

Cytogenetic Response of Vicia faba Plants to
the Herbicide "Treflan"

By

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INTRODUCTION

Trifluralin is a toluidene herbicide (Fig. 1). (α, α, α , trifluoro 2,6-dinitro N,N-dipropyl p-toluidine, Parker and Clyde, 1976). It is used for the control of weeds in the fields of cotton (Kasyanenko and Davydov, 1984), tomatoes (Gurova et al., 1981), and Vicia faba (Zahran, 1982). It is also used in fruit nurseries to control weeds (Alekseeva and Keshtov, 1985).

The aim of the present investigations is to evaluate the genotoxic effect of Treflan in Vicia faba plant.

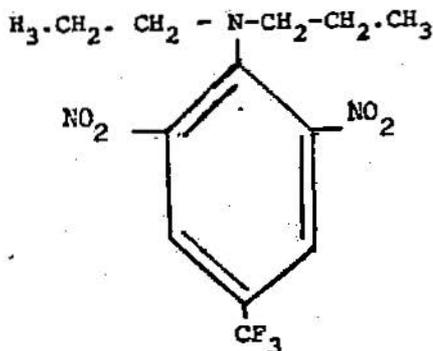


Fig. (1): Treflan

MATERIALS AND METHODS

Sterilized seeds of Vicia faba (var. Giza-2) and pure Treflan were used in this study. Two treatments were carried out.

In the 1st treatment, seeds were sown in pots and irrigated regularly with tap water. Vicia faba plants were sprayed at flowering stage for four successive days with the herbicidal solution (390 ppm). Each plant received daily 7 ml of the experimental agent; the control was sprayed only with distilled water. In the other treatment, the pots were irrigated twice weekly once with Treflan solution and the other with water till the plants have been in the flowering bud stage, the control was irrigated with tap water. For each treatment and control 5 pots with 25 plants (Five plants in each pot) were used (i.e. 100 plants were used for each treatment). Flower buds were gathered 24 hours after the last spray, fixed in 1:3 acetic acid: ethanol and stored in 70% ethyl alcohol for the meiotic study. About (2000 - 3600 PMCs) were analyzed for each treatment from randomly selected anthers of different plants (5-7) and about, 1000 pollen grain viability were analyzed from the same plants to determine pollen grain viability according to conventional acetocarmine technique.

Abnormalities were recorded in the meta-ana, and telophase stages of both meiotic divisions and stainability for determining pollen sterility.

RESULTS AND DISCUSSION

A high percentage of abnormal PMCs/plant, was observed after the treatments with Treflan. Such percentage, however, was higher in the plants sprayed with the herbicide than the other (Table 1).

It is evident from Tables II, III that the highest frequency of abnormal PMCs was that of PMCs undergoing anaphase I. It's percentage reached 12.25% and 19.42 in the plants irrigated and sprayed with Treflan, respectively.

The frequency of PMCs with chromosome stickiness 45.59% (Fig. 2) and that with disturbed meta- and anaphases 22.73% (Figs. 3,4) was relatively higher to the number of scored abnormal PMCs in Vicia faba plants which were sprayed with the herbicide (Table IV).

The most dominant type of chromosome aberrations induced in PMCs, in the two treatments with Treflan was: chromosome stickiness which some times resulted in the formation of sticky bridges in anaphases. It's percentage reached 4.60 ± 1.45 /plant (sprayed plants) compared with 0.32 ± 0.18 in the control and was found to be statistically significant. (Table V) (Amer and Ali, 1983) concluded that, chromosome stickiness is a general effect induced in PMCs after treatment with different chemical agents. Such phenomenon was also observed in the meiosis of Vicia faba after treatment with other pesticides e.g. Phosvel (Amer and Farah,

1980) Dursban (Amer and Farah, 1983), Trichlorophon (Amer and Ali, 1983) and Cypermethrin (Amer et al., 1987). Lagging chromosomes were observed in a considerable percentage in irrigated plants (22.86%) than in sprayed plants (14.20%) and were found in different meiotic stages (Table IV). The presence of a single lagging bivalent in the 1st metaphase stage (e.g. Fig. 5) may be attributed to hinderance of the movement of the bivalent to the equatorial plate (Amer and Ali, 1974), more than one lagging chromosome were present. The presence of lagging chromosomes in the 2nd meiotic division (e.g. Figs. 6,7) may be attributed as in mitosis to hinderance of the prometaphase movement of chromosomes accompanied by adhesion of the centromeres to the adjacent inner surface of the plasma (Barthelness, 1957).

Disturbed meta- and anaphases where the chromosomes spread irregularly in the cell were observed in a considerable percentage after the different treatments with Treflan, but the percentage of the disturbance in the sprayed plants 22.73% are more than it in the irrigated ones (3.81%) (Table IV).

Fragments were observed in a high frequency in PMCs of the plants irrigated with the herbicide (28.57%) (e.g. Fig. 8, Table IV). Fragments and bridges lead to structural changes in the chromosomes. Lagging of chromosomes and their presence as univalents may result in aneuploidy (Schulz-Schaeffer, 1980). As is evident from (Table IV).

Micronuclei were observed in both 1st, and 2nd telophase stages (Table IV) and may result from lagging chromosomes in the 1st ana- and telophases (Amer and Ali, 1974).

The mean percentage of PMCs with fragments and micronuclei reached 0.97 ± 0.24 (sprayed plants) compared with 0.23 ± 0.12 in the control and was found to be statistically significant (Table V).

Anaphase bridges (e.g. Fig. 9), univalents in metaphase I and multipolar telophase II (e.g. Fig. 10) were observed in a low frequency compared with the previously mentioned types of abnormalities (Table IV).

