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MORPHOLOGICAL AND CYTOLOGICAL EFFECTS OF TWO HERBICIDES ON TETRAPLOID WHEAT (*TRITICUM DURUM* L.)

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Abstract

Context: In order to control weeds many chemicals are being used which may change the morphological and cytological constitution of plant.

Objectives: To study the morphological and cytological effects of herbicides on tetraploid wheat.

Materials and Methods: Two herbicides namely, Topstar and Fielder with three different concentrations of each were used along with control in the present study. Seeds of tetraploid wheat were treated for 6 hours with each concentration and some of the treated seeds were allowed to germinate in the petri dishes, while some were sown in the earthen pot. Morphological study was done for both laboratory and pot grown plants. In case of cytological study, mitotic data was recorded using the root tip cells and meiotic data was collected from inflorescence at pre-mature stage of pot grown plant.

Results: In this experiment, data on morphological study such as germination percentage, days required in germination, coleoptile length, root length, plant height, ear length, tiller-number/pot, seed/ear, grain-weight/ear and 100 grain-weight indicated that treatment with Fielder decreased these characters with the increase of doses. In case of plant height, Topstar showed increased result than Fielder and control. Moreover, characters like ear length and seed/ear showed little variation among the doses of Topstar compared to control. Similarly, data on cytological study such as interphase chromosome volume (ICV), mitotic index (MI), % of pollen sterility, mitotic and meiotic abnormalities were found to be increased with increasing concentration of both herbicides. Main chromosomal irregularities were chromosome fragments, bridges, laggards, single and multiple chromatid bridges with laggard or fragment, stickiness etc.

Conclusion: From the study of morphological and cytological effects of two herbicides along with control it can be concluded that these chemicals had adverse impacts which reduced the yield of wheat.

Key words: Tetraploid wheat, Morphological effect, Cytological effect, Herbicides.

Introduction

Weeds are considerable constrain in crop production responsible for heavy yield losses (Anonymous, 2007). To overcome this great problem science has developed many chemicals that are being used worldwide. Many reports showed that using of improper concentration of these chemicals cause morphological and cytological abnormalities of various crop species (Shehata *et al*. 2000). Gul *et al*. (2006) showed that higher concentrations of herbicide Avenoxan significantly induced chromosomal abnormalities in *Allium cepa* L. and *Allium sativum* L. Tetraploid wheat *Triticum durum* (2n=28) is a commercially important crop which is most widely cultivated in developing countries like Bangladesh due to its good yielding traits and produces high quality flour in baking (Shamsi *et al*. 2006). Marwat *et al.* (2006) estimated that weed alone reduced the productivity of wheat by 25-50%. To overcome this loss using of herbicides has become popular among the

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farmers of Bangladesh because of its easy application and rapid action on weed control. In addition, most of the Bangladeshi farmers are illiterate and their indiscriminating use of herbicides regardless of its concentration may have bad impacts on yield and yield traits. Gelang *et al.* (2008) concluded that ozone exposure significantly reduced the harvest index and 1000 grain weights were 10, 28 and 37% lower and the grain yields were 15, 29 and 46% lower.

Considering all these viewpoints, it was attempted to make a comparative study regarding the effects of various concentrations of two herbicides on different morphological and cytological parameters like plant height, tiller-number/pot, seed/ear, grain-weight/ear, ICV, MI, pollen sterility, mitotic and meiotic chromosomal abnormalities related to yield and yield traits of *T. durum*.

