Cytogenetic Studies with Atrazine (2-Chloro-4-Ethyl-Amino-6-Isopropylamino-s-Triazine) on Plants

The herbicidally active s-triazines are specific inhibitors of the Hill reaction[1,2]. Other biochemical processes in plants, such as auxin metabolism, are also affected, besides the photosynthesis[3-5]. Since it was speculated that the triazines may substitute for the pyrimidines, it was investigated whether they could possibly function as base analogs in the nucleic acids[6]. However, it has been shown that herbicidally active triazines and their hydroxy-derivates have no effect on the nucleic acid metabolism of *B. subtilis* and *E. coli* or on the metabolism of viruses in infected bacteria[7]. The triazine herbicides prometryne [2-methylthio-4, 6-*bis*(isopropylamino)-s-triazine] and atrazine (2-chloro-4-ethyl-amino-6-isopropyl amino-s-triazine) are not incorporated into the DNA of an arg-t-u-strain of *E.-coli*[8]. Results of cytogenetic studies with triazine herbicides are available as well. SAWAMURA[9], working with *Tradescantia* hair cells, found no indication of any effect on the chromosomes, nor did STROEV[10] in his investigation with *Hordeum* seeds. WUU and GRANT[11-13] found a slight genetic activity of triazines in tests with *Vicia faba* and *Hordeum* root tip cells and *Hordeum* pollen mother cells, as well as STROEV[14] who performed cytological studies on *Hordeum* root tip cells. LIANG[15,16] had investigated division stages of pollen mother cells in *Sorghum* after application of atrazine in field experiments and reports a small number of aberrant cells. Since the results are somewhat contradictory, it was reasonable to check some of these results and carry out some additional tests.

Vicia faba, Hordeum vulgare and *Sorghum vulgare* were used as experimental material, and atrazine as a representative of the chloro-s-triazines. Maleic hydrazine (MH) was used as a positive control in order to compare the results with those of the above-mentioned authors. First, chromosome analysis was performed on *Vicia faba* (variety 'Aquadulce') lateral root tips after chemical treatment. The method of seedling cultivation and treatment as described by KIHLMAN[17,18] was essentially applied. Atrazine was used as pure substance dissolved in water or in 2% acetone or, for the experiment with high concentrations, formulated as wettable powder (Aatrex® 80 W[19]) in an aqueous suspension. The pH during treatment was between 6 and 8, temperature was around 20 °C and the roots were treated for 24 h. Half the roots were fixed immediately at the end of the treatment; the other roots were fixed after a recovery period of 24 h in water. For all treatments, control experiments with the formulation minus active ingredient were carried out. The concentrations most suitable to use as treatment were determined in preliminary experiments: these were dependent on phytotoxicity and/or solubility of the chemical in water or diluted acetone solutions (maximum 2%, as higher concentrations of acetone are phytotoxic). The fixed roots were prepared as Feulgen squashes. The slides were checked for aberrations mainly by observation of anaphases; fragments and bridges were scored. Each treatment was carried out using 10 beans, in each bean 200 anaphases were evaluated.

The frequencies of anaphase aberrations as observed are recorded in Table I. These data show that the treatment with atrazine did not increase the spontaneous rate of chromosomal aberrations. As anticipated, maleic hydrazide exerts a remarkable effect on the mitotic division, increasing the frequency of abnormal anaphases around 10 times compared to the control. Statistical analysis was significant at the 1% level. The mitotic index was not affected.

Further investigations were performed with *Hordeum vulgare* root tips. The seeds were treated with the chemical, and root tips were analyzed for chromosomal aberrations.

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[19] Aatrex® 80 W = trade name of a herbicide formulation containing 80% of atrazine (2-chloro-4-ethyl-amino-6-isopropylamino-s-triazine) as active ingredient.

Table I. Frequency of anaphase aberrations after treatment of *Vicia faba* root tips with atrazine and maleic hydrazide

Compound	Concentration of the active ingredient (m*M*)	Abnormal anaphases[b] (%)	*t*-test	Mitotic indices[b] (%)
Atrazine[a]	1.0	1.6 (1.0) NS	1.551	3.6 (3.8)
Atrazine[a]	0.5	1.4 (1.7) NS	−0.645	2.8 (2.3)
Atrazine[a]	0.25	1.5 (1.3) NS	0.548	3.2 (2.9)
Atrazine	0.4	1.1 (1.1) NS	−0.153	2.9 (4.1)
Atrazine	0.2	1.2 (1.25) NS	0.150	5.2 (4.6)
Maleic hydrazide	0.01	15.0 (1.25)[c]	16.445	3.5 (4.6)

[a] Atrazine formulated as Aatrex® 80 W. [b] The number in brackets apply to the corresponding controls. [c] Significance at the 1% level.

