Effects of the s-Triazine Herbicide Turbutryn on Mitosis, Chromosomes and Nucleic Acids in Root Tips of *Vicia faba*

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The mitodepressive and chromotoxic activities of herbicides in crop plants are well reported (Ennis 1948, Wu and Grant 1966, Gichner *et al.* 1968, Tobgy *et al.* 1969, Tomkins and Grant 1972, Amer and Farah 1974, 1976, Badr 1979, Badr and Elkington 1982, Badr 1983). The induction of chromosomal anomalies by herbicides has been found to be associated with an action of these chemicals on DNA and RNA (Mohandas and Grant 1972, Cartwright 1976, Chand and Roy 1982, Badr *et al.* 1984). However, studies of the effect of these compounds on cell division and chromosomes in relation to their action on the nucleic acids have not been sufficiently carried out.

Few s-triazine herbicides have been investigated for their ability to affect mitosis and to produce chromosomal anomalies (Liang *et al.* 1967, Wu and Grant 1966, Badr 1983). The effect of none of these compounds on DNA and RNA in dividing cells was not studied. In this paper the cytogenetic activities of the s-triazine herbicide turbutryn and its ability to affect DNA and RNA contents in the root tips of *Vicia faba* are reported.

Materials and methods

Turbutryn is the common name of 4-ethylamino-2-tert butylamino-6-methylthio-s-triazine and controls several mono- and dicotyledonous weeds in a number of crop plants. In this study a formula containing 50% a.i. of turbutryn was used. The Egyptian variety Giza 1 of *V. faba* has been used as a test material in this investigation. Seeds were soaked in tap water for 24 hours and were then transferred to pots filled with moist sand for germination. Actively growing roots were treated with 4 different concentrations for 3, 6, 12 and 24 hours. The concentrations used were 4, 20, 100 and 500 ppm respectively. Control roots were simultaneously treated with tap water.

For cytological preparations root tips were fixed in 3:1 (v/v) ethanol/glacial acetic acid for 24 hours and slides were prepared using the Feulgen squash technique. Six slides from each treatment were examined for the effects on the mitotic index (MI) and for chromosomal anomalies in dividing cells.

DNA and RNA were extracted by crushing 0.5 g. root tips in 4 ml Tris-EDTA buffer (0.05 M Tris.+0.1 M NaCl+0.1 EDTA, pH=8) mixed with 1 ml 1% SDS

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(sodium dodecylsulphate) and 1 ml SSC solution (0.0015 M trisodium citrate + 0.015 M NaCl, pH=7). To remove proteins, fats and other fractions the extract was mixed with 4 ml chloroform and isoamyl alcohol (24:1 v/v) and centrifuged at 4000 rpm. The supernatant contained the nucleic acids.

The amount of DNA was determined by mixing 2 ml of the supernatant with 1 ml 30% perchloric acid and 6 ml of Burton reagent. The mixture was kept at 30°C for 17 hours. (Burton reagent = 3 g. diphenyl amine in 200 ml acetic acid + ml conc. sulphuric acid + 1 ml of 1.6 acetaldehyde). Following this incubation the optical density of the solution was measured using a Spekol Karl-Zeiss spectrophotometer at 595 nm and the amount of DNA was calculated from a standard curve.

For the determination of RNA 1 ml of the extract was mixed with 1 ml 0.1 g. FeCl₃ dissolved in 100 ml 37% HCl and 5 g. orcin. This mixture was then heated in a water bath for 15 minutes, cooled and the volume was adjusted to 4 ml with the buffer used for extraction. The optical density was then measured at 670 nm. The amount of RNA was calculated from a standard curve. The standard error of the mean M1 and the mean amount of DNA and RNA and of the average total chromosomal anomalies was calculated as a measure of variation, in these parameters, within every treatment.

Results and discussion

The effects of the different treatments of turbutryn on M1 and the amounts of DNA and RNA in root tips of *V. faba* are given in Table 1. The 3 h treatments have only resulted in slight increases in M1 values, particularly when the two lower concentrations (4 and 20 ppm) were applied. Of the 3 h treatments only the highest concentration (500 ppm) has slightly reduced the amounts of DNA and RNA. Prolonged treatments, however, resulted in the inhibition of mitotic activity (reduced M1 values) and reduction of the amounts of DNA and RNA in dividing cells of root meristems. The degree of mitotic inhibition is clearly dose dependent. M1 value was reduced as the concentration of the herbicide was increased and the period of treatment was prolonged. The reduction in the amount of DNA and RNA is closely associated with the inhibition of mitotic activity.

