J.K.A.U.: Sci., vol. 5, pp. 15-24 (1413 A.H./1993 A.D.

Cytogenetic Studies on *Vicia faba* Using Some Herbicides Commonly Used in Saudi Arabia

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ABSTRACT. In a previous investigation cytological abnormalities were reported in root tips of *Vicia faba* treated with treflan, fusilade, U-46, and herbstop. A recovery experiment was conducted in this investigation to study the fate of cytological alteration caused by the four herbicides. Treated seedlings with the four herbicides for one hour were left to recover for 24, 48 and 96 hours. Mitotic index reached normality after 48 hours in case of U-46 and herbstop and after 96 hours in case of treflan and fusilade.

After 96 hours of recovery treflan continue to increase metaphase stage, and the four chemical increased prophase stage on the expense of anatelophase stages. These alterations were accompanied by chromosomal aberrations that is bridges, fragments, binucleatic cells, polyploid cells, tripolar nuclei and stickiness of chromosomes. The results suggest direct and indirect effect of the chemicals on genetic material.

Introduction

The universality of the genetic material and of the basis of genetic code, facilitate the use of non-human test system to detect chemical mutagens and clastogens. As a rule there appears to be a good correlation between the chromosome breaking caused by chemicals in plants and cultured animal cells[1]. However, the type of effects may be very different in the two materials[2]. Many workers have reported on the genotoxicity of agriculture chemicals in plants. An extensive work has been carried on to study the herbicides and their genotoxic effects in plants[3-33].

The herbicides: treflan, herbstop, U-46 and fusilade are commonly used in agriculture in Saudi Arabia. The aim of this investigation is to study the fate of cytological alterations induced by the four previous herbicides on subsequent division cycles.

Material and Methods

The chemical designations of the four herbicides used in this investigation are :

Treflan	:	a, a, a trifluoro-2, 6-dinitro-N, N-dipropyl-p-toludine.
Herbstop	:	N-phosphoromethyl glycine
Fusilade ppoog	:	Fluazifop-butyl
U-46	:	Amine salt of 2, 4-dichlorophenoxy acetic acid.

Vicia faba variety Giza was used in this investigation. The four herbicides were prepared in the following concentrations 10, 50, 100, 300 and 500 ppm. Seedlings 8-10 days old were treated with the four chemicals using Kihlman standard method[34]. The seedlings were exposed to the chemicals under investigation for one hour. The acetocarmin technique was used in preparing and staining the slides. Observations were taken about mitotic index, percentage of mitotic phases and chromosomal aberrations. At least 1000 cells were examined for each treatment. The concentration that showed the lowest mitotic index was selected for each herbicide. Seedlings were treated with each herbicide using the selected concentration for one hour. The same seedlings were transferred to fresh water for 3 interval 24, 48, and 96 hours. After each interval the same previous observations were taken. For statistical analysis, the difference between values of different treatments and control were compared with critical values for test involving a difference of two proportions[35].

Results and Discussion

The four herbicides caused a highly significant reduction in mitotic index after one hour treatment (Table 1). This reduction disappeared right after recovery in case of

TABLE Mitotic index in root tip-cells of *Vicia faba* treated with different concentrations of the four herbicides for one hour.

Chemical concentrations (ppm)	Herbicides			
	Treflan	Fusilade	Herbstop	U-46
10	10.46**	8.37**	7.30**	6.90**
50	10.61**	6.56**	5.87**	7.59**
100	8.13**	6.54**	6.87**	6.88**
300	11.19**	5.78**	4.45**	6.14**
500	6.90**	6.93**	5.78**	6.16**
Control for one hour = 12.75				

**Significant at 1% level

herbstop, showing no significant difference than the control through all the recovery periods. However, the reduction in mitotic index disappeared after 48 hours in the other 3 herbicides treflan, fusilade, and U-46 indicating a more permanent effect of the 3 herbicides (Table 2). Wu found that treflan showed a reversible inhibition of mitosis on root tips of *Vicia faba*[36].

TABLE 2. Mitotic index in root tips of *Vicia faba* treated with the four herbicides after recovery for 24, 48 and 96 hours.