Table II. Frequency of anaphase aberrations in *Hordeum* root tip cells after treatment of the seeds with atrazine and maleic hydrazide

Compound	Concentration of the active ingredient (m*M*)	Abnormal anaphases [c] (%)	*t*-test	Mitotic indices [c] (%)
Atrazine [a]	10	2.9 (2.2) NS	0.542	2.5 (2.6)
	5	2.1 (1.4) NS	0.677	2.8 (2.8)
	2.5	2.3 (2.0) NS	0.229	1.9 (2.3)
Maleic hydrazide [b]	2.5	22.0 (1.7 [d]	3.095	0.3 (2.2)
	1	18.7 (1.7) [d]	4.998	0.7 (2.4)
	0.5	9.8 (1.7) [d]	3.106	1.4 (2.9)
	0.25	4.5 (1.6) NS	1.929	2.1 (2.3)

[a] Atrazine formulated as Aatrex® 80 W. [b] Maleic hydrazide formulated as 25 W. [c] The numbers in brackets apply to the corresponding controls. [d] Significance at the 1% level.

Table III. Frequency of aberrant division stages in *Sorghum* pollen mother cells after spray application 3 weeks after emergence with atrazine and maleic hydrazide

Compound	Concentration of the active ingredient (kg a.i./ha)	Abnormal division stages [c] (%)	*t*-test	Mitotic indices [c] (%)
Atrazine [a]	5	1.9 (1.6) NS	0.62	1.3 (1.2)
Maleic hydrazide [b]	3	4.3 (1.9) [d]	3.31	1.0 (1.4)

[a] Atrazine formulated as Gesaprim® 50. [b] Maleic hydrazide formulated as 25 W. [c] The numbers in brackets apply to the corresponding control. [d] Significance at the 1% level.

Dry seeds of *Hordeum* (winter barley, variety 'Astrid') were soaked for 24 h at 20 °C in suspensions of Aatrex® and for comparison in maleic hydrazide of different concentrations. Control treatments without the active ingredient were carried out. After treatment the seeds were rinsed with water and germinated in the dark in Petri dishes. The roots were fixed when they were about 5 mm long. Feulgen squashes were prepared after a 20 min maceration in 4% pectinase. The aberrations were again scored by observation of anaphases. Each treatment was carried out with at least 15 roots; 150–400 anaphases were investigated per treatment. The results are summarized in Table II. They show that the atrazine treatment of the *Hordeum* cells did not significantly increase the spontaneous rate of aberrations, whereas the chromosome-breaking effect of maleic hydrazide is obvious. This is also evident from the mitotic index which remains at a constant level in the atrazine treatments, whereas it greatly decreases when maleic hydrazide is used.

Finally, a field experiment was carried out using *Sorghum vulgare*. After spray treatments of the young plantlets, chromosome analysis in pollen mother cells was performed. The grain Sorghum variety 'Northrup King 110' was used for these investigations, and atrazine (Gesaprim® 50[20]) and maleic hydrazide 25 W were applied at rates of 3 and 5 kg, respectively, i.a./ha, 1 and 3 weeks after seedling emergence. About 8 weeks after seedling emergence the panicles were harvested, fixed in a formol-acetic acid-ethanol solvent mixture and prepared for microscopic examination. The squashes and sections were stained with Feulgen or for the examination of nucleoli with methyl-green-pyronin. About 4 plants per treatment were checked. In each panicle 300 division stages of pollen mother cells were evaluated.

The seedlings treated 1 week after emergence showed that neither maleic hydrazide nor atrazine had any cytogenetic effect. At a later application of the two compounds, atrazine again showed no effect, but the chromosome-breaking effect of maleic hydrazide became obvious and was statistically significant (see Table III). The examination of nucleoli, stained with methyl-green-pyronin, showed no difference between treatment and control.

Our findings (Tables I and II) do not confirm the results obtained by WUU and GRANT[11–13]. The data in Table III do not confirm the results by LIANG[15,16] et al. On the basis of the experiments we have reported here, using atrazine as a representative of the chloro-s-triazine herbicides, there is no evidence of a cytogenetic activity of this compound in plants.

Zusammenfassung. Untersuchungen an *Vicia faba*- und *Hordeum*-Wurzelspitzen sowie an *Sorghum*-Pollenmutterzellen ergeben keine Hinweise für eine zytogenetische Wirksamkeit des Triazin-Herbizides Atrazin in Pflanzen.

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[20] Gesaprim® 50 = trade name of a herbicide formulation containing 50% of atrazine (2-chloro-4-ethyl-amino-6-isopropylamino-s-triazine) as active ingredient.

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