Turkish Journal of Biology

Volume 34 | Number 1 Article 8

1-1-2010

The effects of 2,4-dichlorophenoxy acetic acid and isoproturon herbicides on the mitotic activity of wheat (Triticum aestivum L.) root tips

SANJAY KUMAR

SHASHI KIRAN ARYA

B. K. ROY

ATUL KUMAR SINGH

Turk J Biol
34 (2010) 55-66

doi:10.3906/biy-0802-5

The effects of 2,4-dichlorophenoxy acetic acid and isoproturon herbicides on the mitotic activity of wheat (*Triticum aestivum* L.) root tips

Sanjay KUMAR[1,*], Shashi Kiran ARYA[2], Bijoy Krishna ROY[2], Atul Kumar SINGH[3]

[1]Department of Botany, Nagaland University, Headquarter, Lumami, Nagaland-798601, INDIA

[2]Center of Advance Study in Botany, Banaras Hindu University, Varanasi-221005, INDIA

[3]College of Biotechnology and Allied Science, Allahabad Agricultural Institute, Deemed University, Allahabad-211007, INDIA

Received: 08.02.2008

Abstract: The effects of the herbicides 2,4-dichlorophenoxy acetic acid and isoproturon on 3 wheat (*Triticum aestivum* L.) varieties (HUW 234, HUW 468, and HUW 533) were studied with regards to mitotic abnormalities and chromosomal behavior. Pre-soaked seeds were treated with both herbicides at concentrations of 50-1200 ppm. Both 2,4-D and isoproturon were highly mito-inhibitory and induced chromosomal abnormalities, such as precocious movement, stickiness, and chromosome bridges, with and without laggards and fragments at the anaphase and telophase. The frequency of chromosomal anomalies in almost all the targets used was high at the maximum dose of both herbicides, individually as well as in combination. Isoproturon was more toxic, as it resulted in high-level chromosomal damage in all the varieties. Both herbicides had a dose-dependent impact on the mitotic index (MI) and relative abnormality rate (RAR). These parameters collectively indicate that variety HUW 468 was more susceptible to the mito-depressive and chromotoxic action of the 2 herbicides.

Key words: Isoproturon, 2,4-dichlorophenoxy acetic acid, Tritium aestivum L., chromosomal abnormalities, mitosis, relative division rate (RDR), relative abnormality rate (RAR)

Buğday kök uçlarına (*Triticum aestivum* L.) 2,4-diklorofenoksi asetik asit ve izoproturon herbisitinin etkisi

Özet: Üç buğday (*Triticum aestivum* L.) varyetesi HUW 234, HUW 468 ve HUW 533 'nin mitotik bozukluklar ve kromozomlar üzerine 2,4-diklorofenoksi asetik asit ve izoproturon herbisitinin etkisi çalışılmıştır. Önceden ıslatılmış tohumlar 50-1200 ppm lik herbisit ile muamele edilmiştir. Hem 2,4-D hem de izoproturon mitoz üzerine oldukça inhibitor etkisi olduğu ve erken hareket, stickiness, kromozom köprüleri ve anafaz ve telefazda fragmentler gibi kromozom bozukluklarına sebep olduğu gözlenmiştir. Kromozom anormallikleri her herisit için bireysel veya her ikisnin karşımında en yüksek dozda çok fazla gözlenmiştir. İzoproturon daha toksiktir. Bütün varyetelerde en fazla kromozom anarmalliğine raslanmıştır. Her iki herbisit doza bağlı mitotik indeks (MI) etkisine ve nisbi bozukluk oranına (RAR) neden olmuştur. Parametreler HUW 468 buğday varyetesinin kullanılan herbisitlere karşı daha hassas olduğunu göstermiştir.

Anahtar sözcükler: İzoproturon, 2,4-diklorofenoksi asetik asit, *Tritium aestivum*, kromozom bozuklukları, mitoz, nisbi bölünme oranı, nisbi bozukluk oranı

Introduction

In agricultural practice many herbicides are directly applied to soil to control herbs, weeds, and other competitive plants that grow with the main crop. This is a major problem in developing countries with agro-based economies, including India. Interest in the effects of continued use of these herbicides has increased considerably, as the target organisms develop resistant to herbicides (1,2). Different herbicides, pesticides, or chemicals gradually accumulate in the environment, which may be mutagenic or carcinogenic to non-targeted biological systems (3-5). Various studies have shown that herbicides cause chromosomal abnormalities and inhibit cell division (6,7). Many cytological studies on the harmful effects of various herbicides or chemicals on different plants have been published (8-11). Thus, such herbicides are no longer of unquestionable economic importance, as their side effects alter the very hereditary setup of target and associated organisms (12).

Extensive research has been conducted on the cytogenetic anomalies induced by carbamate herbicides (13,14), trifluralin (15,16), and nitralin (17,18); however, herbicides based on other chemical groups, such as phenoxy (19,20) and substituted urea (21), have not received adequate attention. The increase in utilization of herbicides for crop improvement in modern agriculture has raised the question of whether these chemicals induce any detectable chromosomal damage in the cells of crop plants along with the weeds. The herbicide 2,4-dichlorophenoxy acetic acid (2,4-D) belongs to the phenoxy group, known as growth hormone herbicides, and isoproturon is a member of the urea group. Both herbicides are translocated systemically and act against broad-leaved competitive plants.

The present study aimed to identify cytogenetic anomalies induced by 2,4-D and isoproturon in the root tip cells of 3 wheat varieties (HUW 234, HUW 468, and HUW 533).

Materials and methods

Seeds of *T. aestivum* L. varieties (HUW 234, HUW 468, and HUW 533) were obtained from the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India, presoaked in distilled water for 24 h, and then germinated on moist filter paper in petri dishes. Roots that attained an average length of 1.5-2.0 cm were treated with a common concentration range (50, 100, 200, 400, 800, and 1200 ppm) of 2,4-D or isoproturon alone, or in combination at each concentration (50-1200 ppm, 50%:50%) for 72 h to observe the interacting effect. Root tips (1 mm) were excised, washed, and immediately transferred to colchicine solution (0.02%) for 3 h. The tissue was fixed in a freshly prepared acetic acid and ethanol (1:3) mixture (24 h) and preserved in 70% alcohol (4 °C) for further use. The root tips were hydrolyzed with 1N HCl (5 min) and washed repeatedly with distilled water. For cytological analysis, the root tips were dipped in 2% pectinase enzyme solution (Sigma Chemicals) for 10 min and slides were prepared using the chromosome squash technique with Feulgen stain; untreated sets were used as controls. The root tips were stained with Feulgen for 2 h then squashed in 1% iron acetocarmine to further intensify the stain. Cytological analysis was based on observation of 3 slides of each treatment. Mean frequency percentage of abnormalities was calculated based on the total number of cells at metaphase and anaphase, and the number of cells in division. CurveExpert v.1.3 software was used for regression, standard error, and residual graph bar analyses. The mitotic index (MI) and mitotic inhibition percentage (MIP) were determined according to (22):

$$\text{Mitotic index (MI)} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

$$\text{MIP} = \frac{\text{Mitotic inhibition in control - Mitotic index in treatment}}{\text{Mitotic index in control}} \times 100$$