Materials and Methods

This experiment was conducted in Professor S Alam Cytogenetics Laboratory, Department of Botany, University of Rajshahi, Rajshahi, Bangladesh during the winter of 2011-2012. Seeds of *Triticum durum* L. were used as plant material. Topstar ($C_{15}H_{14}Cl_2N_2O_3$) and Fielder (($C_8H_6C_{12}O_6$) were used as chemical herbicides and distilled water was used for control. Three different concentrations, D_1(0.05%), D_2(0.10%) and D_3(0.25%) of the two herbicides were prepared of which D_1 was the recommended dose while D_2 and D_3 were the higher doses. Before germination approximately 415 dry wheat seeds were taken (according to the suggestions of International Seed Testing Association) for treatment and were soaked in three different doses (aqueous solution) of both the herbicides in separate beakers. After 6 hours, the treated 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°C) whereas, the other part were sown in the pot. Morphological study was done for both the plants grown in laboratory and pot. For mitotic study, germinated roots of 1.0-1.5cm in length were fixed in acetic acid: ethanol (1:3) solution 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 interphase chromosome volume (ICV), mitotic index (MI) and various mitotic abnormalities from different stages of the experiment. The nuclear volume (NV) was calculated using the formula for a sphere, $NV = 4/3\ \pi r^3$ (Nayar *et al.* 1970). The mean nuclear volume divided by the somatic chromosome number gave the ICV.

For meiotic study, young inflorescences from pot plants of various treatments along with control were collected and immediately fixed in Carnoy's fixative (6 ethanol: 3 chloroform: 1 acetic acid). After 48 hours, these inflorescences 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. DMRT estimation of all the parameters was analyzed statistically by MSTAT-C package.

Results

Morphological study

In the present investigation, various characters such as germination percentage, days required for germination, coleoptile length, root length, plant height, ear length, tiller-number/pot, seed/ear, grain-weight/ear and 100 grain-weight/ear were taken under consideration for morphological study. Collected data on these characters were analyzed using DMRT estimation (Table 1). For all the characters, control always showed good performance than the treatment except plant height, in case of which Topstar gave maximum height. Moreover, Fielder showed decreased results than Topstar in all most all cases with the increase of doses.

Table 1. Effect of two herbicides on morphological characters of *T. durum*.

Treatments		Characters											
Chemical	Doses (%)	Germination %		Days req. in germination		Coleoptile length (cm)	Root length (cm)	Plant height (cm)	Ear length (cm)	Tiller no./ pot	Seed no./ear	Grain weight (g)	100 grain weight (g)
		Petri dish	Pot	Petri dish	Pot								
Control	-	94.33a	89.33a	1.66d	7.00e	4.25a	8.19a	61.18a	6.54a	46.45a	30.02ab	1.85ab	6.17a
	0.05 (D_1)	87.00b	83.67a	3.00c	7.33de	3.07b	6.85b	62.87a	6.46ab	40.09b	26.68bc	0.900c	3.37c
Topstar	0.10 (D_2)	78.33c	74.33b	3.33c	7.33de	2.76b	4.80c	62.73a	5.83abcd	35.33cd	23.56cd	0.61de	2.58de
	0.25 (D_3)	64.33de	64.67cd	4.00c	8.66cd	2.07c	3.91c	60.77a	5.40cd	30.88e	20.38de	0.43ef	2.13e
	0.05 (D_1)	70.33d	70.33bc	4.00c	9.66bc	2.96b	2.62d	58.43ab	5.97abc	37.32bc	23.12d	0.75cd	3.26c
Fielder	0.10 (D_2)	61.00e	62.33d	5.33b	10.33ab	1.98c	1.94de	57.31ab	5.52bcd	31.99de	17.96e	0.51ef	2.87cd
	0.25 (D_3)	51.00f	52.33e	7.33a	11.67a	1.68c	1.09e	52.56b	4.86d	24.06f	12.90f	0.29f	2.31de
CV %		3.73	3.83	13.67	7.12	7.30	10.26	4.33	6.84	4.75	6.01	9.16	6.40

Table 2. Effect of two herbicides on cytological characters of *T. durum*.

Treatments		Characters									
Chemical	Doses (%)	ICV (μ^3)	MI	% of Pollen sterility	Mitotic abnormality			Meiotic abnormality			
					%	Bridge	Fragment	%	Bridge	Fragment	Laggard
Control	-	0.094c	6.827a	4.127f	0.363e	0.1815	0.1815	1.672d	1.672	-	-
Topstar	0.05 (D_1)	0.110c	2.965bc	7.100e	2.147b	2.147	-	2.870c	0.870	2.000	-
	0.10 (D_2)	0.113c	1.890b	7.960e	1.040d	-	1.040	3.377c	-	1.335	2.042
	0.25 (D_3)	0.116bc	3.690d	11.870d	2.100b	1.05	1.05	5.330b	1.703	3.627	-
Fielder	0.05 (D_1)	0.113c	3.030bc	14.830c	1.633c	0.105	1.528	2.837c	1.418	-	1.418
	0.10 (D_2)	0.140ab	2.783bcd	21.510b	2.103b	1.051	1.051	5.177b	2.588	-	2.588
	0.25 (D_3)	0.157a	2.070cd	28.320a	3.000a	1.500	1.500	7.320a	2.853	1.467	3.000
CV %		7.01	10.30	7.78	8.43			10.43			

