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CYTOLOGICAL EFFECT OF HERBICIDES ON HEXAPLOID WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

Context: In order to control weeds many chemicals are being used. It is well known that the chromosomal constitution of the cells may be changed as a result of herbicide treatment.

Objectives: This investigation was made to study the cytological effects of herbicides on somatic and germ cells of hexaploid wheat.

Materials and Methods: Two herbicides namely, Fielder and Ronstar were used with three different concentrations along with control. Treatment duration was 12 hours for each of the concentrations. Seeds of hexaploid wheat were treated and the roots emerged from them were studied for mitotic study. A part of the treated seeds were sown in earthen pots and pollen mother cells along with pollen grain were studied from the plants aroused from these treated seeds.

Results: Mitotic index were high as well as low in most of the treatments compared to that of control but did not show any clear relationship based on increasing or decreasing of the doses of herbicides. Interphase chromosome volume was found to decrease in case of Fielder however, increased in case of Ronstar with increasing the concentration of herbicides. Percentage of pollen sterility was found to increase with increasing the concentration of both herbicides. Main chromosomal irregularities were chromosome fragments, bridges, laggards, single and multiple chromatid bridges etc.

Conclusion: Generally it is reported that any chemical might have effect on the plant cells. Mainly investigations have evidenced that the chromosomal anomalies caused by radiation and chemical mutagen is very much common matter now. The herbicides used in the present study showed similar type of radiomimetic effect in the plant cells. Therefore it may be suggested that herbicides should be used in agricultural field maintaining their proper concentration.

Key words: Herbicides, Cytological effect, Wheat.

Introduction

Wheat is the second most important cereal crop in Bangladesh. It provides a source of income and food for millions of farm families. For the last 25 years in Bangladesh, attention has been governed extensively on the higher yields of common wheat (*Triticum aestivum* L.). However, weeds are one of the factors for low yield of wheat (Mukhopadhyah 1992). It has been estimated that weeds reduced productivity of wheat by 15-30% (Mani *et al*. 1998, Jain and Chaubey 1999). In order to control weeds many chemicals are being used. These are either used alone or in combination with other chemicals to eliminate weeds. It is well known that the chromosomal constitution of the cells may be changed as a result of herbicide treatment (Unrau and Corns 1980, Unrau and Larter 1982). Unrau (1983) showed that the herbicide 2,4-dichlorophenoxy acetic acid (2, 4-D) was an effective agent for inducing chromosomal aberrations in meiotic cells of barley and wheat. Unrau and Larter (1982) reported as high as 35% of pollen mother cells of barley and wheat plants with chromosomal abnormalities after spraying with 2,4-D prior to microsporogenesis.

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Alam *et al*. (1980) studied the effects of pesticides, "Carbicron", "Vapona" and "Dimecron" on wheat and herbicide "Ansar" on barley. Different kinds of chromosomal abnormalities were observed by these workers. Al-Najjar and Soliman (1982) reported the effects of 2,4-D and 2,4,5 trichlorophenoxy acetic acid (2,4,5-T) on meiotic cells of hexaploid wheat (*Triticum aestivum* L.) and tetreploid wheat (*Triticum durum*). Percentage of abnormal PMCs induced by two herbicides was significantly higher than control. The kind of abnormalities observed were lagging chromosomes, single and multiple bridges, fragments and asynchronization in the disjunction of chromosomes during second anaphase. Therefore, the purpose of the present investigation was to make a keen and comparative study regarding cytological effects of two herbicides on wheat (*Triticum aestivum* L.).

Materials and Methods

The experiment was conducted in Professor Sultanul Alam Cytogenetics Laboratory, Department of Botany, University of Rajshahi. Seeds of *Triticum aestivum* L. var. Kanchan (2n = 42) was used as material. Two herbicides namely, Fielder and Ronstar with different concentration viz. 0.05%, 0.10% and 0.25% were used as treatment. In addition, distilled water was taken as positive control. Three different concentrations were prepared from each of the herbicides. Dry seeds of wheat were soaked in three different doses (aqueous solution) of Fielder and Ronstar in separate beakers. After 12 hours, the solution was decanted off and the seeds were washed thoroughly with tap water. The seeds for control were also presoaked in distilled water for the same period of time. Part of the seeds were allowed to germinate in the laboratory at room temperature (25-30^{0}C) for mitotic study whereas another part sown in the pot for meiotic study. After emergence of root from Fielder treated wheat seeds at 0.25%, the materials were found to decay gradually and thus, further characters could not be studied. For mitotic study, roots of 1.0-1.5 cm in length were fixed in 1:3 acetic -alcohol for 48 hours and stored in 70% ethanol. Chromosomes were stained with 0.5% haematoxylin following the method of Haque *et al*. (1976). Data were recorded on mitotic index, interphase chromosome volume (ICV) and various mitotic abnormalities from different stages.