The MI is also expressed as the relative division rate (RDR) and relative abnormality rate (RAR), according to (23,24):

$$RDR = \frac{\text{Percentage of dividing cells in treated variant - Percentage of dividing cells in control variant}}{100 - \text{Percentage of dividing cells in control variant}} \times 100$$

$$RAR = \frac{\text{Number of abnormal cells}}{\text{Number of cells observed}} \times 100$$

Results

Mitosis was normal in the control plants (2n = 42); however, varying degrees of chromosome abnormality were observed in the treated root tip cells of all 3 wheat varieties (Tables 1-3). Both 2,4-D and isoproturon reduced the MI (Figures 1a-6a) and their residuals (Figures 1b-6b), as compared to the control, and mitotic inhibition progressively increased (Tables 1 and 2) with increasing doses of herbicide. A mixture of both herbicides showed the same pattern of MI inhibition (Figures 7-9) in all the targeted plants. The mitotic pattern of the treated and untreated seeds of the wheat variety in reference differed with particular reference to chromosomal rearrangement (Figures 10-14).

Table 1. 2,4-D-induced chromosomal anomalies in 3 wheat varieties (HUW-234, HUW-468 and HUW-533) of *Triticum aestivum* L., according to concentration.

	Treatment (ppm)	No. of cells observed	No. of dividing cells	Abnl Cells	MI	MIP	RDR	St (%)	Br (%)	Lg (%)	Mp (%)	RAR	Abnl div. cells (%)
HUW-234	C	907	59	-	6.51 ± 0.21	-	-	-	-	-	-	-	-
	50	541	32	1	5.91 ± 0.11	9.21	−0.64	0.18	-	-	-	0.18	3.12
	100	534	30	2	5.62 ± 0.05	13.67	−0.95	0.19	0.18	-	-	0.37	6.66
	200	527	28	2	5.31 ± 0.09	18.43	−1.28	0.19	-	0.18	-	0.37	7.14
	400	500	25	5	5.00 ± 0.10	23.19	−1.61	0.30	0.29	0.20	0.20	0.99	20.0
	800	458	22	7	4.81 ± 0.05	26.11	−1.81	0.54	0.38	0.22	0.38	1.52	31.8
	1200	448	20	9	4.47 ± 0.07	31.33	−2.18	0.91	0.41	0.25	0.44	2.01	45.0
HUW-468	C	353	37	-	10.4 ± 0.98	-	-	-	-	-	-	-	-
	50	346	33	2	9.56 ± 0.24	8.69	−1.02	0.29	0.28	-	-	0.57	6.06
	100	332	30	4	9.06 ± 0.33	13.46	−1.57	0.60	0.30	0.30	-	1.20	13.33
	200	300	27	6	9.00 ± 0.10	14.04	−1.64	0.66	0.66	0.34	0.33	1.99	22.22
	400	290	26	8	8.97 ± 0.06	14.32	−1.68	1.00	0.68	0.71	0.36	2.75	30.76
	800	260	22	9	8.46 ± 0.11	19.10	−2.24	1.75	0.68	0.40	0.53	3.46	40.90
	1200	217	18	10	8.31 ± 0.06	20.63	−2.41	2.68	0.47	0.46	1.00	4.61	55.53
HUW-533	C	423	67	-	15.83 ± 1.23	-	-	-	-	-	-	-	-
	50	403	56	-	13.1 ± 0.45	12.19	−2.28	-	-	-	-	-	-
	100	327	35	2	10.2 ± 0.61	32.28	−6.07	0.31	0.30	-	-	0.61	5.71
	200	293	29	2	9.91 ± 0.29	37.39	−7.03	0.34	0.30	-	-	0.68	6.89
	400	273	23	3	8.42 ± 0.31	46.80	−8.80	0.52	0.37	-	-	1.09	13.04
	800	252	20	5	7.93 ± 0.34	49.90	−9.38	0.80	0.40	0.41	0.37	1.98	25.00
	1200	244	14	7	5.73 ± 0.39	63.80	−11.9	1.20	0.50	0.54	0.42	2.86	50.00

No.: Number; Abnl: abnormal; MI: mitotic index; MIP: mitotic inhibition percentage; RDR: relative division rate;
St: stickiness; Br: bridges; Lg: laggards; Mp: multipolarity; RAR: relative abnormality rate; div: dividing; C: control.

Table 2. Isoproturon-induced chromosomal anomalies in 3 wheat varieties (HUW-234, HUW-468 and HUW-533) of *Triticum aestivum* L., according to concentration.

	Treatment (ppm)	No. of cells observed	No. of dividing cells	Abnl Cells	MI	MIP	RDR	St (%)	Br (%)	Lg (%)	Mp (%)	RAR	Abnl div. cells (%)
HUW-234	C	298	46	-	15.4 ± 0.78	-	-	-	-	-	-	-	-
	50	291	39	1	13.4 ± 0.57	12.97	−2.36	0.34	-	-	-	0.34	2.56
	100	277	35	2	12.6 ± 0.18	18.15	−3.31	0.36	0.36	-	-	0.72	5.71
	200	267	31	2	11.5 ± 0.17	24.83	−4.52	0.37	-	0.37	-	0.74	6.45
	400	214	23	3	10.7 ± 0.12	30.35	−5.53	0.48	0.46	-	0.46	1.40	13.04
	800	205	20	5	9.77 ± 0.04	36.64	−6.68	1.00	0.48	0.48	0.48	2.44	25.0
	1200	174	15	7	8.62 ± 0.18	44.09	−8.03	2.28	0.57	0.57	0.60	4.02	46.6
HUW-468	C	383	34	-	8.87 ± 0.89	-	-	-	-	-	-	-	-
	50	378	32	-	8.47 ± 0.07	4.50	−0.43	-	-	-	-	-	-
	100	360	30	2	8.33 ± 0.10	6.08	−0.59	0.28	0.27	-	-	0.55	6.66
	200	345	28	3	8.11 ± 0.07	8.56	−0.83	0.29	0.29	0.28	-	0.86	10.71
	400	300	24	4	8.01 ± 0.18	9.69	−0.94	0.68	0.32	0.32	0.68	2.00	25.00
	800	291	20	5	6.88 ± 0.15	22.43	−2.18	1.36	0.36	0.36	0.34	2.40	35.00
	1200	147	10	6	6.82 ± 0.09	23.11	−2.24	2.20	0.69	0.69	-	5.45	60.00
HUW-533	C	375	44	-	11.73 ± 1.23	-	-	-	-	-	-	-	-
	50	356	39	2	10.5 ± 0.54	6.64	−0.88	0.56	-	-	-	0.56	5.12
	100	286	31	3	10.2 ± 0.26	7.75	−1.03	0.68	0.36	-	-	1.04	9.67
	200	284	30	3	10.6 ± 0.11	9.97	−1.32	0.70	0.18	0.17	-	1.05	10.0
	400	263	26	4	9.87 ± 0.28	15.85	−2.10	0.85	0.26	0.40	-	1.51	15.38
	800	262	24	6	9.17 ± 0.09	21.82	−2.90	1.00	0.52	0.39	0.38	2.29	25.00
	1200	193	17	8	8.80 ± 0.21	24.97	−3.31	2.55	0.53	0.53	0.53	4.14	47.05