Cytological study

Some important cytological parameters such as ICV, MI, % of pollen sterility, mitotic and meiotic abnormalities were also examined in this study and the collected data were analyzed using DMRT estimation (Table 2). For ICV 0.25% of Fielder treated wheat seeds gave maximum value (0.157µ³), whereas control gave minimum (0.094µ³) which was also identical with seeds treated with the three doses of Topstar and 0.05% of Fielder (Table 2). Among the two herbicides and control, Fielder was more effective for ICV (Fig. 1) and in case of different doses, maximum ICV was found in 0.25% dose while minimum was found in 0.05% dose (Fig. 2) for both herbicides.

Mitotic index was found to be of highest value (6.827%) for control while the lowest value (2.070%) was observed when 0.25% of Fielder was used (Table 2). The highest value for mitotic index in root tip cells was due to the use of Fielder rather than Topstar (Fig. 1). Besides that the lowest and highest value for mitotic index was produced by 0.05% and 0.25% doses, respectively (Fig. 2).

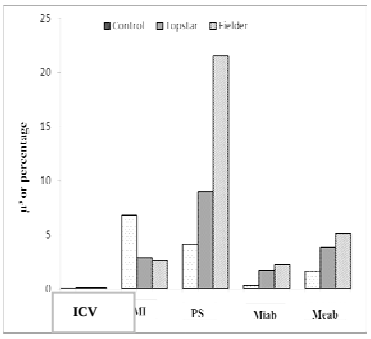

Fig.1. Effect of herbicides along with control on cytological character of *T. durum*.

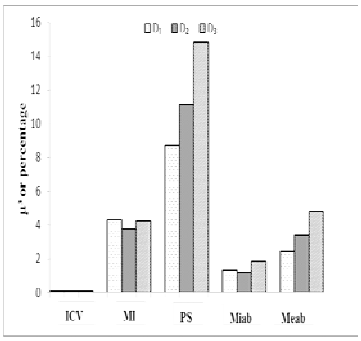

Fig.2. Effect of doses of herbicides on cytological character of *T. durum*.

Plants grown from 0.25% Fielder treated seeds showed the highest percentage of pollen sterility (28.32%) and the lowest (4.12%) was found in control (Table 2), and between two herbicides Fielder caused maximum percentage of pollen sterility (Fig. 1). Among the three concentrations, 0.25% dose produced the highest percentage, whereas 0.05% dose produced the lowest percentage of pollen sterility (Fig. 2 and Plate 1).

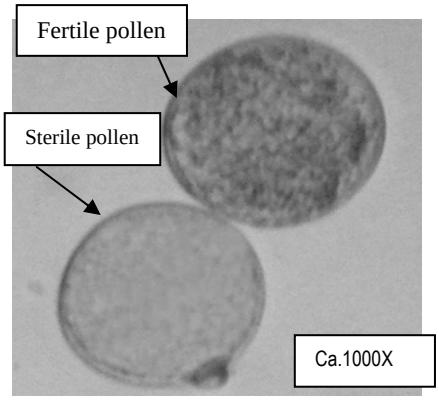

Plate 1. Photograph of sterile and fertile pollen grain in case of percentage of pollen sterility observation.