The nuclear volume (NV) was calculated using the formula for a sphere, NV = 4/3 πr^3 (Nayer *et al*. 1970). The mean nuclear volume divided by the somatic chromosome number gave the ICV. For meiotic study, young inflorescence from plants of various treatments along with control were collected and immediately fixed in modified carnoy's fixative (6:3:1: ethanol: chloroform: acetic acid). After 48 hours the ear heads were transferred to 70% ethanol. To collect data on meiotic abnormalities and pollen sterility, temporary slides were prepared from suitable anthers by acetocarmine smear technique. The findings obtained from the experiment were analyzed statistically.

Results and Discussion

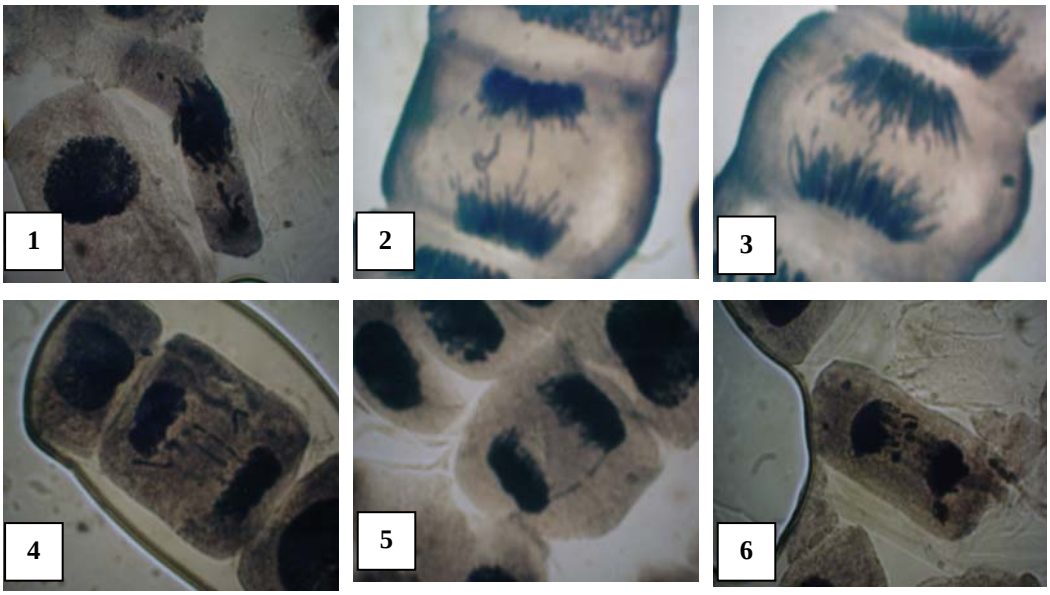
Data regarding comparative effects of two herbicides on mitotic index (MI), interphase chromosome volume (ICV), mitotic and meiotic abnormalities and pollen sterility are shown in Tables 1-2 and Figs. 1-12. Mitotic index was found to be highest (6.75%) in the roots emerged from seeds treated with 0.10% of Fielder and the value was found to be significantly different from that of all other treatments (Table 1). On the other hand, the lowest value for mitotic index (2.10%) was observed due to 0.05% of Fielder and this value was significantly different from that of all other treatments. It was observed that the mitotic index (MI) in most of the treatments higher than that of the control. In lower doses of both the herbicides, mitotic index was reduced significantly than that of the control. Higher doses of Ronstar showed almost similar mitotic index with as control, however the mitotic index was significantly higher from that of the control in 0.10% Fielder treatment (Table 1). Similar observation was reported by Khan (1983). Kabir (1981) also reported that the mitotic index was considerably reduced due to insecticides and mycotoxin treatment. In the present findings, no clear relationship between increasing mitotic index in root tip cells and or decreasing of the doses of herbicides from the recommended concentration was found.

Table 1 indicates that the maximum value for interphase chromosome volume (1.696 μ^3) was observed due to 0.25% of Ronstar treated wheat seeds and it was significantly different from that of all other treatments. On the other hand, the minimum interphase chromosome volume (0.272 μ^3) was observed in 0.10% of Fielder which also significantly different from that of all other treatments. The concentration of Ronstar and ICV was directly proportional whereas it was inversely proportional in case of Filder (Table 1). Kabir (1981) observed that ICV was increased with an increase of the doses of two insecticides. Interphase chromosome volume is generally caused due to alternation of cell membrane configuration, modification of chromosomal proteins and changes in sensitivity of chemicals. Yamakawa and Sparrow (1968) found that increase in mutation rate per roentgen in petals of five higher plants was highly correlated with an increase in both ICV and DNA content per chromosome.