No.: Number; Abnl: abnormal; MI: mitotic index; MIP: mitotic inhibition percentage; RDR: relative division rate;
St: stickiness; Br: bridges; Lg: laggards; Mp: multipolarity; RAR: relative abnormality rate; div: dividing; C: control.

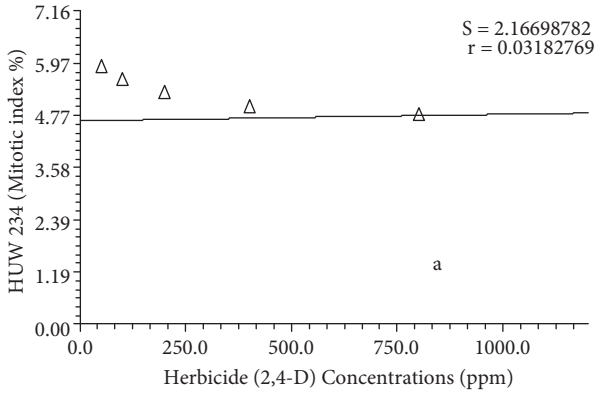

Figure 1a. Coefficient correlation (r), standard error (s), and mitotic index percentage (MI%) in the 2,4-D-treated wheat variety HUW 234.

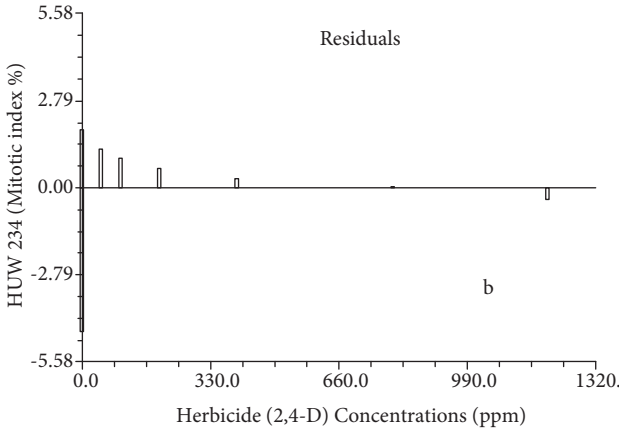

Figure 1b. Residual bar graph of r, s, and MI% in the 2,4-D-treated wheat variety HUW 234.

Table 3. Chromosomal anomalies induced by the mixture of 2,4-D and isoproturon in 3 wheat varieties (HUW-234, HUW-468 and HUW-533) of *Triticum aestivum* L., according to concentration.

	Treatment (ppm)	No. of cells observed	No. of dividing cells	Abnl Cells	MI	MIP	RDR	St (%)	Br (%)	Lg (%)	Mp (%)	RAR	Abnl div. cells (%)
HUW-234	C	590	52	-	8.81 ± 1.21	-	-	-	-	-	-	-	-
	50	534	40	1	7.49 ± 0.16	14.98	−0.14	0.18	-	-	-	0.18	2.50
	100	448	32	2	7.14 ± 0.28	18.95	−1.83	0.22	0.10	-	-	0.44	6.25
	200	387	27	4	6.97 ± 0.02	20.88	−2.01	0.50	0.27	0.26	0.20	1.03	14.81
	400	364	25	5	6.86 ± 0.19	22.13	−2.13	0.81	-	0.28	0.28	1.37	20.00
	800	323	20	7	6.19 ± 0.05	2973	−2.87	1.20	0.32	0.32	0.32	2.16	35.00
	1200	300	18	9	6.00 ± 0.39	31.89	−3.08	1.65	0.45	0.90	0.45	3.00	50.00
HUW-468	C	358	32	-	8.93 ± 1.23	-	-	-	-	-	-	-	-
	50	344	30	2	8.72 ± 0.19	2.35	−0.23	0.29	0.29	-	-	0.58	6.66
	100	338	25	2	7.39 ± 0.46	17.24	−1.69	0.30	0.15	0.29	0.14	0.59	8.00
	200	328	22	4	6.70 ± 0.16	24.97	−2.44	0.45	0.31	0.30	0.15	1.21	18.18
	400	280	18	5	6.42 ± 0.07	28.10	−2.75	1.05	0.37	-	0.36	1.78	27.77
	800	274	17	6	6.20 ± 0.03	30.57	−2.99	1.15	0.37	0.30	0.36	2.18	35.29
	1200	222	13	8	5.85 ± 0.13	34.49	−3.38	2.05	0.45	0.45	0.55	3.60	61.53
HUW-533	C	344	50	-	14.53 ± 0.98	-	-	-	-	-	-	-	-
	50	322	40	1	12.42 ± 0.36	14.52	−2.46	0.31	-	-	-	0.31	2.50
	100	314	32	3	10.11 ± 0.57	29.86	−5.07	0.62	0.33	-	-	0.95	9.37
	200	300	30	4	10.00 ± 0.33	31.17	−5.30	0.66	0.34	0.33	-	1.33	13.33
	400	258	25	6	9.68 ± 0.12	33.37	−5.67	1.14	0.40	0.39	0.39	2.32	24.00
	800	257	23	7	8.94 ± 0.09	38.47	−6.54	1.52	0.40	0.40	0.40	2.72	30.43
	1200	200	15	8	7.50 ± 0.46	48.38	−8.82	2.25	0.50	0.50	0.75	4.00	53.33

No.: Number; Abnl: abnormal; MI: mitotic index; MIP: mitotic inhibition percentage; RDR: relative division rate; St: stickiness; Br: bridges; Lg: laggards; Mp: multipolarity; RAR: relative abnormality rate; div: dividing; C: control.