Mitotic study of the root tip cells of *Triticum durum* emerged from the seeds treated with different doses of two herbicides along with control showed various types of mitotic abnormalities (Plate 2) such as single or double bridge, with or without fragment and laggard etc. The highest percentage (3%) of mitotic abnormality was observed in 0.25 % of Fielder and the lowest (0.363 %) was found in control (Table 2) and overall, Fielder induced more abnormality than Topstar (Fig. 1). On the other hand, 0.05% dose was responsible for the highest percentage of mitotic abnormality and 0.10% dose resulted in lowest percentage of mitotic abnormality (Fig. 2).

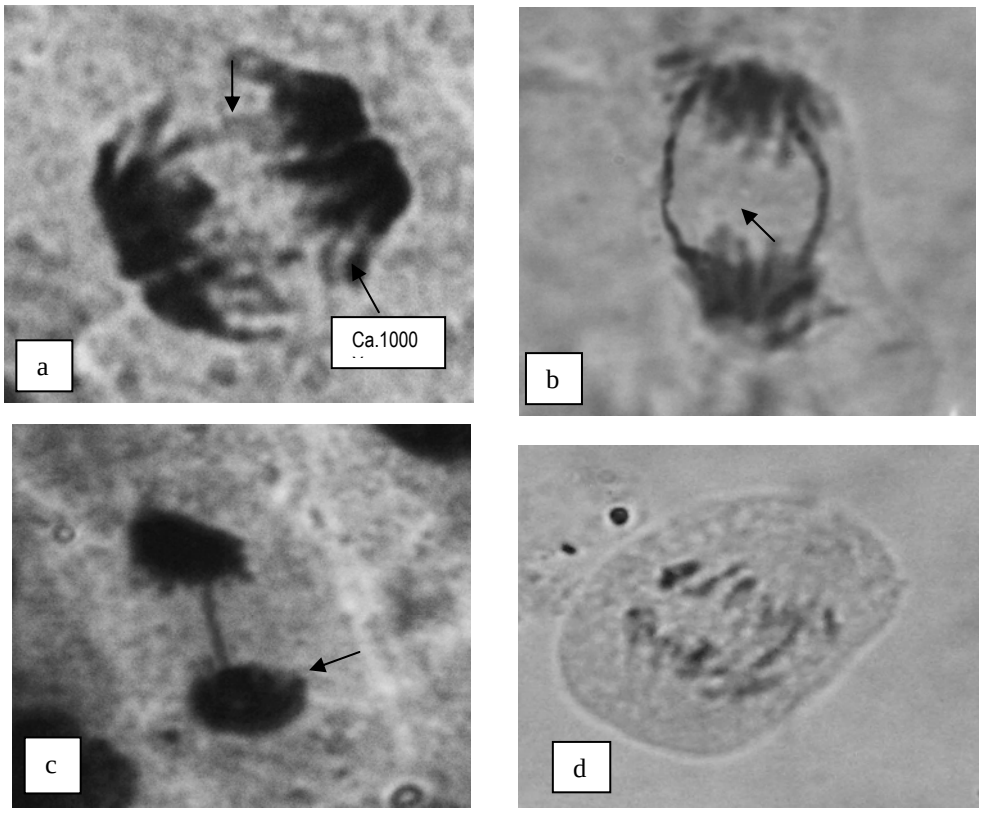

Plate 2. Different types of aberrations induced by the two herbicides in mitotic cell of *Triticum durum*. a. Anaphase with chromatid bridge and fragment (due to 0.10 % of Topstar), b. Early telophase with double chromatid bridge (due to 0.05 % of Topstar), c. Telophase with chromatid bridge (due to 0.25 % of Fielder), d. Anaphase with chromatid bridge (due to 0.25 % of Fielder).

Meiotic study in the pollen mother cells (PMCs) of *Triticum durum* plants grown from the seeds treated with different doses of herbicides showed various types of chromosomal aberration such as fragment, bridge, laggard, stickiness etc. (Plate 3). These type of aberrations were more or less found in both Meiosis I and Meiosis II. DMRT estimation indicated that the highest percentage of meiotic abnormality (7.320%) was observed in 0.25% of Fielder whereas, the lowest value (1.672%) was found in control (Table 2), and Fielder caused more abnormalities than Topstar (Fig.1). On the other hand, the highest percentage of meiotic abnormalities was found in case of dose 0.05% and the lowest in 0.10% dose (Fig. 2). Moreover, the

frequency of pollen sterility has relationship with meiotic abnormality and the regression co-efficient value was 3.896 which were highly significant at 1% level (Fig. 3). So, the regression analysis revealed that pollen grain sterility was increased with the increase of meiotic abnormality in each treatment.