Type of treatment	Concentration ppm	1 hr treatment	Period of recovery hr		
			24	48	96
Control	Control	12.75	8.44	7.14	7.73
Treflan	500	6.90**	6.15*	6.03	8.17
Fusilade	300	5.78**	5.36**	5.54	8.13
Herbstop	300	4.45**	7.17	7.91	7.55
U-46	300	6.14**	6.16**	7.55	9.88

**Significant at 1% level.
*Significant at 5% level.

The reduction of mitotic index in the 24 hours period of recovery in case of treflan, fusilade and U-46 may be correlated with the significant increase of total abnormalities caused by the same herbicides in the same period (Tables 7-9). It was found in previous investigation that the four herbicides reduced the DNA synthesis and suggested to affect G_2 period reducing number of cells entering prophase, and consequently reduced mitotic index[37]. It can be assumed that, cells when removed from the herbicides and left to recover in tap water, increased their mitotic activity either by reincreasing number of cells entering prophase or by shortening the G_2 period and lengthening duration of mitosis or releasing the blocked DNA synthesis of the affected cells. Any one of these assumptions would require extra effort and would need more time for the cells to reach normality.

Effect of the herbicides on altering frequency of mitotic phases was temporary and disappeared from the first period of recovery in all cases except in treflan and herbstop (Tables 3-5). In treflan the effect on increasing metaphase stage due to for-

TABLE 3. Percentage of mitotic cells in prophase in root-tips of *Vicia faba* treated with the four herbicides after recovery for 24, 48 and 96 hrs.

Type of treatment	Concentration ppm	1 hr treatment	Period of recovery hr		
			24	48	96
Control	Control	47.29	36.89	32.29	30.55
Treflan	500	33.30**	25.30	23.55	35.51
Fusilade	300	33.88**	34.61	43.75	35.51
Herbstop	300	38.66	33.65	37.71	49.81*
U-46	300	54.94	32.29	33.33	38.28

**Significant at 1% level.
*Significant at 5% level.

mation of arrested metaphase was non-significant after 24 hours but a significant increase in the same stage appeared after 48 hours and continued up to 96 hours but was non-significant comparing up to 96 hours but was non-significant comparing with

the control (Table 4). These results indicated a more permanent effect on inhibition of spindle formation. Spindle is thought to be formed of protein and RNA[38]. RNA are synthesized and accumulated essentially in three steps: (one immediately post-telophase, one during the S period, and one pre-prophase period[39]. Rates of protein synthesis are high during interphase and mitosis with the highest degree at G_2 period and prophase[40]. The effect of treflan on the spindle may be due to its effect on RNA or on protein synthesis. Young and Camper demonstrated that trifluralin inhibited RNA, and protein synthesis in tobacco[41]. Reduction of RNA or protein synthesis by treflan may affect formation of the spindle fibers in the new cells and thus forming arrested metaphase in the next cycle.

TABLE 4. Percentage of mitotic cells in metaphase in root-tips of *Vicia faba* treated with the four herbicides after recovery for 24, 48 and 96 hours.

Type of treatment	Concentration ppm	1 hr treatment	Period of recovery hr		
			24	48	96
Control	Control	14.86	32.03	37.50	37.96
Treflan	500	48.95**	45.78	54.33**	42.99
Fusilade	300	49.58**	42.30	28.75	33.64
Herbstop	300	25.33**	34.61	31.57	24.50
U-46	300	25.27**	32.29	32.35	36.71

**Significant at 1% level.
*Significant at 5% level.

TABLE 5. Percentage of mitotic cells in ana-telophase in root-tips of *Vicia faba* treated with the four herbicides after 3 periods of recovery.

Type of treatment	Concentration ppm	1 hr treatment	Period of recovery hr		
			24	48	96
Control	Control	37.83	31.06	30.20	31.48
Treflan	500	17.70**	28.91	21.50	21.49
Fusilade	300	16.51**	23.07	27.50	30.84
Herbstop	300	36.00	31.73	30.70	26.47
U-46	300	19.78	35.41	34.31	25.00

**Significant at 1% level.

In case of herbstop, the herbicide showed significant decrease of metaphase after 96 hours of recovery comparing with the control (Table 4), and in the same time a highly significant increase of prophase. The results in this investigation indicated that the cells when treated with the herbicide showed arrested metaphase owing to spindle poisoning. By removing cells from herbstop, the spindle formed and cells proceed to ana-telophase reducing frequency of metaphase until it reached the control after 48 hours of recovery. In the same time cells may overcome the effect of her-

bicide by shortening the duration of metaphase and lengthening the duration of prophase ending up with high frequency of prophase and low frequency of metaphase after 96 hours of recovery. Another possibility is the indirect effect of the herbicide on delaying breakdown of nuclear membrane, causing the high frequency of prophase type.