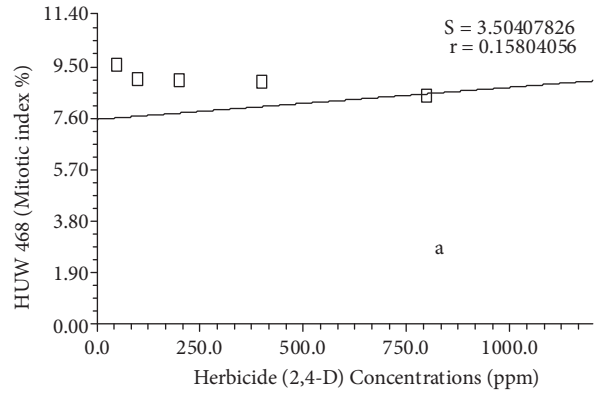

Figure 2a. Coefficient correlation (r), standard error (s), and mitotic index percentage (MI%) in the 2,4-D-treated wheat variety HUW 468.

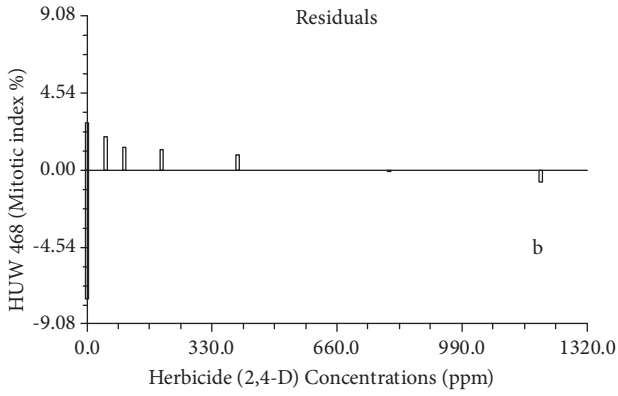

Figure 2b. Residual bar graph of r, s, and MI% in the 2,4-D-treated wheat variety HUW 468.

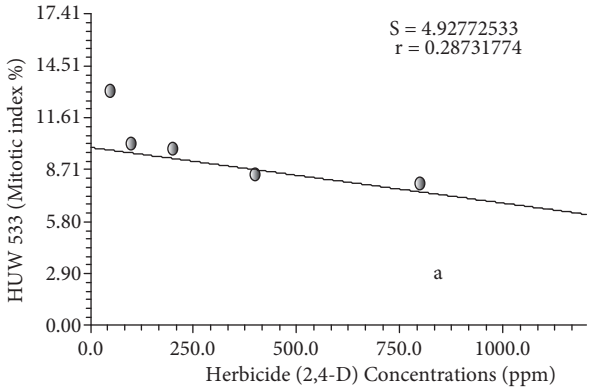

Figure 3a. Coefficient correlation (r), standard error (s), and mitotic index percentage (MI%) in the 2,4-D-treated wheat variety HUW 533.

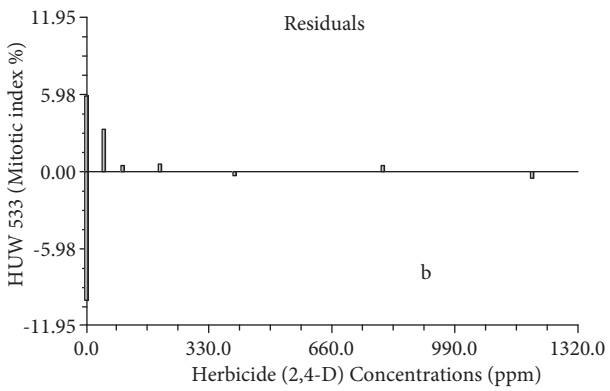

Figure 3b. Residual bar graph of r, s, and MI% in the 2,4-D-treated wheat variety HUW 533.

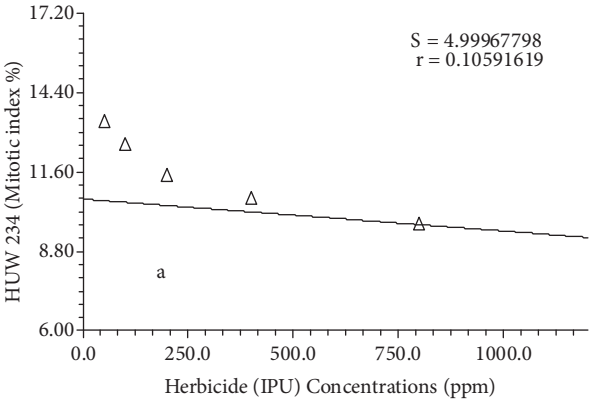

Figure 4a. Coefficient correlation (r), standard error (s), and mitotic index percentage (MI%) in the isoproturon (IPU)-treated wheat variety HUW 234.

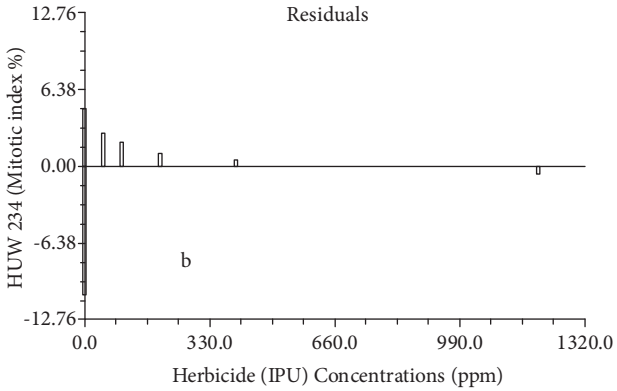

Figure 4b. Residual bar graph of r, s, and MI% in the IPU-treated wheat variety HUW 234.

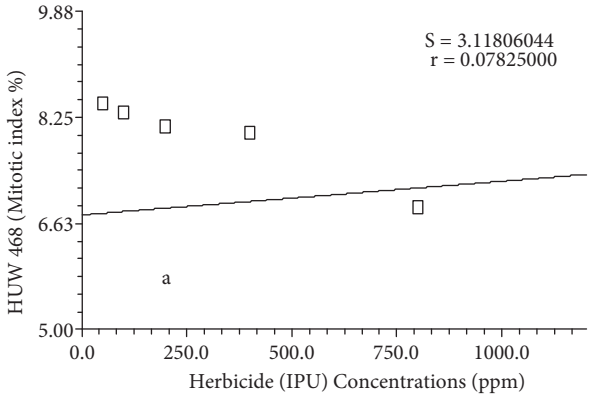

Figure 5a. Coefficient correlation (r), standard error (s), and mitotic index percentage (MI%) in the isoproturon (IPU)-treated wheat variety HUW 468.

