $V_4$ and $V_5$; $D_1$, $D_2$, $D_3$ and $D_5$ in $V_2$ also showed non-significant variation from that of control ($D_0$). Results indicate that there is no significant variation among the different varieties, different doses and also in their interaction. Among the five varieties the highest ICV were found in $V_3$ and $V_4$ and lowest in $V_5$ (Fig. 3). The highest interphase chromosome volume was found in 24 h treatment duration followed by 12 and 6 h (Fig. 7).

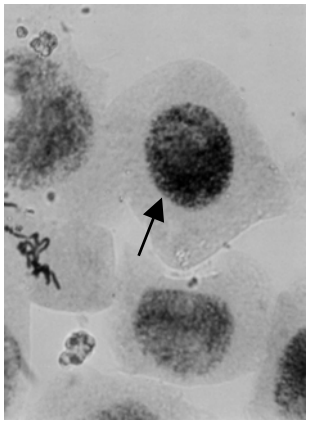
Chromosomal abnormalities (Plate.1, B–I) in root tip cells of five wheat varieties treated with different doses of EG were determined. In case of 6 h treatment duration percentage of chromosomal abnormalities were found to vary among the different doses. It revealed that, abnormalities due to $D_1$, $D_2$, $D_3$ and $D_4$ in $V_1$, $V_3$, $V_4$; $D_1$ in $V_2$; $D_1$, $D_2$, $D_3$ in $V_5$ showed non-significant variation in comparison to that of control ($D_0$). Results showed non-significant variation among the different varieties (Table 2). In case of different doses there is highly significant variation. Among five varieties, the highest chromosomal abnormalities were found in $V_3$ and $V_5$ and lowest in $V_2$ and $V_4$ (Fig. 4).

Chromosomal abnormalities were also found to vary among the different doses of EG for 12 h treatment duration. Abnormalities due to $D_5$ in $V_1$, $V_2$, $V_4$ and $V_5$; $D_4$ and $D_5$ in $V_3$ were found to be highest and showed significant variation in comparison to that of control ($D_0$). Abnormalities due to $D_1$, $D_2$, $D_3$ in $V_1$ & $V_3$; $D_1$ and $D_4$ in $V_2$; $D_1$ and $D_2$ in $V_4$; $D_2$ and $D_3$ in $V_5$ showed non-significant variation. Analysis of variance (Table 2) showed that there is no significant variations among the varieties and also in case of the interaction between varieties and doses. But among the doses there was highly significant variation. Among five varieties the highest abnormalities were found in $V_5$ and lowest in $V_3$ (Fig. 5).

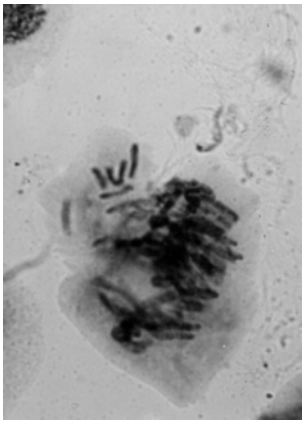
For 24 h treatment duration chromosomal abnormalities were also found to vary among the different doses of EG. Abnormalities due to $D_1$, $D_2$, $D_3$ in $V_1$; $D_1$ in $V_2$ and $V_3$; $D_1$, $D_2$, $D_3$, $D_4$ in $V_5$ showed non-significant variation.

Results (Table 2) indicated that non-significant variations were found in varieties but highly significant variations were found among the different doses. Interaction between varieties and doses also showed no significant variation. The highest

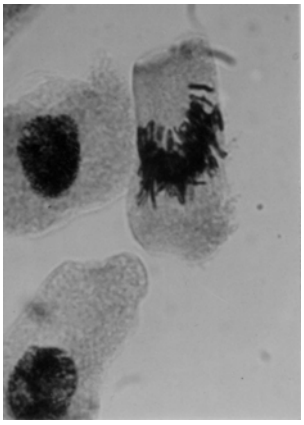
chromosomal abnormalities were found in $V_5$ and lowest in $V_2$ (Fig. 6). Among the three treatment durations the highest chromosomal abnormalities were found in 24 h treatment duration followed by 12 h and 6 h (Fig. 8). It was also found that the abnormalities increased with the increasing doses of EG and also with the increase of treatment durations.