Spraying Vicia faba plants with 390 ppm Treflan for four successive days induced a statistically significant percentage of sterile pollen grains/plant (Table I). Such sterility may be due to the presence of abnormal PMCs in telophase II (Amer and Ali, 1988).

The present results indicate that "Treflan" is genotoxic in the meiosis of Vicia faba plant.

It induced a statistically significant percentage of chromosome abnormalities/plant and a statistically significant percentage of stickiness and fragments + micronuclei/plant.

Accordingly much care should be taken in using Treflan in agricultural aspects.

SUMMARY

The meiotic effects of the herbicide α,α,α , trifluro 2,6-dinitro N,N-dipropyl p-toluidine "Treflan" were studied. Vicia faba plants (var. Giza 2) were sprayed with aqueous saturated. "Treflan" solution (390 ppm) at the flowering stage, and the another treatment, the plants irrigated weekly with the same herbicide solution let the plants be in the flowering stage. The two treatments induced a significant percentage of abnormal pollen mother cells (PMCs/plant), but in the pollen grain viability the sprayed plants only induced a significant percentage.

The most dominant types of the observed anomalies was chromosome stickiness and lagging in the different meiotic stages.

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Table I: Mean percentage of abnormal PMCs and PGS Vicia faba/plant after treatment with 390 ppm Treflan solution.

Treatments	No. of invest. plants	Mean		Mean	
		% PMCS	± S.E.	% PGS	± S.E.
Irrigated control	5	1.45 ± 0.40	0.04	0.04	± 0.04
Irrigated plants	6	5.56 ± 1.23*	0.11	0.11	± 0.05
Control	6	1.55 ± 0.26	0.12	0.12	± 0.08
Plants sprayed for four successive days at 35 days	7	8.65 ± 2.00*	0.52	0.52	± 0.10*

(* Significant at 0.05 level (t-test).)

Table II: Number and percentage of the abnormalities occurring in the 1st meiotic division after treatment with 390 ppm Treflan solution.

Treatments	No. of count. PMCs in the 1st division	Metaphase I		Anaphase I		Telophase I	
		No. of PMCs	% of abn. PMCs	No. of PMCs	% of abn. PMCs	No. of PMCs	% of abn. PMCs
Irrigated control	1534	553	1.27	349	4.01	632	0.63
Irrigated plants	963	417	6.48	147	12.25	399	2.51
Control	881	276	1.81	202	2.97	403	0.99
Plants sprayed for four successive at 35 days.	1126	409	13.20	206	19.42	511	2.35

Table III: Number and percentage of the abnormalities occurring in the 2nd meiotic division after treatment with 390 ppm. Treflan solution.

Treatments	No. of count. PMCs in the 2nd division	Metaphase II		Anaphase II		Telophase II	
		No. of PMCs	% of abn. PMCs	No. of PMCs	% of abn. PMCs	No. of PMCs	% of abn. PMCs
Irrigated control	2090	617	1.30	314	1.59	1159	0.60
Irrigated plants	1139	274	8.76	117	5.93	748	2.54
Control	1317	272	2.21	377	1.33	668	0.75
Plants sprayed for four successive days at 35 days	1277	278	15.11	132	5.30	867	2.42

Table IV: Percentage of abnormalities* occurring in the meiosis Vicia faba plant treatment with 390 ppm Treflan solution.

Treatments	PMCs		Percentage of the different types of abnormalities relative to No. of abn. PMCs							
	No.	No. of abn.	Stick.	Lag.	Frag.	Univ.	Dist.	Brid.	Multi.	Micro.
Control	3624	45	6.67	26.67	24.44	-	4.44	24.44	8.89	6.67
Irrigated plants	2102	105	27.62	22.86	28.57	3.81	3.81	4.76	6.67	7.62
Control	2198	31	16.13	32.26	6.45	-	6.45	22.58	9.68	12.90
Plants sprayed for four successive days at 35 days	2403	176	46.59	14.20	7.95	-	22.73	7.95	6.25	6.25

* In the analysis of the different types of abnormalities, a cell containing more than one type of abnormalities was recorded under those types in the same time (e.g. a cell with stickiness and fragments).

Table V : Mean percentage of sticky, fragmentation and micronuclei PMCs Vicia faba/plant after treatment with 390 ppm Treflan solution.

Treatments	No. of invest. plants	Mean		Mean
		% of st. ± S.E. PMCS	% of frag. ± S.E. micro.PMCS	
Irrigated control	5	0.05 ± 0.05	0.45	± 0.22
Irrigated plants	6	1.95 ± 1.20	1.72	± 0.44
Control	6	0.32 ± 0.18	0.23	± 0.12
Plants sprayed for four successive days at 35 days	7	4.60 ± 1.45*	0.97	± 0.24*

* Significant at 0.05 level (t-test).



(2)



(3)



(4)

Figs. (2-4): Metaphase I with chromosome stickiness and Lagging bivalent (2), disturbed metaphase I (3) and disturbed metaphase II (4), after spraying (2,4) and irrigated (3) Vicia faba plants with 390 ppm Treflan solution.



(5)



(6)



(7)

Figs. (5-7): Metaphase I with lagging bivalent (5), metaphase II with two lagging chromosomes⁽⁶⁾, and telophase II with lagging chromosomes⁽⁷⁾ after irrigated (5) and sprayed (6,7) Vicia faba plants with 390 ppm Treflan solution.



(8)



(9)



(10)

Figs. (8-10): Metaphase I with two fragments (8), Anaphase II with bridge (9) and pentapolar telophase II (10), after spraying Vicia faba plants with 390 ppm Treflan solution.