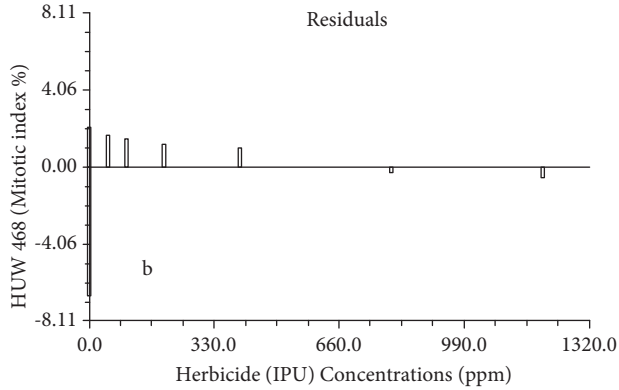

Figure 5b. Residual bar graph of r, s, and MI% in the IPU-treated wheat variety HUW 468.

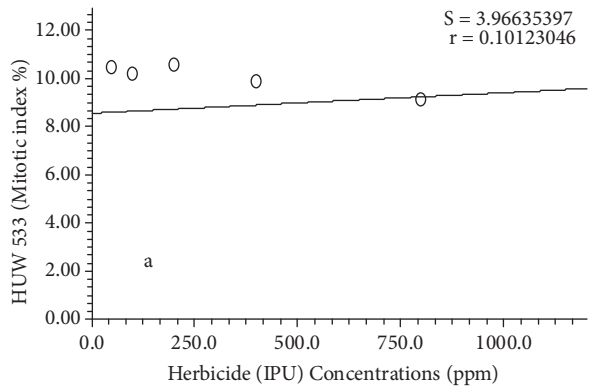

Figure 6a. Coefficient correlation (r), standard error (s), and mitotic index percentage (MI%) in the isoproturon (IPU)-treated wheat variety HUW 533.

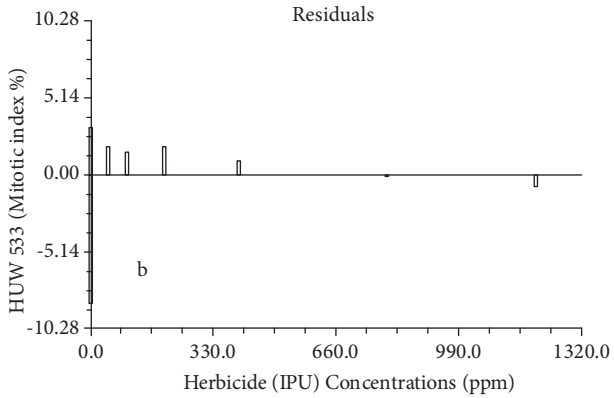

Figure 6b. Residual bar graph of r, s, and MI% in the IPU-treated wheat variety HUW 533.

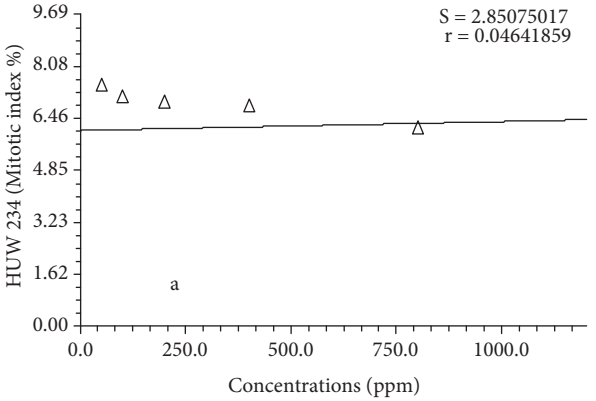

Figure 7a. Coefficient correlation (r), standard error (s), and mitotic index percentage (MI%) in the 2,4-D and isoproturon (IPU)-treated wheat variety HUW 234.

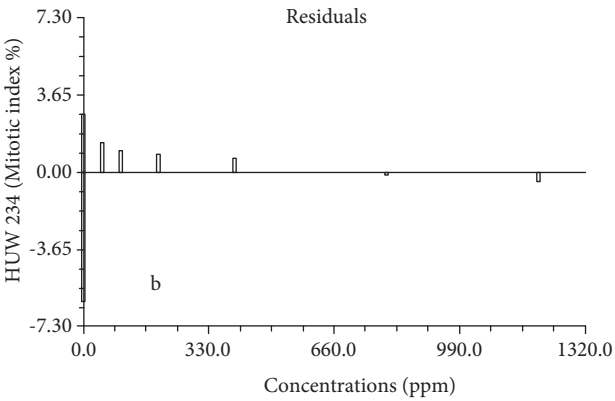

Figure 7b. Residual bar graph of r, s, and MI% in the 2,4-D and IPU-treated wheat variety HUW 234.

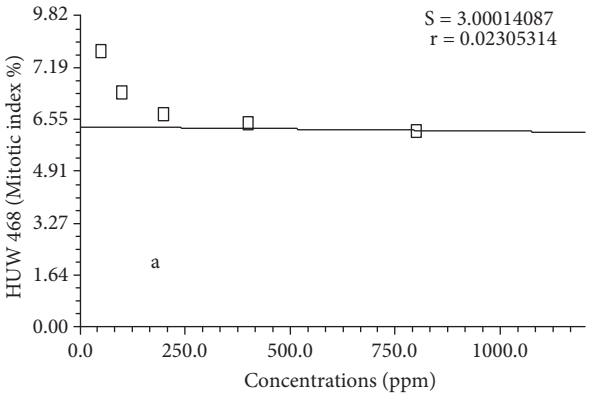

Figure 8a. Coefficient correlation (r), standard error (s), and mitotic index percentage (MI%) in the 2,4-D and isoproturon (IPU)-treated wheat variety HUW 468.

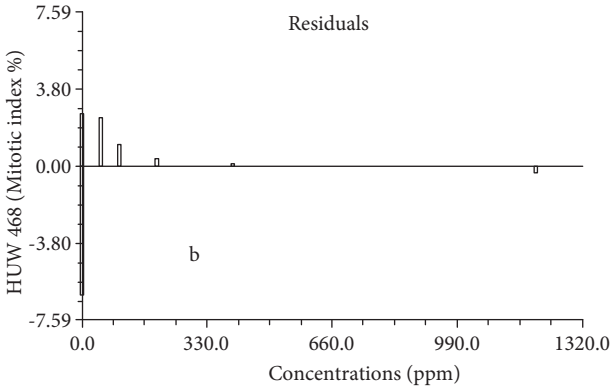

Figure 8b. Residual bar graph of r, s, and MI% in the 2,4-D and IPU-treated wheat variety HUW 468.