For both the morphological and cytological study, the results of analysis of variance (ANOVA) revealed that all characters were highly significant and the F-value ranged from 6.172-574.358 whereas, germination percentage in pot, days required for germination in pot, plant height and ear length were non-significant in case of chemical × dose interaction with the range of F-value (1.053-2.508).

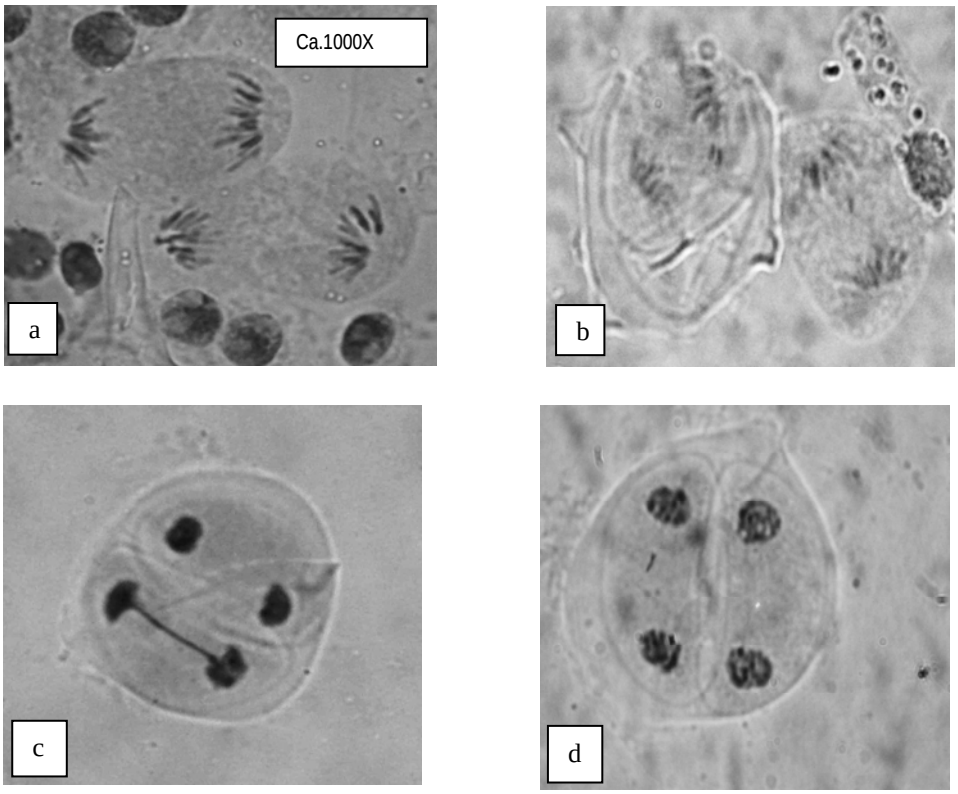

Plate 3. Different types of aberrations induced by the two herbicides in meiotic cell of *Triticum durum*. a. Normal anaphase- II (control), b. One of dyad of anaphase-II with chromosome fragment (due to 0.25 % of Fielder), c. Telophase-II with chromatid bridge (due to 0.25 % of Topstar), d. Telophase-II with laggard chromosome (due to 0.10% of Fielder).

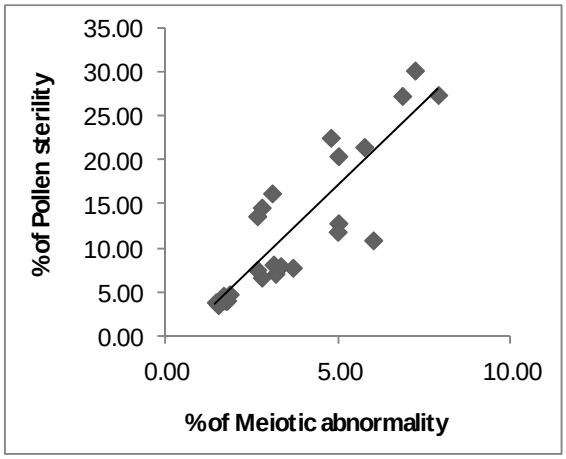

Fig. 3. The relationship of pollen sterility with meiotic abnormality in *T. durum*.