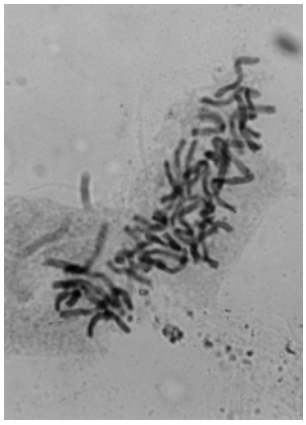
A. Interphase



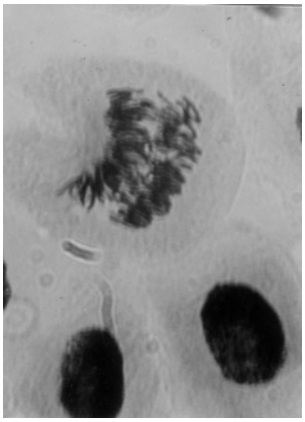
B. Disoriented metaphase chromosomes



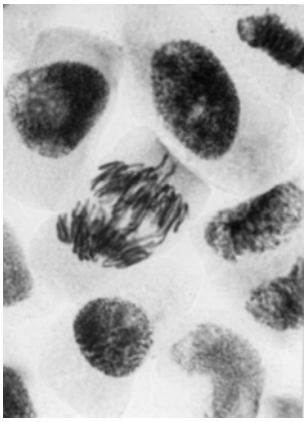
C. Desynchronized metaphase chromosomes



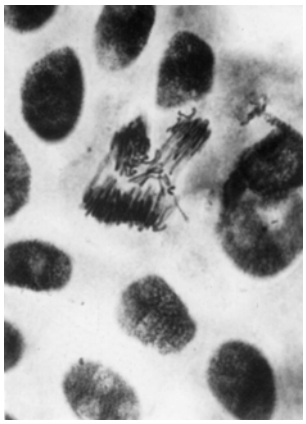
E. Inhibited metaphase chromosomes

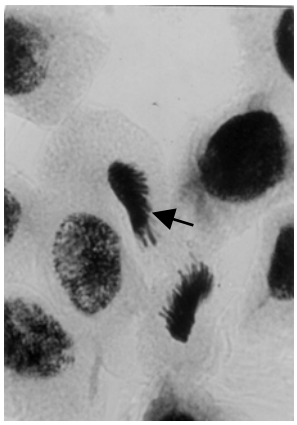


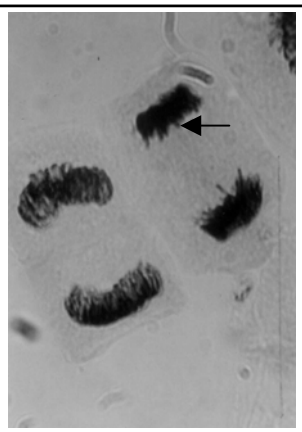
F. Irregular arrangement of anaphase chromosomes