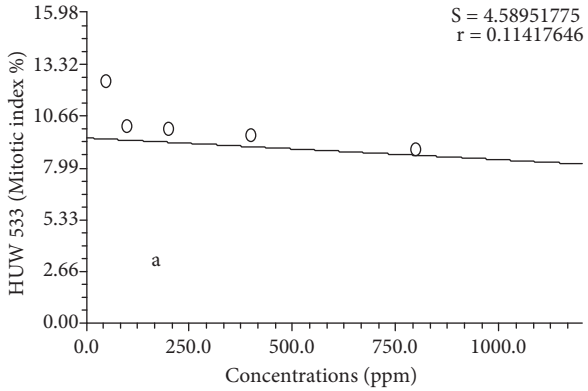

Figure 9a. Coefficient correlation (r), standard error (s), and mitotic index percentage (MI%) in the 2,4-D and isoproturon (IPU)-treated wheat variety HUW 533.

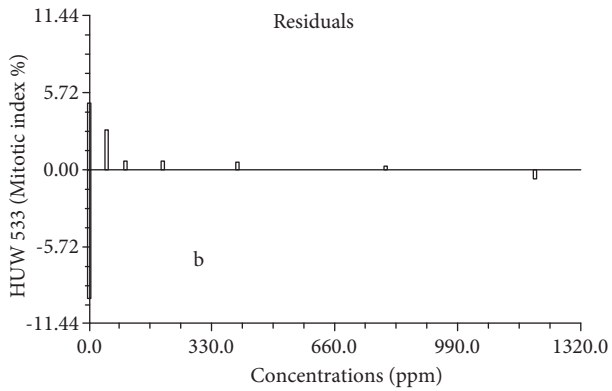

Figure 9b. Residual bar graph of r, s, and MI% in the 2,4-D and IPU-treated wheat variety HUW 533.

The maximum frequency of abnormal cells was observed at 1200 ppm of 2,4-D in HUW 468, followed by the mixture of both herbicides in HUW 234. Induction of chromosomal abnormalities was dose-dependent in all treatments. The percentage of mitotic inhibition increased progressively in response to increasing doses of either herbicide or the combination of both. The roots of HUW 468 treated with the high-concentration (1200 ppm) mixture of both herbicides showed maximum chromosomal abnormality (61.53%) of dividing cells. The unoriented bivalents, precocious movement, and multipolarity were all pronounced at metaphase in the treated plants. Anaphase was characterized by stickiness, laggards, bridges, and multipolarity (Figures 10-14). The most common chromosomal aberrations caused by 2,4-D and isoproturon, alone or in combination, were stickiness and bridges.

Mostly single, and occasionally double and triple bridges were observed in response to the herbicides at all concentrations. The highest frequency of bridges (0.69%) was observed at 1200 ppm isoproturon in variety HUW 468, and the least (0.10%) and same were applicable to the lowest concentration (100 ppm) of both herbicides. Chromosomal abnormalities (55.53% and 60%) in the same wheat variety were observed in response to 2,4-D and isoproturon, respectively. The percentage of stickiness was high at the 1200 ppm concentration of both herbicides, individually and in combination. The highest percentage (2.68%) was observed in response to 2,4-D in variety HUW 468 and the lowest (0.18%) was recorded at the lowest concentration (50 ppm) in HUW 234. The magnitude of such abnormalities and treatment of the wheat varieties are given in Tables 1-3.

Multipolarity, with considerable frequency, was also noted at the maximum herbicide dose at metaphase and telophase in all the wheat varieties. Chromosome bridges were sometimes accompanied by fragments, although their occurrence was mostly independent of each other. Chromosome laggards occurred at the highest frequency in response to the highest herbicide concentration in all 3 wheat varieties. Isoproturon caused greater reductions in the MI than 2,4-D or the control treatment. The mitotic pattern of the plants treated with both herbicides and the control plants, with respect to the arrangement and behavior of chromosomes, was quite apparent (Tables 1-3 and Figures 1-14). It was also observed that RDR gradually decreased and RAR increased in response to increasing concentrations of both herbicides, alone and in combination. These results suggest that HUW-234 could be designated as the most tolerant genotype and HUW-468 as the most sensitive.

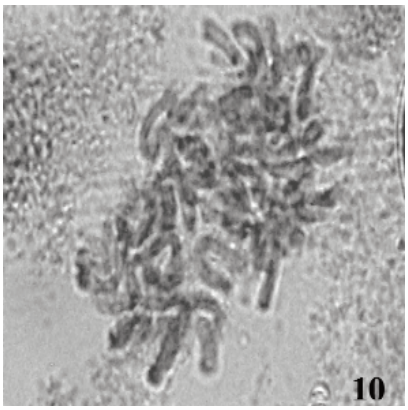

Figure 10. Sticky and irregular arrangement of chromosomes at metaphase.

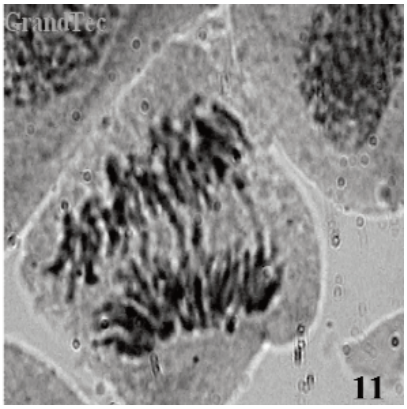

Figure 11. Chromosome bridges at anaphase.

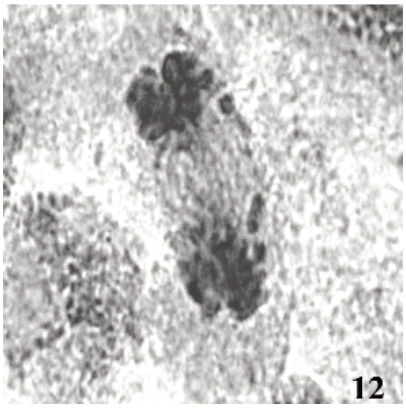

Figure 12. Lagging chromosomes during late anaphase.