Discussion

In this experiment, data on morphological characters indicated that Fielder decreased the characteristic values with the increase of doses compared to seeds treated with water. In case of plant height (cm) Topstar showed higher value than Fielder and control. Similar results were also obtained by Bibi *et al.* (2008) and Xue-Xie *et al.* (2009).

The interphase is sometimes termed as metabolic state of nucleus at which the nuclear size generally increased by any chemical or physical agent that may be caused rapidly and precede cell deaths. The present study showed that with the increase of herbicides concentration there was an increase in interphase chromosome volume in case of both the Topstar and Fielder than the control. This result showed resemblance with Akhter *et al.* (2009) who stated that interphase chromosome volume of hexaploid wheat was found to be increased in case of Ronstar with increasing the concentration of herbicides.

It was found in the present investigation that mitotic index was decreased with the increased doses of herbicide Fielder and found to be the highest in control. The fall of mitotic index may due to the inhibition of hormones, enzymes and nucleic acids synthesis. The result of this present study was in good agreement with Sanjay (2010) who reported that both 2, 4-D and Isoproturon reduced the MI and their residuals as compared to the control and mitotic inhibition progressively increased with the increasing doses of herbicide on *Triticum aestivum*.

Percentages of sterile pollens in all the treatments were somewhat higher than the control in the present findings. This percentage of pollen sterility was also found to be increased with an increase of herbicidal concentration. The present observation was in accordance with Deepak *et al.* (2007) who reported that among the gametocides the pollen sterility was achieved maximum in case of maleic hydrazide (MH) and the percentage of pollen sterility increased as the concentration increased in case of okra (*Abelmoschus esculentus*).

It has been suggested that stickiness of chromosomes might be a result of the action of herbicides (Labbauf *et al*. 1997) and may lead to many kinds of chromosomal abnormalities. Sticky chromosomes might be used as a measurement of the cytotoxicity of the herbicides which ultimately might lead to cell death (Boehringer and Mannheim 1998). The present work revealed increased percentage of mitotic abnormality with the

increase of herbicidal concentration and the frequency of abnormal cells recorded at anaphase and telophase stage was higher than other stages. Zaman and Saleh (2005) also obtained that the chemical mutagen Ethylene Glycol was found to increase the frequency of chromosomal changes like chromatid bridges, chromosome fragments, laggards etc. with the higher doses on wheat which showed much more resemblance with the present investigation.

The meiotic abnormalities were observed in meiosis-I and meiosis-II and the percentage of abnormalities were found to be increased with an increase of herbicidal concentration in compare to control. Presence of bridge without fragments and lagging chromosome in the cells indicated that the former was due to stickiness of the chromosome and the latter was due to the breakage and reunion of the chromosome. The results obtained by Atef *et al.* (2011) also showed that all concentrations and treatment periods of Telliton and Dithane M-45 pesticides on *Vicia faba* induced a number of chromosomal aberrations in PMCs as stickiness, bridges, laggards, micronuclei and multinucleate which is in good agreement with the result of the present study.

Abnormalities occurring in meiosis are very important because they cause sterility in pollens. The present study revealed a positive relationship between chromosomal damages and pollen sterility which was also found by Çali (2009) on fungicidal effect of tomato.

Conclusion

From the foregoing results and discussion, it can be concluded that the higher concentration of chemical like herbicides has various bad impacts on morphological and cytological characters of wheat (*Triticum durum* L.) which are strongly related to the loss of yield and yield traits. A sense of awareness should be build up among our farmers to keep the use of herbicides at minimum level. Moreover, alternative measures like biological control of weeds should be made popular among the farmers which is environmentally friendly and have no such adverse effects on crop plants.

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