The inhibition of mitotic activity induced by turbutryn resembles the action of several other herbicides. These include some substituted urea compounds (Wuu and Grant 1966, Tomkins and Grant 1972, El-Sadek and Ashour 1978, Badr and Elkington 1982), some substituted phenols (Mohandas and Grant 1972, Chand and Roy 1981), carbamates (Ennis 1948, Amer and Farah 1974), nitratin (Badr 1979), amitole (Bartels and Wolf 1965, Mohandas and Grant 1972), maleic hydrazide (Evans and Scott 1964) and some others. Tomkins and Grant (1972) and Badr and Elkington (1982) concluded that the substituted urea herbicides metobromuron and isopromuron may inhibit DNA synthesis which results in lower M1 values. Similarly Scott (1968) suggested that maleic hydrazide inhibits DNA replication. Chand and Roy (1981) proposed that 2,4-dinitrophenol inhibits DNA and RNA synthesis by reducing the oxidative phosphorylation in plants resulting in lower levels of ATP. This supports the hypothesis of Ashton and Crafts (1973) that

herbicides that reduce ATP are strong inhibitors of RNA and protein synthesis.

Turbutryn induced a wide range of chromosomal anomalies in the root tips of *V. faba*. The percentage cells showing abnormalities is also given in Table 1. The 3 h and 6 h treatments were indifferent with regard to the percentage of total chromosomal abnormalities produced, but 12 h and 24 h treatments resulted in higher percentage of abnormal dividing cells. In general the percentage of dividing cells showing chromosomal anomalies increased with the increase of herbicide concentration. Considerable increase of abnormalities was recorded as the period of treatment was prolonged from 6 h to 12 h and from 12 h to 24 h (Table 1).

Table 1. Effects of the different treatments of turbutryn on M1, the amounts of DNA and the percentage of chromosomal abnormalities in root tips of *V. faba*

Treatment conc. ppm	M1 $\pm$ SE ⁺	DNA μ g $\pm$ SE g	RNA μ g $\pm$ SE g	Chrom. abn. percentage $\pm$ SE
3 h Cont.	10.9 0.32	165 4.5	286 5.2	2.4 0.44
4	12.1 0.29	168 5.3	286 6.4	28.6 0.94
20	11.8 0.35	167 4.3	277 5.1	34.2 1.16
100	10.9 0.22	164 5.7	285 4.7	41.1 2.35
500	10.6 0.32	158 6.1	278 5.4	40.8 1.48
6 h Cont.	10.5 0.22	172 3.5	280 4.3	2.0 0.44
4	10.0 0.32	168 2.8	270 3.5	26.1 1.64
20	10.2 0.62	162 4.3	265 2.8	34.5 1.16
100	9.4 0.22	151 4.7	255 4.7	38.6 2.14
500	8.4 0.32	141 3.2	242 4.7	40.3 1.48
12 h Cont.	9.7 0.35	169 5.2	295 5.4	1.8 0.32
20	7.7 0.22	151 4.3	271 4.2	40.1 1.16
100	6.1 0.32	146 3.8	268 4.2	42.4 2.56
500	5.8 0.35	122 2.6	248 4.1	50.2 1.48
24 h Cont.	10.2 0.22	176 4.4	292 4.4	2.6 0.32
4	8.2 0.22	144 4.5	262 3.8	41.0 2.32
20	6.8 0.32	138 3.6	254 3.8	45.4 2.16
100	5.9 0.35	115 2.7	230 2.9	49.5 1.48
500	5.1 0.35	107 2.1	216 3.4	53.4 2.34

⁺ SE=Standard error.

The types and frequency of chromosomal anomalies produced by the different treatments of turbutryn are shown in Table 2 and photographs of these abnormalities are illustrated in Figs. 1–10. The colchicine type metaphase configurations (Fig. 1) were the major abnormality produced by all treatments. Multipolar anaphase and telophase figures (Fig. 2) were also observed in considerable frequency by all treatments. C-anaphase configurations (Fig. 3) in which the two chromatids of C-metaphase chromosomes are separated and chromosome lagging (Fig. 4) were recorded in lower frequencies by all treatments, whereas, polyloidy (Fig. 5) was only observed following the 24 h treatments. Chromosomal bridges (Fig. 6) and chromosome breakage (Fig. 7) were induced by all treatments; their frequency increased with increased concentration and prolonged period of treatment. Chromosomal stickiness (Fig. 8) was not observed after the 3 h treatments, but was recorded in considerably

Figs. 1–10. Types of chromosomal abnormalities induced by the herbicide turbutryn in the root tips of *V. faba*. 1, C-metaphase 3 h; 4 ppm. 2, multipolar anaphase 6 h; 4 ppm. 3, C-anaphase 6 h; 4 ppm. 4, chromosome lagging 6 h; 20 ppm. 5, S: polyploidy 24 h; 100 ppm. 6, chromosomal bridges and stickiness 24 h; 500 ppm. 7, chromosome breakage 12 h; 100 ppm. 8, stickiness 24 h; 100 ppm. 9, 9, micronuclei 24 h; 20 ppm. 10, chromatin bridge 24 h; 100 ppm.

high proportions following both the 12 h and 24 h treatments. Abnormal figures not included in one of these types have been referred to as disturbed figures; their frequency is given in Table 2. Some abnormal interphase cells were observed following the 6 h, 12 h and 24 h treatments. These were comprised of multinucleated cells and cells containing micronuclei (Fig. 9) and chromatin bridges (Fig. 10). The percentage of abnormal interphase cells is also given in Table 2.