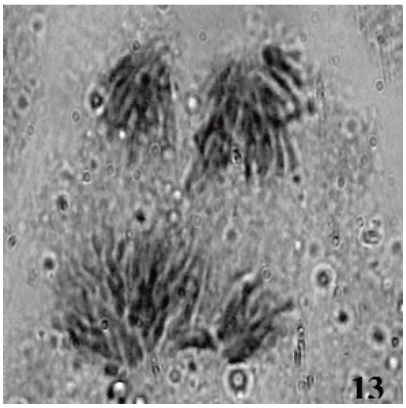

Figure 13. Multipolar spindle at telophase.

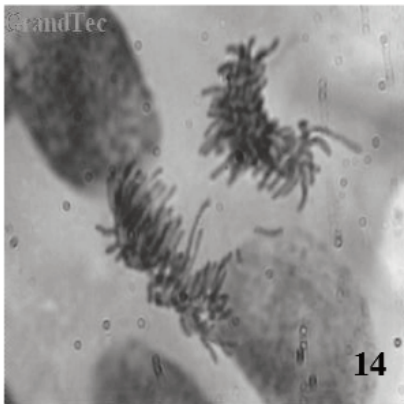

Figure 14. Chromosome fragments at anaphase.

Discussion

In recent years most herbicides have been commercially applied to control weeds that affect crop productivity. The life cycle of wheat (*T. aestivum*) is short, but the crop has multipurpose utility owing to its richness in carbohydrates, proteins, starch, and vitamins. The widespread use of herbicides in crop fields has also stimulated study of their cytogenetic

effects on targeted and non-targeted plants. Approximately 25% of all herbicides marketed belong to the mitotic disrupter herbicide group. Reductions in mitotic activity caused by the herbicides isoproturon and 2,4-D observed in the present study are similar to the effects of other urea-substituted herbicides, including potent mutagens (25). The mitotic inhibition and formation of chromosomal abnormalities observed in the present study are similar to earlier observations of other common herbicides (26-28). Herbicide-treated plants clearly revealed that the number of dividing cells and MI decreased in a dose-dependent manner (29,30); such reductions in mitotic activity have been attributed to inhibition of DNA synthesis, and formation of irregular and disorganized phragmoplasts (31).

In the present study RDR gradually decreased with subsequent increases in RAR in response to increasing concentrations of both herbicides in the 3 wheat varieties. It is suggested that both herbicides, individually and in combination, played a major role in the induction of chromosomal abnormalities, such as stickiness, bridges, fragmentation, laggards, and multipolar arrangement, in a fashion similar to that of dinitroaniline and carbamate herbicides (32,33).

Chromosome stickiness was reported to be due to genetic or environmental factors, and genetically induced in *Hordeum vulgare* (34) and wheat (35); however, Gaulden (36) postulated that chromosome stickiness was due to the effect on chromosomal proteins or disturbances in the functioning of specific non-histone protein(s) essential for chromatid separation and segregation.

Fragments at metaphase may be due to the failure of broken chromosomes to recombine with the same locus bearing chromosome, leading to the formation of dicentric chromosomes (37). Chromosome breakage has been linked to DNA synthesis, which is sensitive to many chemicals (38). Chromosomal bridges mainly arise due to the non-disjunction of sticky chromosomes or to breakage and reunion during separation at anaphase (39). Chromosome bridges, with or without laggards or fragments, may be the direct consequence of herbicide treatment, mainly due to fusion of broken chromatids. The observed laggards and multipolarity might have been caused by inhibited spindle formation or the destruction of microtubular protein (40). Similar chromosomal abnormalities can also be observed in cases of physical and chemical mutagen exposure (41).

Comparison of the frequency of such abnormalities in all 3 wheat varieties in the present study suggests that HUW 468 cells were more susceptible to chromosomal damage and mitotic disturbances caused by the 2 herbicides alone or in combination. Nonetheless, the 3 wheat varieties collectively exhibited considerable damage caused by both herbicides, and in combination they seemed to be neither synergistic nor antagonistic, at least in terms of chromotoxicity. This implies that the herbicides studied may have altered the pattern of chromosomal organization in a dose-dependent pattern and that such cell abnormalities may eventually affect the vigor, yield, fertility, and competitive ability of the exposed crop plants (42,43).

It is quite evident that both herbicides are potent mutagens, as indicated by the extent of chromosomal damage observed, which could also result in genetic variability in the otherwise natural wheat gene pool. The maximum level of chromosomal anomaly was recorded in HUW 468, suggesting that this inbred line was more susceptible and, thus, the least tolerant, while the others exhibited a degree of resistance. As chromosomal damage indicates mutation (44,45), isoproturon and 2,4-D should be studied further via mutagenicity testing at the molecular level. It is clear that both herbicides used in agricultural practice are lethal to mitotic activity because of the induced cytological disturbances in root tip cells. All the herbicide concentrations used in the present study induced mitotic abnormalities, and the frequency of abnormalities increased in a concentration-dependent manner. To conclude, such herbicides seem to be capable of inflicting irreversible cytological damage in plants if used consistently.

Acknowledgement

The authors are thankful to the Institute of Agricultural Sciences, Banaras Hindu University, for providing seeds. Thanks are due to the Head of the Department of Botany, Banaras Hindu University for providing the necessary facilities. The authors are also thankful to Professor S. P. Singh, Emeritus Scientist for reviewing the manuscript.

Corresponding author:

Sanjay KUMAR
Department of Botany,
Nagaland University,
Lumami, Nagaland-798601,
INDIA
E-mail: ksanjay79@gmail.com

