



Cytological effect of UV radiations and chemical mutagens on *Pisum sativum* L. and *Hordeum vulgare* L.

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Received for publication: September 29, 2015; Revised: October 11, 2015; Accepted: October 22, 2015

Abstract: The present work provides a comparative account of cytological effect of mutagens on two plant models *Pisum sativum* L. and *Hordeum vulgare* L. Both UV radiations and chemical mutagens induce chromosomal anomalies at various dose and concentrations respectively. Varieties of anomalies were seen in *H. vulgare* as compared to *P. sativum*. In general, the anomalies increased as the concentration of the mutagen increased.

Key words: Mutagens; UV; Chromosomal anomalies; *P. sativum*; *H. vulgare*.

INTRODUCTION

Genetic variations in the germ plasm are the outcome of effective techniques of selection during plant breeding. Mutagenesis can be beneficially utilized for tailor-made varieties of crop plants. Many researchers have worked on different mutagens and their effects on different crops. While many researchers like Rao and Rao (1983), Kumar and Dubey (1998), Dhanayanth and Reddy (2000) and Bhat *et al.*, (2005) found chemical mutagens to be more effective than physical ones, others like Tarar and Dnyansagar (1980), Zeerak (1991) and Singh (2003) found the reverse case. Chemical mutagens provide a good scope for selection, as a tool for alteration in the genotype to enhance the variability of characters.

Natrajan (1993) stated that genotoxic agents induce chromosomal alterations such as bridge formations, micronuclei, laggards both *in vitro* and *in vivo*. Ionizing radiations are very effective inducers of chromosomal aberrations. Chemical mutagens also play important role in inducing mutagenesis in crop plants.

Even though all mutagenic changes are not beneficial for human welfare, Researchers have worked to extract maximum beneficial potency in mutation breeding programmes to incorporate beneficial changes in agriculture and crop improvement.

In the current investigation physical mutagen such as UV radiations and varied concentrations of two potent chemical mutagens 1, 4-dichlorobenzene (PDB) and colchicine, were used to treat *Pisum sativum* and *Hordeum vulgare*.

MATERIALS AND METHODS

Sample Collection: Healthy and viable seeds of *Pisum sativum* and *Hordeum vulgare* were obtained from local market and authenticated in Department of Botany, Bhavan's college, Andheri West.

Mutagen Treatment

The seeds were subjected to treatment by four different concentrations (0.05, 0.1, 0.3 and 0.5 %) of PDB (Paradichlorobenzene) and colchicine after presoaking of 12 hours. For UV radiations the seeds were exposed to radiations for a period of 2 and 4 hours. 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 M1 generation.

Microscopic Investigation

For mitotic studies root tips were randomly selected. The apical meristem of the tips were fixed in freshly prepared Carnoy's fixative (Absolute alcohol, Chloroform and Acetic acid in 6:3:1 ratio) for 20 minutes, washed and preserved in 70% alcohol. The apical meristems of the tip were squashed in 2% aceto-carmine and kept for 15 minutes. The stained tips were then mounted in Canada balsam and dried at 45°C. Microphotographs were taken from freshly prepared slides.

RESULTS AND DISCUSSION

Effect of UV light on *Pisum sativum*:

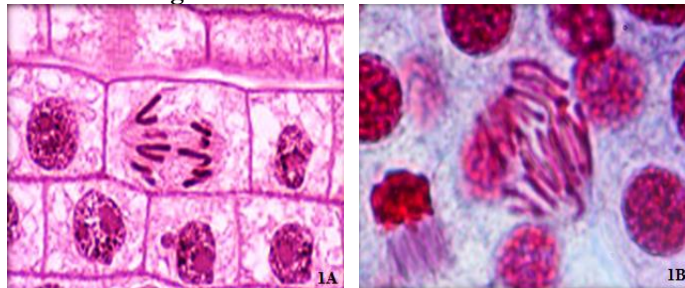


Figure 1A: Ill-defined movement of chromosomes at 2 hours exposure.