The percentage of total aberrations produced by each fungicide after one hour treatment was highly significant than control (Table 6). However, the frequency of the total aberrations did not show any significant increase than control during the periods of recovery with 2 exceptions: after 24 hours of recovery in case of treflan, fusilade, and U-46, and after 48 hours in case of treflan. The first case may explain the reduction of mitotic index that appeared after 24 hours of recovery in case of treflan, fusilade, and U-46. The second case, increasing total aberrations after 48 hours from recovery of treflan may be due to the significant increase of metaphase in this period, since most abnormalities appear in metaphase or anaphase stages.

TABLE 6. Percentage of total abnormalities and different types of abnormalities in root-tips of *Vicia faba* after one hour–treatment of each herbicide.

Type of treatment	Types of abnormalities									Total percentage abnormalities
	B	L	S	C	T	PP	F	B&F	Bi	
Control	1.35	1.35	–	–	–	–	–	0.67	–	4.05
Treflan 500 ppm	–	1.04	8.33	12.5	–	–	–	–	–	21.87**
Fusilade 300 ppm	–	3.3	16.52	11.57	3.3	–	1.65	–	3.3	39.66**
Herbstop 300 ppm	5.33	–	9.33	–	1.33	–	2.66	8.0	–	26.66**
U - 46 300 ppm	1.09	2.19	4.39	4.39	–	–	–	2.19	4.39	8.68**

B : Bridges L : Laggard S : Stickiness of chromosomes
C : C-metaphase T : Tripollar cell PP : Polyploid cell
F : Fragment B & F : Bridge + fragments Bi : Binucleate cell.

**Significant at 1% level

Concerning types of aberrations induced during recovery periods, the four herbicides induced one or more of the following aberrations: bridges, fragments, laggards, C-metaphase, stickiness of chromosomes, tripolar cells, polyploid cells, bridges + fragment, contraction of chromosome, binucleate cells + bridges, and wide poles (Tables 7-9) and (Fig. 1). Contraction of chromosomes was produced in this investigation using treflan, fusilade and U-46. This aberration may be a result of an indirect effect of the herbicides on nucleoprotein fibrils causing more coiling of chromatids. Fragmentation was caused by treflan and U-46, indicating an indirect effect of the 2 herbicides causing chromosome breakage. Binucleate cells were formed during recovery of treflan and herbstop. These results indicated an indirect effect of the two herbicides on cytokinesis. Laggards were produced using treflan. Tripolarity was induced by treflan and by U-46, wide poles were formed using herbstop. Tripolarity and wide poles indicated the indirect effect of herbicides on spindle formation. Polyploid cells were obtained during recovery using either, herbstop, or U-46. The

TABLE 7. Percentage of total and different types of abnormalities in root-tips of *Vicia faba* treated with the four herbicides after recovery for 24 hrs (Period of treatment 1 hr).

Type of treatment	Concentration in ppm	Type of abnormalities												Total abnormalities
		B	F	L	C	S	T	PP	B&F	Bi	Con	Bi&B	Wi	
Control	Control	1.94	–	0.97	1.94	–	–	–	2.91	–	–	–	–	7.76
Treflan	500	2.4	2.4	1.25	3.75	–	–	–	–	–	4.8	–	–	19.27**
Fusilade	300	–	1.28	2.56	–	2.56	–	–	–	5.12	10.25	1.28	–	23.07**
Herbstop	300	4.8	0.96	0.96	–	1.92	–	–	2.88	1.92	1.92	–	0.96	16.34**
U-46	300	5.2	1.04	1.04	–	–	4.16	1.04	4.16	2.08	2.08	–	–	20.83**

Con : Contraction of chromosomes Bi & B : Binucleate cell and bridges Wi : Wide poles
**Significant at the 1% level.

