Cytological Effects of Herbicides

I. Effect of 2, 4-D, and 2, 4, 5-T on meiotic cells of wheat and two related species

Nahla R. Al-Najjar and Atef S. Soliman¹

Department of Biology, College of Science, University of Baghdad, Iraq

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The sodium salts of 2,4-D(2,4-dichlorophenoxyacetic acid) and 2,4,5-T(2,4,5-trichlorophenoxyacetic acid) are unique weed killers. They have several herbicidal actions. Many investigators reported on their actions for example, Crafts and Robbins (1973) indicated that the application of 2,4-D and 2,4,5-T in herbicidal concentrations caused many changes to broad leaved plants. The two herbicides caused differential growth rates in petioles, pulvini, and elongating region of the stem. Moreover they caused thickening of leaves and stems, increase in turgor, and death of tissues followed by browning and drying of stems and leaves. Other research workers reported that 2,4-D and 2,4,5-T proved to be safe to be used in wheat fields. For example Mitchel and Popay (1978) reported that 2,4-D caused minor effects on yield in wheat and other cereals. Pop *et al.* (1978) found that the herbicide Icedin (2,4-D + dicamba) had no effect on wheat plants and showed no phytotoxicity when applied during tillering time. Many investigators reported on cytological effects caused by 2,4-D and 2,4,5-T in cereals and other crops. Saalbach and Koblitiz (1977) found many caryological irregularities caused by 2,4-D in haploid barley. Logvinenko and Morgun (1978) found that spraying wheat plants with 2,4-D and its sodium and amine salts in various concentrations increased frequency of chromosome aberrations in root tip cells up to five times. The chromosomes irregularities observed were bridge formation, spindle suppression, polyploidy, micronuclei formation, stickiness of chromosomes, accumulation of metaphases, and other cytological changes. Most of these changes were observed in root tip squashes.

Our objectives were as follows: First the study of cytologic effects of two well known herbicides as 2,4-D and 2,4,5-T in the meiotic cells of hexaploid wheat *Triticum aestivum* L., a tetraploid wheat *T. durum* Desf., and a diploid related species *Aegilops ligustica* L. Second the study of the relationship between level of ploidy and frequency of chromosomal aberrations. Third the study of dose effect on the frequency of aberrations by the use of 2,4,5-T as a herbicide and *Ae. ligustica* as a chosen species.

Materials and methods

Pure dry seeds of the following species *Triticum aestivum* L. cultivar Saber beg, *T. durum* Desf. cultivar Johri, and pure selection of the species *Ae. ligustica* L., were

¹ Present address: Faculty of Cotton Science at Alexandria Bulkely, Alexandria, Egypt.

grown in three randomized complete block design in the Botanical Garden, College of Science, University of Baghdad, Iraq. Three plots were chosen at random and treated with the sodium salt of the herbicide 2,4-D 0.1% solution at tillering time. Three other plots were treated with the second herbicide in the same concentration, and the remainder three plots in each block were left as control. The plants were treated agronomically as usual in regard to irrigation and thinning. At heading time young spikes were chosen at random from each replicate and were fixed in 6:3:1 solution (six parts absolute alcohol, three parts glacial acetic acid, and one part chloroform) for 24 hours at room temperature. Fixed spikes were washed with 70% alcohol and kept in the same concentration of alcohol under refrigeration until used. Anthers of suitable size were smeared in 2% acetocarmine stain. Slides were made permanent according to Darlington and La Cour (1976) mounted in D. P. X. mountant. Pollen mother cells were examined microscopically and total number of abnormal cells were recorded, as well as the different kinds of aberrations during the following stages: metaphase I, anaphase I, metaphase II, and anaphase II of meiosis. Analysis of variance was made to study the effect of herbicides on chromosomal abnormalities observed. Also the same analysis was applied to study dose effect on the frequency of abnormal cells. Besides the kinds of chromosomal aberrations were recorded for each treatment during the above mentioned stages of meiosis.