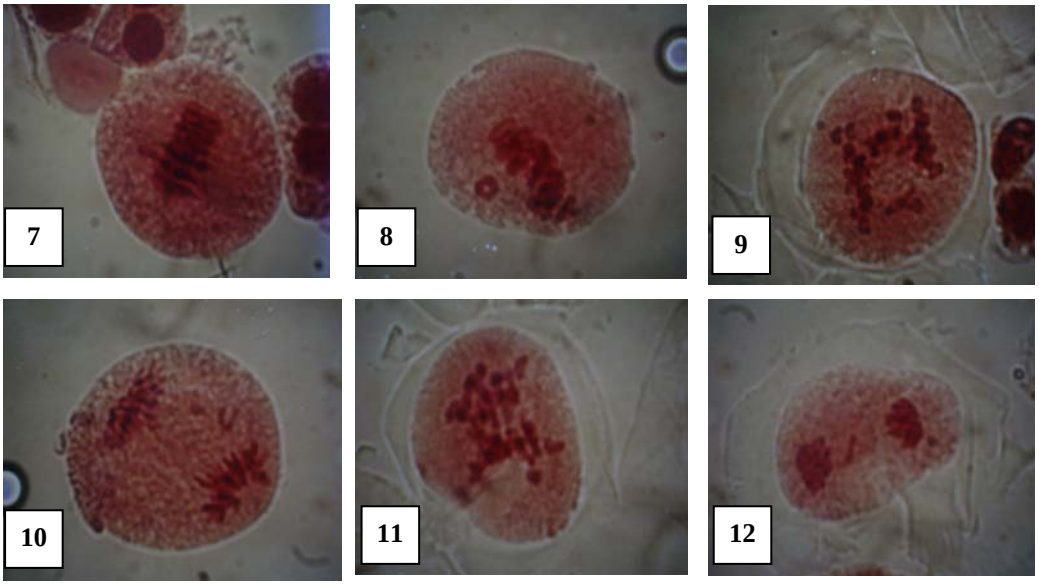
Table 1. Effect of herbicides on mitotic index (MI), interphase chromosome volume (ICV) and mitotic abnormalities in the root tip cells of *Triticum aestivum* L.

Treatments		MI (%)	ICV (μ^3)	Mean No. of cells studied	Mean abnormality (%)	% of different abnormalities		
Chemical	Dose (%)					Bridges	Lagards	Fragment
Water	-	4.11b	0.619c	161.55	0.068c	0.0	0.0	0.068
	0.05	2.10c	0.397d	131.33	0.722bc	0.352	0.332	0.315
Fielder	0.10	6.75a	0.272e	57.33	2.325a	0.437	1.531	0.421
	0.25	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	0.05	3.74b	0.603c	92.67	0.77bc	0.218	1.332	2.425
Ronstar	0.10	4.15b	1.314b	83.78	0.912ab	0.51	0.52	0.21
	0.25	4.10b	1.696a	73.11	1.564ab	0.552	0.571	1.490
CV (%)		18.03	19.06	-	12.36	-	-	-

Means followed by the same letter(s) do not statistically differ at 5% level tested by DMRT.



Figs 1-6. Photomicrographs showing different types of mitotic abnormalities in the root tip cells of *Triticum aestivum* induced by different doses of herbicides (Ca 900x). 1. Metaphase with laggards, 2. Anaphase with broken bridge, fragments and laggards, 3. Anaphase with bridge, 4. Telophase with fragments and laggards, 5. Telophase with broken bridge and 6. Telophase with bridges and fragments.



Figs 7-12. Photomicrographs showing different types of meiotic abnormalities in *Triticum aestivum* due to different doses of herbicides (Ca 650x). 7. Normal metaphase I, 8. Metaphase I with a ring, 9. Anaphase I with bridge and laggard, 10. Anaphase I with laggard, 11. Irregular separation of bivalents at anaphase I and 12. Fragment and laggard in one of the dyad at anaphase II.

Mitotic study of the root tip cells of *Triticum aestivum* emerged from the seeds treated with different doses of herbicides showed various types of chromosomal abnormalities viz. chromosome fragments, lagging chromosomes, bridges etc (Figs. 1-6). In case of certain doses, some cells showed diverse types of chromosomal abnormalities such as bridges and fragments, bridges and lagging chromosomes, fragments and lagging chromosomes in a single cell. The bridges were single, double, multiple with or without fragment and lagging chromosome.