References

1. Weed Science Society of America. DCPA. In: Herbicide handbook of the Weed Science Society of America, fifth edition. Weed Science Society of America, Champaign, Illinois, USA; pp. 144-146: 1983a.
2. Weed Science Society of America. Propham. In: Herbicide handbook of the Weed Science Society of America, fifth edition. Weed Science Society of America, Champaign, Illinois, U.S.A.; pp. 413-419: 1983b.
3. Chauhan LKS, Sundraraman V. Effect of substituted ureas on plant cells I. Cytological effects of isoproturon on the root meristem cells of *Allium cepa*. Cytologia 55: 91-98, 1990.
4. Wuu KD, Grant WF. Morphological and somatic chromosomal aberrations induced by pesticides in barley (*Hordeum vulgare*). Can J Genet Cytol 8: 481-501, 1966.
5. İnceer H, Ayaz S, Beyazoğlu O et al. Cytogenetic effects of copper chloride on the root tip cells of *Helianthus annus* L. Turk J Biol 27: 43-46, 2003.
6. Hilmann G, Ruthmann A. Effect of mitotic inhibitors on the ultrastructure of root meristem cells. Planta 155: 124-132, 1982.
7. Holmsen JD, Hess FD. Growth inhibition and disruption of mitosis by DCPA in oat (*Avena sativa*) roots. Weed Sci 32: 732-738, 1984.
8. Bovery RW, Meyer RE. Effects of 2, 4, 5-T, Triclopyr and 3, 6-Dichloropicolinic acid on crop seedlings. Weed Sci 29: 256-261, 1981.
9. DePrado R, Scalla R, Gaillardon P. Differential toxicity of simazine and diuron to *Torilus arvensis* and *Lolium rigidum*. Weed Res 30: 213-221, 1990.
10. Lerda D. The effects of lead on *Allium cepa* L. Mutat Res 281: 89-92, 1992.
11. Yoshida Y, Nakamura K, Hiura A. Contraction of chromosomes and depression of RNA synthesis by isopropyl N-(3-chlorophenyl) carbamate (CIPC) in *Vicia faba* root tip cells. Cytologia 48: 707-714, 1983.
12. Strachan SD, Hess FD. The biochemical mechanism of the action of the dinitroaniline herbicide oryzaline. Pestic Biochem Physiol 20: 141-150, 1983.
13. Coss RA, Pickett-Heaps JD. The effects of isopropyl N-phenyl carbamate on the green alga *Oedogonium cardiacum* I. Cell division. J Cell Biol 63: 84-98, 1974.
14. Jackson WT. Regulation of mitosis II. Interaction of isopropyl N-phenylcarbamate and melatonin. J Cell Sci 5: 745-755, 1969.
15. Lignowski EM, Scott EG. Effect of trifluralin on mitosis. Weed Sci 20: 267-270, 1972.
16. Delcourt A, Deysson G. Effects de la trifluralin sur les meristemes radiculaires d' *Allium sativum* L. Cytologia 41: 75-84, 1976.
17. Vaughn KC, Lehnen LP Jr. Mitotic disrupter herbicides. Weed Sci 39: 450-457, 1991.
18 Gentner WA, Burk LG. Gross morphological and cytological effects of nitralin on corn root tips. Weed Sci 16: 259-260, 1968.
19. Darwent AL, Elliot CR, Clark JM. Effect of 2, 4-D, Dicamba and 2, 4, 5-T on creeping red fescue seed production. Can J Plant Sci 57: 451-456, 1977.
20. Bovey RW. Hormone like herbicides in weed control. Econ Bot 25: 385-400, 1971.
21. Gichner T, Gaul H, Omura T. The influence of post- treatment, washing and redrying of barley seeds on the mutagenic activity of N-methyl-N-nitrosourea and N-ethyl-N-nitrosourea. Radiat Bot 8: 499-507, 1968.
22. Rekha K, Kak SN, Langer A. Cytological abnormalities in *Artemisia pollens* Wall. Induced by gamma rays. Indian J Forestry 27: 355-359, 2004.
23. Egami N, Hyodo-Taguchi Y. Dominant lethal mutation rates in fish *Oryzias latipes* irraditated at various stages of gametogenesis In: Schroder JH. ed. Genetics and Mutagenesis in Fish, Springer Verlag, pp. 75-81, 1973.
24. Kumar G, Kumar R. Chromotoxic and mito-inhibitory effects of pesticides in *Trigonella foenum-graecum* L. J Cytol Genet 1: 11-15, 2000.
25. Merlin G, Nuret F, Ravanel P et al. Mitosis inhibition by a N-(1, 1-dimethylpropyl) benzamide series. Phytochem 26: 1567-1571, 1987.

26. Amer S, Ali EM. Cytological effects of pesticides- V. Effects of some herbicides on *Vicia faba*. Cytologia 39: 633-643, 1974.

27. Morrison JW. Cytological effects of the herbicides avadex. Can J Plant Sci 42: 78-81, 1962.

28. Topaktas M, Rencuğullari E. Cytogenetic effects of herbicides gesegard and igran in barley. Cytologia 56: 419-424, 1991.

29. Elkhodary S, Habib A, Haliem A. Cytological effect of the herbicide garlon-4 on root mitosis of *Allium cepa*. Cytologia 54: 465-472, 1989.

30. Badr A. Mitodepressive and chromotoxic activities of two herbicides in *Allium cepa*. Cytologia 48: 451-457, 1983.

31. Lehnen LP, Vaughan MA, Vaughn KC. Terbutol affects spindle microtubule organizing centers. J Exp Bot 41: 537-546, 1990.

32. Vaughn KC. Cytological studies of dinitroaniline-resistant *Eleucine*. Pestic Biochem Physiol 26: 66-74, 1986.

33. Tissut M, Nurit F, Ravanel P et al. Herbicidal modes of action depending on substitution in a phenylcarbamate series. Physiol Veg 24: 523-535, 1986.

34. Badr A, Elkington TT. Antimitotic and chromotoxic activities of isoproturon in *Allium cepa* and *Hordeum vulgare*. Environ and Exp Bot 22: 265-270, 1982.

35. Johanson NG, Muzik TJ. Some effect of 2, 4-D on wheat yield and root growth. Bot Gaz 122: 188-194, 1961.

36. Gaulden ME. Hypothesis: Some mutagens directly alter specific chromosomal proteins (DNA topoisimerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagenesis 2: 357-365, 1987.

37. Evans HJ, Scott D. The induction of chromosome aberrations by N- mustard and its dependence on DNA synthesis. Proc R Soc B Biol 173: 491-512, 1969.

38. Kumar G, Singh V. Studies in M1 and M2 generations of azide induced medium-strong desynaptic mutant of pearl millets. The Nucleus 47: 159-163, 2004.

39. Koduru PRK, Rao MK. Cytogenetics of synaptic mutants in higher plants. Theor Appl Genet 59: 197-214, 1981.

40. Morejohn LC, Fosket DE. Inhibition of plant microtubule polymerization in vitro by the phosphoric amide herbicide amiprophosmethyl. Sci 224: 874-876, 1984.

41. Zeerak NA. Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in brinjal (*Solanum melongena* L.). J Nuclear Agri Biol 21: 84-87, 1992.

42. Grant WF. The present status of higher plant bioassays for the detection of environmental mutagens. Mutat Res 310: 175-185, 1994.

43. World Health Organisation. Guide to short term tests for detecting mutagenic and carcinogenic chemicals. Environ Health Criteria 51: Geneva, pp 208, 1985.

44. Grant WF. Chromosomal aberration in plants as a monitoring system. Environ Hlth Persp 27: 37-43, 1978.

45. Oraler G, Gözukırmızı N, Olgun A. Mutagenic effects of some pesticides in different organisms. Doğa Bilim Dergisi 8: 105-111, 1984.