TABLE 8. Percentage of total and different types of abnormalities in root-tips of *Vicia faba* treated with the four herbicides after recovery for 48 hrs (Period of treatment 1 hr).

Type of treatment	Concentration in ppm	Type of abnormalities												Total abnormalities
		B	F	L	C	S	T	PP	B&F	Bi	Con	Bi&B	Wi	
Control	Control	2.08	–	2.08	2.08	2.08	1.04	–	1.04	–	–	–	–	10.41
Treflan	500	3.22	2.15	1.07	2.15	7.52	1.07	–	1.07	–	5.37	–	–	23.55*
Fusilade	300	–	1.25	–	–	2.5	–	–	–	8.75	3.75	–	–	18.75
Herbstop	300	2.63	1.75	–	1.75	2.63	–	0.87	2.63	2.63	3.5	0.87	–	19.29
U-46	300	4.9	0.98	–	0.98	0.98	–	–	2.94	1.96	2.94	–	–	15.68

*Significant at the 5% level.

TABLE 9. Percentage of total and different types of abnormalities in root-tips of *Vicia faba* treated with the four herbicides after recovery for 96 hrs (Period of treatment 1 hr).

Type of treatment	Concentration in ppm	Type of abnormalities												Total abnormalities
		B	F	L	C	S	T	PP	B&F	Bi	Con	Bi&B	Wi	
Control	Control	6.48	0.92	–	0.92	1.85	–	–	4.62	–	–	–	–	14.81
Treflan	500	2.8	–	1.86	0.93	–	0.93	–	–	2.8	4.67	–	–	15.88
Fusilade	300	1.86	1.86	–	–	1.86	–	–	0.93	6.54	2.8	0.93	–	16.82
Herbstop	300	1.96	0.98	–	1.96	1.96	1.96	–	4.9	1.96	–	–	0.98	16.66
U-46	300	2.34	0.78	–	–	2.34	1.56	–	2.34	3.12	–	–	–	12.50

appearance of polyploid cells was probably a result of division of chromosomes in C-metaphase formed before recovery, in the case of U-46.

From the previously mentioned results, we can conclude that the four herbicides acted directly and indirectly on genetic material.