The highest percentage of mitotic abnormality (2.325%) was observed in 0.10% Fielder treatment. This value was statistically identical with that of 0.10 and 0.25% of Ronstar. The lowest mitotic abnormality was found in 0.05% Fielder and Ronstar which was statistically similar to control (0.068%). The percentage of mitotic abnormality increased with an increasing of the concentrations of both herbicides. Similar results were also reported by Bruhin and Wanner (1995) using insecticides 5, 5-dimethyl-dyhydro resorcin dimethyl carbonate on *Vicia faba.* Bhattacharya and Lenskens (1985) reported that 0.001 to 0.1% concentration of Systox also caused chromosome fragments in root tip cells of *Vicia faba*. Metaphase, anaphase and telophase fragments along with other abnormalities have been reported by Ahmed and Grant (1982) during their study on the cytological effects of the insecticide “Phosdrin” and the herbicide “Bladex” in root tip cells of *Tradescantia* and *Vicia faba.*

Meiotic study in the pollen mother cells (PMCs) of *Triticum aestivum* plants grown from the seeds treated with different doses of herbicides showed various types of chromosomal aberrations such as fragments, bridges and lagging chromosomes (Table 2, Figs. 7-12). The highest percentage of abnormality (6.062%) was observed in 0.05% of Ronstar treatment, which was significantly different from that of other treatments. The lowest percentage of abnormality was observed in all the treatments except 0.05% of Ronstar (Table 2). The lowest percentage of abnormality was statistically similar to control (0.21%). The results of meiotic study indicated that chromosome fragment, lagging chromosome and bridges were the main chromosomal

aberrations in PMCs. Presence of bridges without fragments or ana-bridges with fragments and lagging chromosome in the some cells indicated that the former is due to stickness of the chromosome and the latter is due to the breakage and reunion of the chromosome. Similar anaphase bridge were also noticed after treatment with the insecticide "Urethans" and "Carbon tetrachloride" in *Vicia faba* by Reddy and Rao (1969). Sharma and Mukherjee (1985) in their study on the effect of "Hydroquinone" and "Resorcinol" on the PMCs of *Allium cepa* founds that laggards, fragments and bridges were the chief induced abnormalities.

Percentage of pollen sterility was found to increase with an increasing of the concentrations of the herbicides. Pollen sterility in wheat plants raised from seeds treated with 0.10% of Fielder showed highest percentage of pollen sterility (11.33%) and that was significantly different from that of other treatments (Table 2). In the present findings, percentage of sterile pollens in all the treatments were somewhat higher than the control. This percentage of pollen sterility was also found to be increased with an increasing of the herbicidal concentrations. Similar result was observed by Alam and Kabir (1983) due to the treatment with insecticides on barley. In the present investigation, the frequency of pollen sterility appeared to be in close agreement with the frequency of meiotic abnormalities of the corresponding treatment as reported by Alam and Kabir (1983).

Table 2. Effect of herbicides on meiotic abnormalities and pollen sterility in *Triticum aestivum* L.

Treatments		Mean No. of cells studied	Mean abnormality (%)	% of different abnormalities				Pollen sterility (%)
Chemical	Dose (%)			Bridges	Lagards	Fragment	Others	
Water	-	51.556	0.21b	0.0	0.0	0.21	0.0	2.67d
	0.05	54.889	1.286b	0.438	0.428	0.445	0.0	10.67b
Fielder	0.10	50.778	2.427b	0.445	1.632	0.457	0.0	11.33a
	0.25	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	0.05	46.556	6.062a	0.338	1.432	2.625	1.711	6.00c
Ronstar	0.10	50.44	2.06b	0.560	0.760	0.22	0.441	6.67c
	0.25	63.0	2.966b	0.529	0.621	1.488	0.352	6.67c
CV (%)		-	15.21	-	-	-	-	16.60

Means followed by the same letter(s) do not statistically differ at 5% level tested by DMRT.

Conclusion

Generally it is reported that any chemical might have effect on the plant cells. Mainly investigations have evidenced that the chromosomal anomalies caused by radiation and chemical mutagen is very much common matter now. The herbicides used in the present study showed similar type of radiomimetic effect in the plant cells. Therefore it may be suggested that herbicides should be used in agricultural field maintaining their proper concentration. Otherwise, chemical hazardness may cause many sort problems in the life processes of crop plants.

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