Figure 1B: Disturbed Anaphase at 4 hours exposure

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Effect of UV light on *Hordeum vulgare*:

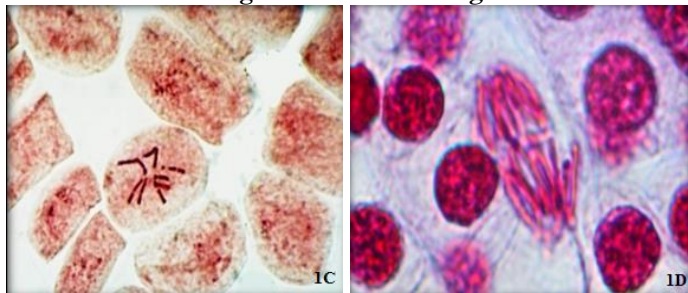


Figure 1C: Prominent centromere visible and arrested metaphase at 2hours exposure
Figure 1D: Late anaphase and lagging chromosome at 4hours exposure

Effect of PDB on *Pisum sativum*:

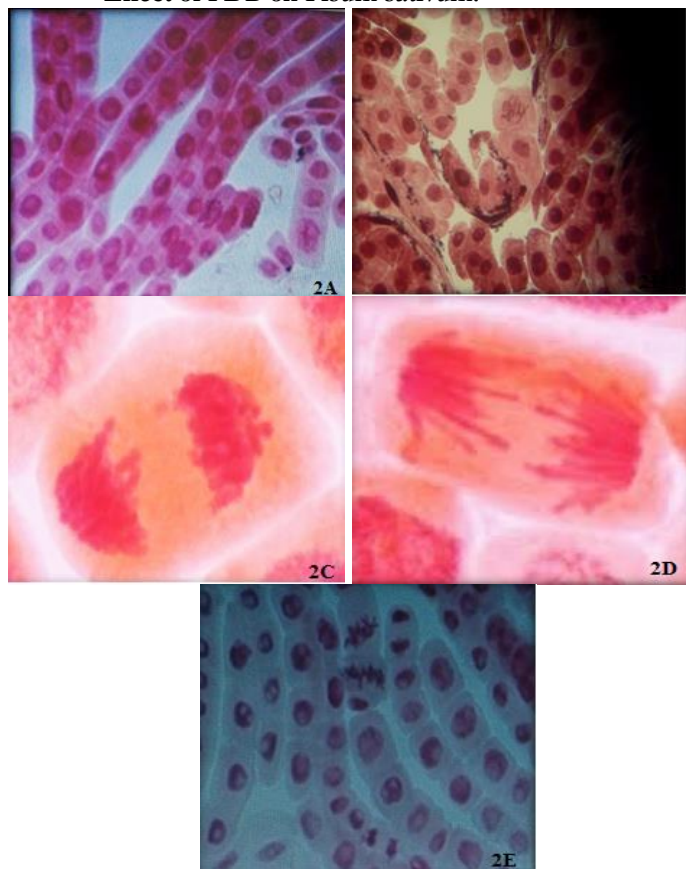


Figure 2A: Vacuole formation at 0.05%
Figure 2B: Vacuole formation and disturbed metaphase at 0.1%
Figure 2C: Lagging chromosome at 0.1%
Figure 2D: Incomplete Bridge formation at 0.3%
Figure 2E: Metaphase and micronucleus formation in anaphase at 0.3%.

Effect of PDB on *Hordeum vulgare*:

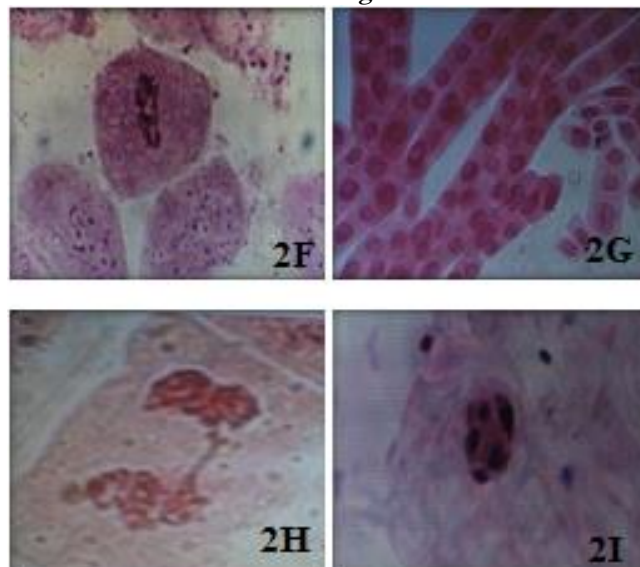


Figure 2F: Nuclei elongation and stickiness at 0.1%
Figure 2G: Vacuole formation and streaming movement of nuclei at 0.1%,
Figure 2H: Bridge formation and stickiness at 0.3%,
Figure 2I: Micronuclei formation at 0.5%