Experimental results

Table 1 presents the total number of cells observed in the different stages of meiosis, and the number of abnormal cells observed in each stage and their percentages. The results indicated that the number of abnormal cells was higher in the treated plants than the control plants. The herbicide 2,4,5-T caused a higher percentages of abnormal PMC's than 2,4-D with the exception of metaphase II cells. Besides there were a higher percentages of abnormal cells in the hexaploid wheat than in the other two species but that difference was significant only in anaphase I and metaphase II (Table 2). From Table 2 there was a highly significant difference between treatments as far as abnormal cells were concerned. Table 3 shows the different kinds of chromosomal aberrations. It is worthy to note a higher percentages of lagging chromosomes during M I, A I, M II in pollen mother cells from treated plants. Chromosomal stickiness was higher in anaphase II in all treated plants (Table 3). Table 3 shows the total percentages of each aberration. Figures 1, 2 show the lagging chromosome during metaphase I as result of the treatment of hexaploid wheat with 2,4-D and 2,4,5-T. Figure 3 shows a single bridge in hexaploid wheat treated with 2,4,5-T. Asynchronization and chromosome stickiness were common in hexaploid wheat treated with 2,4-D (Fig. 4, 5). The aberrations observed in *Triticum aestivum* were significantly higher than in *T. durum* and *Aegilops ligustica* L. but there were clear chromosomal aberrations in the latter two species (Figs. 6, 7, 8, and 9). The figures in this paper show some of the common aberrations observed during this study, and the same aberrations were observed when higher doses were applied but in higher frequencies. It is clear from Table 4 that the number of abnormal cells increased with the increase of 2,4,5-T dose used. Table 5 shows the

Figs. 1-6. 1, a lagging chromosome in metaphase I of *T. aestivum* treated with 2, 4-D. 2, a lagging chromosome in metaphase I of *T. aestivum* treated with 2,4,5-T. 3, a single bridge in anaphase II of *T. aestivum* treated with 2,4,5-T. 4, asynchronization in anaphase II of *T. aestivum* treated with 2,4-D. 5, multiple bridges in anaphase II of *T. aestivum* L. treated 2,4,5-T. 6, lagging chromosomes in anaphase I in *T. durum* treated with 2,4,5-T.

Table 1. Number of cells and the percentages of abnormal cells in different stages of meiosis of hexaploid wheat *T. aestivum*; tetraploid wheat *T. durum*; and *Aegilops ligustica* treated with 2,4-D and 2,4,5-T

Treatment	Species	Total number of cells	Metaphase I			Anaphase I			Metaphase II			Anaphase II		
			Number of cells observed	No. of ab-normal cells	%	Number of cells observed	No. of ab-normal cells	%	Number of cells observed	No. of ab-normal cells	%	Number of cells observed	No. of ab-normal cells	%
2,4-D	T.	2435	1379	319	23.13	259	97	37.45	472	99	20.97	325	118	36.31
2,4,5-T	<i>aestivum</i>	2147	343	98	28.57	266	104	39.10	1149	221	19.23	389	116	29.82
Control		3117	1409	121	8.59	346	35	10.12	1128	112	9.93	234	39	16.67
2,4-D	T.	2817	1912	492	25.73	470	124	26.38	247	55	22.27	188	22	11.70
2,4,5-T	<i>durum</i>	2400	696	154	22.13	153	31	20.26	777	101	13	774	219	28.29
Control		2693	318	13	4.1	740	15	2.03	1004	27	2.69	631	34	5.39
2,4-D	<i>Ae.</i>	3244	968	181	18.7	672	41	6.1	1093	98	8.97	511	46	9.00
2,4,5-T	<i>ligustica</i>	3145	1046	214	20.46	271	19	7.01	1116	81	7.26	712	49	6.88
Control		5136	1019	14	1.37	1574	7	0.44	923	25	2.71	1620	31	1.91

Table 2. Statistical analysis for the observed abnormal cells in different meiotic stages in *T. aestivum*, *T. durum* and *Aegilops ligustica* treated with 2,4-D and 2,4,5-T