Table 2. Types and percentage of chromosomal abnormalities produced by the different treatments of turbutryn in root tips of *V. faba*

Treatment conc.	C-M	C-A	Multi. A+T	Lagg.	Poly-ploidy	Bridge	Break	Stick	Dist.	Abn. int.
3h Cont.									100	
4	82.1	3.5	10.4	2.9	0.0	0.0	0.0	0.0	2.1	0.0
20	78.6	2.4	12.3	2.6	0.0	2.1	0.0	0.0	1.7	0.0
100	79.2	1.4	11.5	2.7	0.0	2.4	0.0	0.0	2.8	0.0
500	81.7	2.7	10.3	2.0	0.0	1.8	0.0	0.0	1.5	0.0
6 h Cont.									100	
4	73.4	1.7	12.1	5.0	0.0	3.8	1.0	0.0	2.0	0.3
20	72.6	2.2	11.6	4.2	0.0	5.6	1.0	1.0	2.0	0.2
100	64.3	3.2	15.4	4.3	0.0	6.8	1.5	2.6	2.2	0.2
500	58.8	2.8	17.4	4.9	0.0	8.3	1.2	5.2	1.8	0.2
12 h Cont.									100	
4	64.5	2.2	15.5	3.4	0.0	3.2	2.1	8.0	1.9	0.4
20	57.2	3.2	17.2	3.5	0.0	5.8	1.7	10.2	3.0	0.5
100	52.8	2.7	16.4	2.6	0.0	6.8	3.0	16.6	2.7	0.5
500	52.3	3.4	22.6	4.1	0.0	12.4	3.4	10.5	2.1	0.6
24 h Cont.									100	
4	60.5	1.7	15.1	2.1	2.1	6.1	2.7	12.0	2.0	2.4
20	55.2	2.3	16.1	1.8	2.4	8.6	3.1	16.4	2.8	3.6
100	52.8	2.8	18.2	2.9	2.0	9.8	3.6	25.8	2.8	4.7
500	48.6	2.8	19.6	3.1	3.0	14.7	3.5	29.7	3.8	5.4

The types of chromosomal anomalies produced by turbutryn may be grouped in three classes. The first is often referred to as mitotic abnormalities and is the result of an action on the mitotic apparatus (Brogger 1977, Grant 1978). These include C-metaphase, C-anaphase, multipolar mitosis, polyploidy and chromosome lagging. These types of abnormalities have been induced by several herbicides (Ennis 1948, Crocker 1953, Liang *et al.* 1967, Amer and Farah 1974, Badr 1979, Badr and Elkington 1982, Badr 1983). Another class of anomalies observed is chromosomal stickiness which is also induced by several agricultural chemicals (Grant 1978). Stickiness is generally regarded as a physiological effect on chromosomes during division (Savage 1975) and has been considered as a category of chromatid aberration (Klasterska *et al.* 1976). The third class of chromosomal anomalies produced by turbutryn is chromosome aberrations including chromosome breakage. In this respect turbutryn resembles some other herbicides including substituted ureas, substituted phenols and s-triazine compounds (Wuu and Grant 1966, Ahmed and Grant 1972, Mohandas and Grant 1972, Badr and Elkington 1982, Badr *et al.* 1984).

Chemicals which induce chromosome breakage are known to exert a clastogen action on chromosomes which is generally regarded to involve an action on DNA (Grant 1978).

A number of herbicides, which produced chromosomal anomalies similar to those produced by turbutryn, have been reported to induce mutation in higher plants. These include the s-triazine compounds atrazine and simazine (Wuu and Grant 1966), the substituted urea herbicides N-ethyl N-nitroso urea and N-methyl N-nitroso urea (Gichner *et al.* 1968), EMS (Tomkins and Grant 1972) and maleic hydrazide (Gichner *et al.* 1982). The present results, therefore, add a new compound to the list of agricultural chemicals which, when used widely, may result in harmful damage to crop plants. The application of these chemicals must be strictly controlled and their capacity to induce cytogenetic activities in crop plants should be extensively studied.

Summary

Actively dividing root tips of *Vicia faba* were treated with 4 different concentrations of turbutryn for 3, 6, 12 and 24 hours. The concentrations used were 4, 20, 100 and 500 ppm of the herbicide. Almost all treatments exerted a mitodepressive action and resulted in reductions in the amounts of DNA and RNA. Turbutryn produced a number of chromosomal anomalies. The types of abnormalities induced are comprised of mitotic abnormalities, which indicate an effect on the spindle apparatus, chromosomal stickiness and chromosomal aberrations including chromosome breakage. The mitodepressive action of turbutryn is remarkably associated with the ability of this herbicide to reduce the amounts of DNA and RNA and its capacity to induce chromosomal anomalies.

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