G. Anaphase with chromatid bridge







H. Disorganized telophase with chromatid bridge

I. Depolarised telophase chromosomes

J. Telophase with chromosome fragment

**Plate. 1 (A-J).** Mutagenic effects of ethylene glycol on somatic chromosomes of hexaploid wheat.

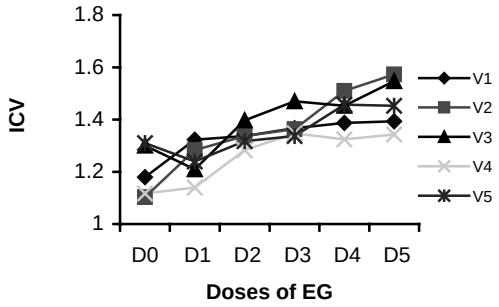



**Fig. 1.** Interphase chromosome volume (ICV) in root tip cells of wheat treated with different doses of EG for 6h.

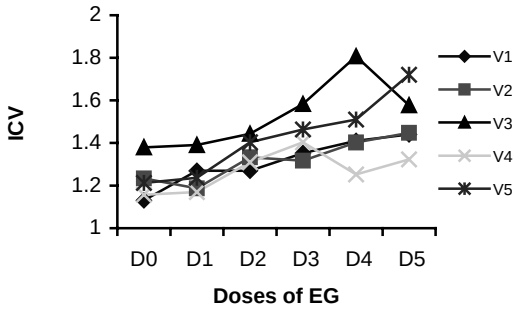



**Fig. 2**. Interphase chromosome volume (ICV) in root tip cells of wheat treated with different doses of EG for 12h.

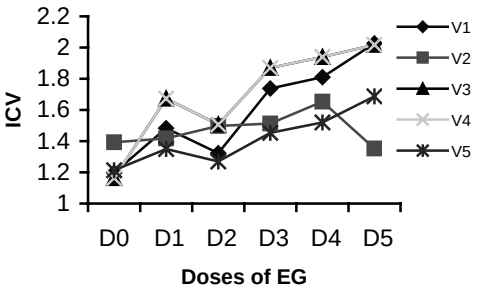



**Fig. 3**. Interphase chromosome volume (ICV) in root tip cells of wheat treated with different doses of EG for 24h.

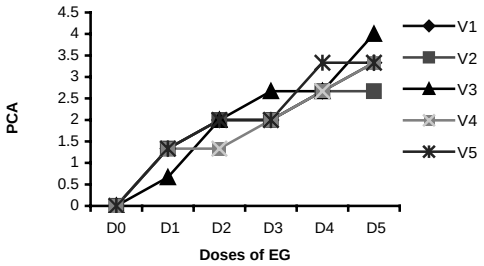



**Fig. 4**. Percent chromosomal abnormalities (PCA) of wheat treated with different doses of EG for 6h.

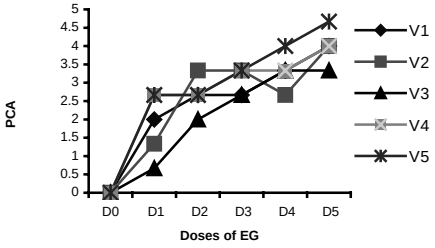



**Fig. 5**. Percent chromosomal abnormalities (PCA) of wheat treated with different doses of EG for 12h.

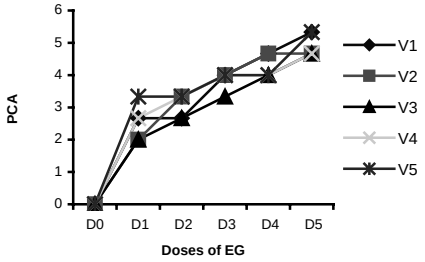
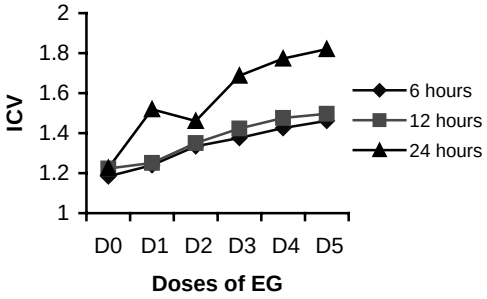



**Fig. 6**. Percent chromosomal abnormalities (PCA) of wheattreated with different doses of EG for 24h.



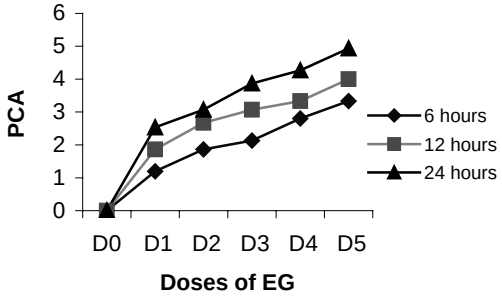



**Fig. 7**. Average Interphase chromosome volume (ICV) in root tip cells of wheat treated with different doses of EG for 3 different durations.

**Table 1.** Analysis of variance for interphase chromosome volume of wheat treated with EG for three treatment durations (6, 12 & 24 hours).

| Duration (h) | Source of Variation | df | SS | MS | F |
|---|---|---|---|---|---|
| 6 | Replication | 2 | 1.5682 | 0.7841 | 2.920 |
| | Variety (V) | 4 | 0.1868 | 0.0467 | 0.173 |
| | Dose (D) | 5 | 0.8407 | 0.1681 | 0.626 |
| | V X D | 20 | 0.2319 | 0.0116 | 0.043 |
| | Pooled error | 58 | 15.5735 | 0.2685 | |
| | Total | 89 | 18.4011 | | |
| 12 | Replication | 2 | 0.4884 | 0.2442 | 0.773 |
| | Variety (V) | 4 | 0.8208 | 0.2052 | 0.649 |
| | Dose (D) | 5 | 1.0215 | 0.2040 | 0.645 |
| | V X D | 20 | 0.3893 | 0.0195 | 0.061 |
| | Pooled error | 58 | 18.3209 | 0.3159 | |
| | Total | 89 | 21.0409 | | |
| 24 | Replication | 2 | 1.5627 | 0.7814 | 1.937 |
| | Variety (V) | 4 | 3.3661 | 0.8415 | 2.087 |
| | Dose (D) | 5 | 2.7011 | 0.5402 | 1.339 |
| | V X D | 20 | 1.3924 | 0.0696 | 0.172 |
| | Pooled error | 58 | 23.3873 | 0.4032 | |
| | Total | 89 | 32.4096 | | |

**Table 2.** Analysis of variance for chromosomal abnormalities of wheat treated with EG for three treatment durations (6, 12 & 24 hours).