Source	Degrees of freedom	Metaphase I			Anaphase I			Metaphase II			Anaphase II		
		Mean squares	F		Mean squares	F		Mean squares	F		Mean squares	F	
Replicates	2	28.037	0.032 ns		0.482	0.008 ns		2.333	0.0125 ns		7.7035	0.03 ns	
Species	2	1736.7	1.97 ns		806.64	12.53++		2119	11.388++		811.37	3.202 ns	
Herbicides	2	20187.7	22.85++		1168.48	18.16++		1623.4	8.725++		2302.37	9.086++	
Error	20	883.5			64.34			186.072			253.404		

ns = $p > 0.05$

++ = $p < 0.01$

Table 3. Analysis of the occurring abnormalities percentage in the different meiotic stages as occurring after treating *T. aestivum*, *T. durum*, and *Ae. ligustica* with 2,4-D and 2,4,5-T

Treatment	Species	Metaphase I				Anaphase I				Metaphase II				Anaphase II			
		Lag.	Stick.	Frag.	Lag.	Stick.	Frag.	Single brid.	Multiple brid.	Lag.	Stick.	Frag.	Single brid.	Lag.	Stick.	Frag.	Multiple brid.
2,4-D	<i>T. aestivum</i>	96.87	1.25	1.88	39.18	21.65	2.06	18.56	18.55	93.94	2.02	4.04	13.56	41.53	1.69	18.64	24.58
2,4,5-T		91.84	6.12	2.04	39.42	23.08	3.85	18.27	15.38	96.83	2.26	0.91	3.45	41.38	—	19.83	35.34
2,4-D	<i>T. durum</i>	98.78	1.02	0.20	36.29	12.9	3.23	23.39	24.19	94.55	3.62	1.82	13.64	22.73	—	31.82	31.81
2,4,5-T		94.81	3.9	1.29	29.03	19.35	—	22.58	29.04	93.07	3.96	2.97	20.09	25.57	—	22.37	31.97
2,4-D	<i>Ae. ligustica</i>	98.11	1.89	—	33.27	7.75	—	42.10	16.88	93.12	5.90	0.98	30.25	48.10	1.2	16.86	3.59
2,4,5-T		97.15	2.64	0.21	61.08	18.30	1.06	13.54	6.02	92.93	6.41	0.66	22.18	46.7	—	26.36	4.76

Table 4. Number of cells and the percentages of abnormal cells in different stages of meiosis of *Aegilops ligustica* treated with different doses of 2,4,5-T

Treatment	Species	Total number of cells	Metaphase I			Anaphase I			Metaphase II			Anaphase II		
			Number of cells observed	No. of abnormal cells	%	Number of cells observed	No. of abnormal cells	%	Number of cells observed	No. of abnormal cells	%	Number of cells observed	No. of abnormal cells	%
A ₁	<i>Aegilops</i>	3145	1046	214	20.46	271	19	7.01	1116	81	7.26	712	49	6.88
A ₂	<i>ligustica</i>	4531	2370	706	29.79	932	482	51.72	557	217	38.96	672	172	25.59
A ₃		7498	2259	1718	76.05	1495	1274	85.22	1699	959	56.45	2065	1640	79.42

A₁ = one dose

A₂ = two doses

A₃ = three doses

Table 5. Statistical analysis for the observed abnormal cells in different meiotic stages in *Aegilops ligustica* treated with different doses of 2,4,5-T

Source	Degrees of freedom	Metaphase I			Anaphase I			Metaphase II			Anaphase II		
		Mean squares	F		Mean squares	F		Mean squares	F		Mean squares	F	
Replicates	2	13.775	0.218 ns		10.11	0.365 ns		110.33	1.141 ns		38.11	1.441 ns	
Doses	2	196012.5	3105.7 ⁺⁺		134258.8	4847.8 ⁺⁺		74441.3	770.1 ⁺⁺		261190.8	9876.8 ⁺⁺	
Error	4	63.113			27.695			96.67			26.445		

ns = $p > 0.05$ ⁺⁺ = $p < 0.01$ Table 6. Analysis of the occurring abnormalities percentage in the different meiotic stages as occurring after treating *Aegilops ligustica* with different doses of 2, 4, 5-T