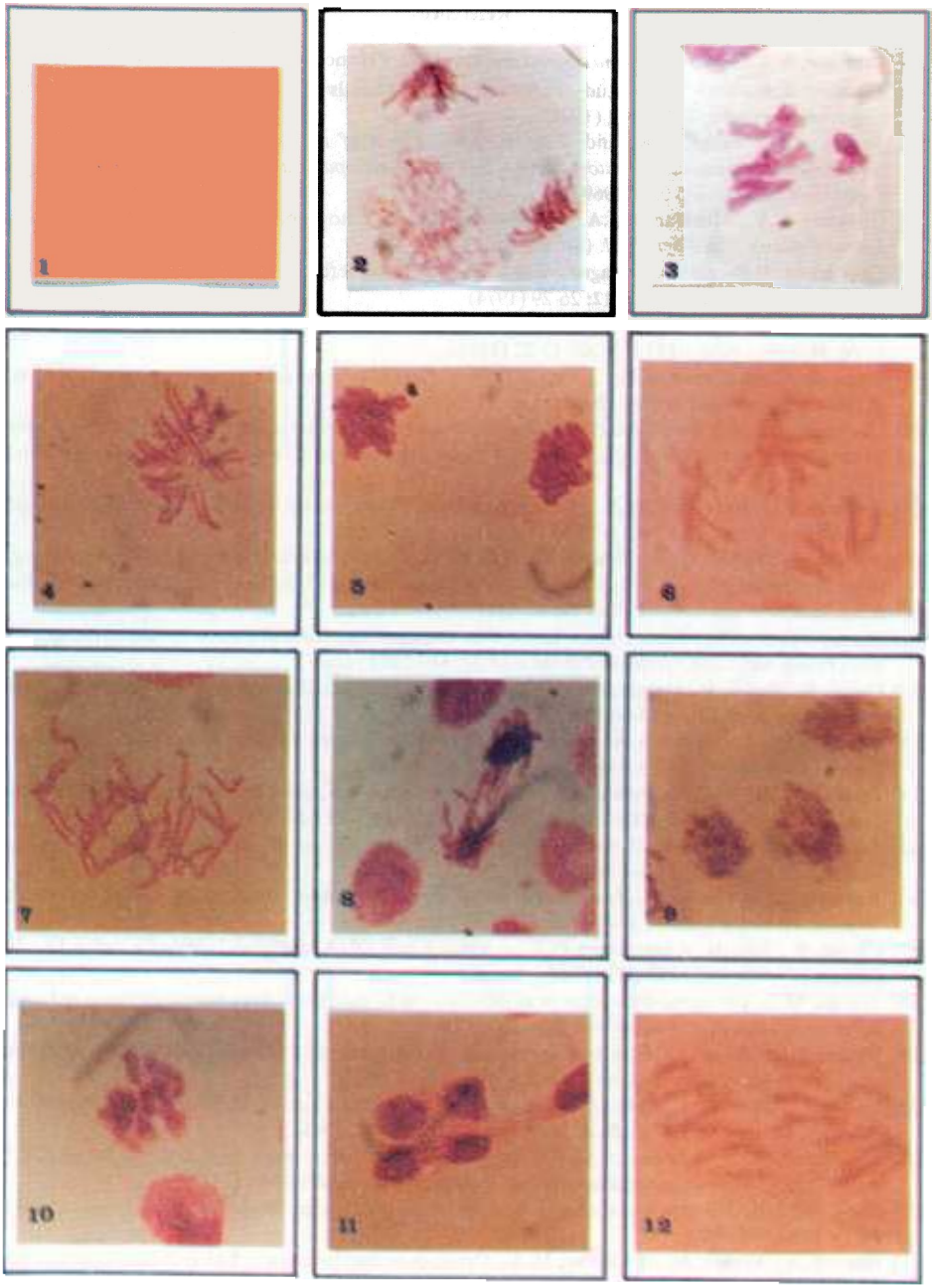

FIG Chromosomal aberrations induced by the four herbicides before and after plant recovery. 1. bridge, 2. fragments, 3. laggard, 4. C-metaphase, 5. stickiness of chromosomes, 6. tripolar cell, 7. polyploid cell, 8. bridges + fragment, 9. binucleate cell, 10. contraction of chromosomes, 11. binucleate cells + bridges, 12. wide poles.

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دراسة وراثية خلوية في نبات الفول باستخدام بعض مبيدات الحشائش المستخدمة في المملكة العربية السعودية

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المستخلص . لوحظ في بحث سابق بعض التشوهات السيتولوجية في خلايا القمم النامية لجذور نبات الفول المعاملة بالمبيدات العشبية التالية : الترفلان ، الفيوزيلاد ، ي-٤٦ و الهربستوب . وفي هذا البحث تم دراسة هذه التشوهات في الدورات الميتوزية المتعاقبة حيث عوملت بادرات الفول بالمبيدات الأربعة سالفة الذكر بتركيزات مختلفة لمدة ساعة ثم تركت لفترات ٢٤ و ٤٨ و ٩٦ ساعة لإزالة آثار المبيد . وأظهرت النتائج أن معدل الانقسام الميتوزي يتأتي بعد ٤٨ ساعة عند استعمال كل من ي-٤٦ والهربستوب وبعد ٩٦ ساعة عند استعمال كل من الترفلان والفيوزيلاد .

وبعد ٩٦ ساعة أدى الترفلان إلى استمرارية زيادة الدور الاستوائي كما أن المبيدات الأربعة نتج عنها زيادة الدور التمهيدي على حساب الدورين الانفصالي والنهائي . هذا وقد صاحبت هذه التغيرات شواذ كروموسومية مثل جسور كروموسومية وشظايا كروموسومية وخلايا ذات نواتين وخلايا عديدة المجموعة الكروموسومية وأنوية ثلاثية الأقطاب ولزوجة الكروموسومات . وهذه النتائج توضح حدوث تأثيرات مباشرة وغير مباشرة لهذه المواد الكيماوية على المادة الوراثية بالخلايا .