| Duration (h) | Source of Variation | df | SS | MS | F |
|---|---|---|---|---|---|
| 6 | Replication | 2 | 12.9617 | 6.9809 | 1.9683 |
| | Variety (V) | 4 | 0.8832 | 0.2208 | 0.0622 |
| | Dose (D) | 5 | 105.2927 | 21.0585 | 5.9376* |
| | V X D | 20 | 6.0438 | 0.3022 | 0.0852 |
| | Pooled error | 58 | 205.7079 | 3.5466 | |
| | Total | 89 | 330.8893 | | |
| 12 | Replication | 2 | 19.0182 | 9.5091 | 3.1222 |
| | Variety (V) | 4 | 7.8209 | 1.9552 | 0.6419 |
| | Dose (D) | 5 | 149.1623 | 29.8325 | 9.7952* |
| | V X D | 20 | 10.8416 | 0.5421 | 0.1779 |
| | Pooled error | 58 | 176.6476 | 3.0456 | |
| | Total | 89 | 363.4906 | | |
| 24 | Replication | 2 | 14.4205 | 7.2103 | 2.6936 |
| | Variety (V) | 4 | 3.1074 | 0.7769 | 0.2902 |
| | Dose (D) | 5 | 228.6219 | 45.7244 | 17.0817* |
| | V X D | 20 | 6.4891 | 0.3245 | 0.1212 |
| | Pooled error | 58 | 155.2578 | 2.6768 | |
| | Total | 89 | 407.8967 | | |

* = significant at 1%

## Discussion

**Fig. 8**. Average chromosomal abnormalities of wheat treated with different doses of EG for 3 different durations.

It was found that there was an increase in interphase chromosome volume with the increasing doses of EG. With some exceptions, interphase chromosome volumes were greater than that of control. The main cause of increasing the interpahse chromosome volume might be due to alteration of cell membrane configuration, modification of chromosomal proteins and changes in sensitivity to ethylene glycol. Yamakawa and Sparrow (1965, 1966) studied the interphase chromosome volume as a reliable index of radiosensitivity in plants. Underbrink et al. (1973) studied the roles of ICV, NV and ploidy on the degree of pollen abortion induced by radiation. Uddin (2005) reported that there was an increase in interphase chromosome volume with an increasing dose of different hormones in onion (*Allium cepa*). Similar results were also obtained by Khatun (2004) in pigeon pea (*Cajanus cajan* L.) treated with different doses of gamma ray, EMS and EG.

Interphase chromosome volume is considered as a reliable index of mutagen sensitivity in plant cell and the effect caused due to the action of mutagen may be helpful to estimate the mutation rate. Mutagen generally induces a high frequency of chromosomal changes and mitotic abnormalities. In broad sense, all types of changes in the chromosome structure and number are the chromosomal abnormalities. In structural changes both chromatids of the chromosomes are involved, as opposed to chromatid or sub-chromatid aberration. Presplit aberrations normally arise spontaneously or by application of mutagens in the not yet reduplicated chromosomes of pre- synthetic interphase nucleus. Certian accidents or irregularities are caused due to application of certain chemicals also.

The actions of ionizing radiations and chemical mutagens on somatic cell division have been reported by some investigators in a number of plant species (Clowes and Hall 1970; Reiger and Michaelis 1972). All of them observed mitotic irregularities such as abnormal distribution of chromosome, laggards, anaphase bridges, unequal separation of chromosomes, clumpings etc.

In present study the major structural changes of chromosomes were inhibited chromosomes, bridges, fragments, laggards, desynchronized chromosomes etc. Abnormalities were recorded from metaphase, anaphase and telophase stages of mitosis. It was also found that the frequency of structural changes increased with an increased dose of EG and also with increasing the treatment duration.

Different mutagen induces different types of structural changes, like inhibited chromosomes, fragments, bridges etc. (Bose and Banerjee 1977; Bandyopadhyay and Bose 1979,1980). Natarajan and Upadhya (1964) and Mikaelson et al. (1968) observed fragements with EMS. Jana et al. (1974) observed inhibited chromosomes and Nicoloff et al. (1971) observed a similar inhibition with broken chromosome with maleic hydrazide. Bridges at anaphase and telophase stages observed in this study were also reported by Prasad (1972), Kalloo (1972) and Singh and Godward (1974). Anaphase bridges were observed in *Vicia faba* with different types of chemicals by Gopalan and Njagi (1984). Due to sticky nature of chromosomes chromatin bridges may be found at anahase and telophase stages (Jain and Sarbhoy, 1987) and that is also caused due to mutagenesis.

In the present study chromosomal abnormalities were found to increase with increasing the doses of EG. Similar results were also reported by Alam *et al.,* (1980) and Khatun (2004).

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