Treatment	Species	Metaphase I			Anaphase I			Metaphase II			Anaphase II						
		Lag.	Stick.	Frag.	Lag.	Stick.	Frag.	Lag.	Stick.	Frag.	Lag.	Stick.	Frag.	Single brid.	Multiple brid.		
A ₁	<i>Aegilops</i>	97.15	2.64	0.21	61.08	18.3	1.06	13.54	6.02	92.93	6.41	0.66	22.18	46.70	—	26.36	4.76
A ₂	<i>ligustica</i>	97.74	1.98	0.28	53.33	16.0	3.33	13.56	13.78	92.17	6.91	0.92	61.05	11.62	0.58	19.19	7.56
A ₃		47.38	51.92	0.70	32.18	55.65	1.10	3.69	7.38	95.72	3.13	1.15	62.93	23.29	1.10	6.89	5.79

A₁ = one doseA₂ = two dosesA₃ = three doses

analysis of variance for the effect of dose on the frequency of abnormal cells and it showed a highly significant difference between treatments. The different kinds of chromosomal aberrations are shown in Table 6. It is apparent that lagging chromosomes represented higher percentages than other kinds of aberrations with the excep-

Figs. 7-9. 7, a lagging chromosome in anaphase II of *T. durum* treated with 2,4-D. 8, a lagging chromosome in metaphase II of *Ae. ligustica* treated with 2,4,5-T. 9, a lagging chromosome in anaphase II of *Ae. ligustica* treated with 2,4-D.

tion of anaphase II in plants treated with single dose. It seems that stickiness of chromosomes increase with the increase of 2,4,5-T dose in M I, and A I but other irregularities remained almost without major change.

Discussion

The results obtained in the present study showed that the two herbicides 2,4-D and 2,4,5-T increased the percentages of abnormal pollen mother cells during meiotic

division. Generally the increase in chromosomal aberrations was similar to what was obtained by the use of other pesticides (Soliman and Al-Najjar 1980). The results obtained indicated that the two herbicides with the concentration 0.1% caused major chromosomal aberrations in wheat and the two species used in the present study. From previous work by Mac Key (1959, 1967 and 1968) which showed that the number of spontaneous chromosomal mutation per cell as random events increase with the number of chromosomes per cell. The above findings may explain the relative increase in total abnormal meiotic cells in the control of *T. aestivum* in the present study (Table 1). Chromosome stickiness which was common in the present investigation, caused many chromosomal aberrations such as single bridges, multiple bridges and other irregularities. All abnormalities observed in the present study confirmed the assumption that the two herbicides with 0.1% concentration acted as chemical mutagens. Besides the results obtained with the use of different doses of the herbicide 2,4,5-T on *Ae. ligustica* were in favor of that assumption. From the results on the treated plants *T. aestivum* showed higher percentages of abnormal cells than the other two species during most stages of meiosis. These observations were in accordance with our previous work with some fungicides (Soliman and Al-Najjar 1980). The chromosomal aberrations observed in the present study might lead to sterility and subsequent effects such as yield reduction and quality inferiority in the following generations. These effects need to be studied in the future. Moreover a study is needed to find better alternatives to control weeds in wheat fields rather than the two herbicides 2,4-D and 2,4,5-T for that purpose.

Summary

The meiotic effects of 2,4-D and 2,4,5-T on hexaploid wheat *T. aestivum* L., tetraploid wheat *T. durum* Desf. and a diploid related species *Ae. ligustica* L. The two herbicides increased the percentages of abnormal meiotic cells significantly. The kinds of irregularities observed were lagging chromosomes, single and multiple bridges, fragments and asynchronization in the disjunction of chromosomes during second anaphase. It was evident that chromosomal stickiness was the cause of most of the aberrations observed. The increase in dose of 2,4,5-T caused significant increase in the percentages of meiotic cells with chromosomal aberrations in *Ae. ligustica* L.

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