Effect of Colchicine on *Pisum sativum*:

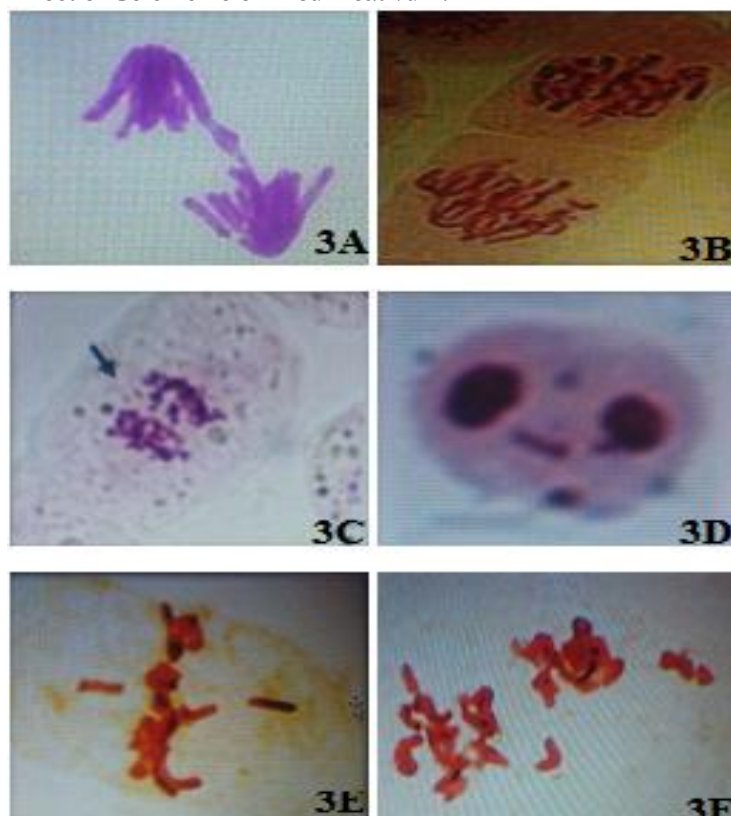
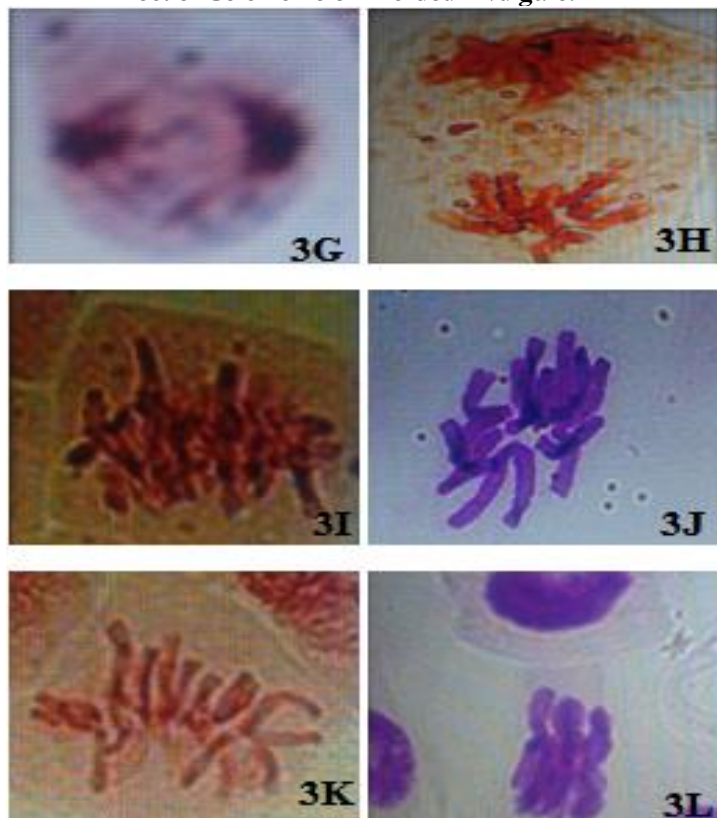


Figure 3A: Chromosomal bridge with umbrella shaped chromosome at 0.3%
Figure 3B: Disturbed Metaphase at 0.5%
Figure 3C: Laggard formation at 0.1%
Figure 3D: Binucleate cell at 0.3%
Figure 3E: Clumping of chromosomes and stickiness and vagrant chromosome 0.3%
Figure 3F: Clumping and vagrant chromosome at 0.1%

Effect of Colchicine on *Hordeum vulgare*:**Figure 3G:** Binucleate condition at 0.3%**Figure 3H:** Early Telophase and Clumping of chromosomes at 0.1%**Figure 3I:** Stickiness at metaphase at 0.05%**Figure 3J:** Arrested metaphase with distinct 14 chromosomes at 0.3%**Figure 3K:** Disturbed metaphase at 0.1%**Figure 3L:** Stickiness of chromosomes at late prophase at 0.1%

The effect of physical and chemical mutagens is summarized in table 1, 2 and 3 for UV radiations, PDB and Colchicine respectively. In these two species all three types of mutagens were successful in inducing chromosomal anomalies and alterations. There was a variety of anomalies in *H. vulgare* in comparison to *P. sativum*. From the study it is clear that with the increase in dosage in terms of concentration or exposure there is increase in aberrations. Different types of chromosomal abnormalities observed during the present investigation as laggard, bridges, binucleate condition, micronuclei, chromosomal stickiness and unsynchronised movement have been reported by various workers in different plant materials after treated with physical and chemical mutagens (Ahmed, 1993; Kumar and Dubey, 1998; Dhamayanthi and Reddy 2000; Bhat *et al.*, 2005). The laggards observed might be due to the delayed terminalization stickiness of chromosomal ends or because of failure of the chromosomal movement (Jayabalan Rao, 1987; Soheir *et al.*, 1989). Induction of such mutations may have applications in crop improvement programmes. Even though all mutations are not beneficial it's the skill of geneticist and plant breeder to select the appropriate type for the betterment of crop improvement.

Table 1: Effect of UV light on *Pisum sativum* and *Hordeum vulgare*

S.no	Time of exposure	Effect on <i>P. sativum</i>	Effect on <i>H. vulgare</i>
1.	2 hours	Ill-defined chromosomes	Arrested metaphase with prominent centromere
2.	4 hours	Disturbed anaphase	Late anaphase with lagging chromosomes

Table 2: Effect of PDB on *Pisum sativum* and *Hordeum vulgare*

S. no	Concentration (in Percentage)	Effect on <i>P. sativum</i>	Effect on <i>H. vulgare</i>
1.	0.05	Vacuole formation	No visible damage
2.	0.1	Vacuole formation and disturbed metaphase, lagging chromosomes	Nuclei elongation, stickiness, vacuole formation, streaming movement of nuclei.
3.	0.3	Incomplete bridge formation, micronuclei formation during anaphase	Bridge formation and stickiness
4.	0.5	Chromosome breaks	Micronuclei formation

Table 3: Effect of Colchicine on *Pisum sativum* and *Hordeum vulgare*

S. no	Concentration (in percentage)	Effect on <i>P. sativum</i>	Effect on <i>H. vulgare</i>
1.	0.05	No visible damage	Stickiness at metaphase
2.	0.1	Laggard formation and clumping of chromosomes	Disturbed metaphase, Early telophase with clumping of chromosomes
3.	0.3	Chromosomal bridge with umbrella shaped polar chromosomes, formation of binucleate cell, clumping stickiness of chromosomes	Binucleate cell, arrested metaphase.
4.	0.5	Disturbed metaphase	Chromosomal breaks

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CITE THIS ARTICLE AS:

Kakoli Dassharma, Rohan Gavankar, Basil DMello, Priyanka Kesur. Cytological effect of UV radiations and chemical mutagens on *Pisum sativum* L. and *Hordeum vulgare* L. *International Journal of Bioassays* 4.12 (2015): 4586-4589.

Source of support: Nil

Conflict of interest: None Declared