Acta Biologica Szeged. *21* (1—4), pp. 141—146 (1975)

THE EFFECT OF SODIUM 2.4-DICHLOROPHENOXYACETIC ACID ON DOMESTICATED PLANTS

MÁRIA M. HORVÁTH and J. KERTÉSZ

Genetics Group, Attila József University, Szeged

(Received March 28, 1975)

Abstract

In high concentration sodium 2.4-dichlorophenoxyacetic acid (2.4-D, Dikornit) inhibited the growth of experimental plants. Its effect also remains in the T generation. Increase of the 2.4-D concentration increased the amount of soluble protein and the activity of peroxidase (with the exception of maize). The hydroxylation processes too are influenced by 2.4-D, the amounts of the derivatives being increased at low concentration.

GRANT (1972) considers the herbicides to be local promoters of evolution. Even if only 1 p.c. of the domesticated plants undergoes significant change in a genetic balance on herbicide accumulation, it serves as the starting basis for another hereditary pathway. All this can be measured in the treated generations the structural and numerical changes in the cromosomes, and morphological, physiological and biochemical indices. The current increase of chemization in plant protection presents us with both theoretical and practical research tasks.

In the present paper a study is made of the effects of a herbicide, sodium 2.4-dichlorophenoxyacetic acid (2.4-D, Dikonirt) on some plant species. In accordance with their hormonal ground substance, at low concentrations the herbicides stimulate growth, and at high concentrations inhibit it (VEINBRANTS, 1972). WUU and GRANT (1966a, b) observed chromosomal aberrations in the meiosis of barley root-tips on the action of herbicides, and the pollen mother cells of the treated second generation also exhibited abnormal meiosis. When applied in various concentrations to wheat, sunflower and bean, 2.4-D enhances the activities of the enzymes participating in the oxidative metabolism (VORT, 1964).

Materials and Methods

The experimental plants were MV 530 hybrid maize, broad bean, pea, pearl bean, cucumber, musk melon and water melon. The outdoor experiments were performed on 1 m plots. The 2.4-D dose was 1, 2, 4 or 10 g per plot for maize, and 0.05 or 0.26 g per plot for the other plants. The herbicide was employed as a preemergent simultaneously with the sowing, together with a sufficient number of control sowings. In the cytological and genetic examinations the plants were also grown in breeding vessels under semiconditioned circumstances for the observation of the development of the shaping of the genetic system variation. The controls were grown on tap-water and on 2.4-D solution with concentrations corresponding to those of the various outdoor experiments. A study was made of the modifying effect of 2.4-D, compared to the control, in the mitotic division, and of the changes occurring in the meiosis. The development of the protein content was measured by the method of LOWRY et al. (1951), and the changes in the pigments by the method of SMITH 1963). The peroxidase activity was determined by the procedure of SOLYMOSY—FARKAS (1964), and the indole hydroxylation was investigated by the method of HORVÁTH (1975).

Results and Discussion

In the outdoor experiments it was observed that when 2.4-D was used as a preemergent herbicide the increase of its amount was paralleled by increasing inhibition or even complete cessation of the germination of the seeds. For maize, 10 g/m^2 was the highest dose applied, and this inhibited growth of the roots. The effect of 2.4-D was also apparent in the growth of the shoots and in the development of the number of leaves. The time of tassel-silking too was shifted, and the yield decreased. The one or two grains present were larger and heavier than the grain yield of the normally-developed control plant (KERTÉSZ, 1974).

The other experimental plants reacted with different sensitivities to the increase of the 2.4-D concentration. In these plants the highest concentration of the herbicide used was 0.25 g/m^2, and this completely inhibited the germination of musk melon and water melon seeds. Pearl bean, pea and broad bean plants grew to a lesser extent. The greater the amount of 2.4-D given in the preemergent stage, the more reduced was the length of the shoots and roots, and the fewer the leaves formed; further, these latter ones were more distorted in shape. Only a few seeds developed here too.

The gourds reacted with varying sensitivity to 2.4-D. Cucumber well endured even the highest 2.4-D concentration employed, and the growth and development of the shoots and roots were only slightly irregular. At a lower concentration no change could be observed compared to the control. On the contrary this same lower concentration led to much damage among musk melons and water melons, many plants dying before the reproductive stage. On this basis we see that there is a correlation between the water — demand and 2.4-D sensitivity, and this is why cucumber can well endure this herbicide. Our results are supported by the data in the following Table 1 measured on the meiosis of the cells of the root-tip meristem.

Table 1. Effect of 2,4-D on the meiosis of root-tip meristem cells of seedlings treated in the preemergent stage.

Variant	No. of cells examined	No. of meiotic cells	Prophase	Metaphase	Anaphase	Telophase
MV 530 maize						
Control	2892	1068	906	51	57	50
Low conc.	2360	877	853	8	11	6
High conc.	2110	686	671	5	6	4
Pearl bean						
Control	1160	521	278	98	78	67
Low conc.	547	41	11	13	9	8
High conc.	398	17	6	4	4	3
Broad bean						
Control	448	196	171	9	6	10
Low conc.	1158	107	96	4	3	4
High conc.	1045	11	8	1	0	2

It can be seen from the Table that 2.4-D exerted a very considerable deceleratory influence on the course of the mitosis in all three plants, at the higher concentration almost eliminating the meiosis and thus the growth of the root.

The effect of 2.4-D could also be seen in the meiotic division of the T_0 plants treated. The arrangement of the chromosomes was irregular (ROJIK, 1973).

As regards the seeds of T_0 maize plants, it was found that the initial growth was more intensive than that of the control plants (KERTÉSZ, 1972).

The study of the T_0 and T_1 generations of broad bean led to a similar result as observed for maize. KOVÁCS (1972) found that 2.4-D is accumulated in the seeds. The extent of the accumulation rose with the increase of the concentration of the herbicide.

2.4-D inhibits the growth of seedlings; this is well illustrated in Table 2 by the higher amount of soluble protein in pearl-bean seedlings which underwent a slight degree of growth destruction.

Table 2. Change in the amount of total soluble protein in pearl bean seedlings on preemergent 2,4-D treatment (γ/g fresh weight).

Age of seedling in days	Control			Treated with low conc.			Treated with high conc.		
	Shoot	Root	Coty-ledon	Shoot	Root	Coty-ledon	Shoot	Root	Coty-ledon
3	4050	2205	4140	4120	2750	4400	4200	3850	5100
4	3375	1900	4320	3400	2600	4350	3800	3200	4300
5	3210	1885	4515	3170	2350	4010	3450	2840	4210
6	3105	1860	4650	2800	2250	3600	3260	2610	4130

Increase of the amount of 2.4-D led to a fall in the green pigment components of the experimental plants; this is connected with the hydrolysis of the proteins. There is barely any change in the amount of yellow pigment. These results were reported earlier (HORVÁTH, 1970). The herbicide increased the peroxidase activity in these plants, with the exception of maize seedlings. The results of the enzymatic measurements are shown in the following Table 3.

It can be seen that the peroxidase activity increased in parallel with the damage. Maize endured without damage the 2.4-D concentration employed here.

2.4-D causes damage in metabolism according to the above results, but it presumably also stimulates the functioning of an enzyme complex, as shown by the formation of hydroxy derivatives of indole. The derivatives are produced in larger amount at low 2.4-D concentration; at higher 2.4-D concentration the enzyme complex is directed to the elimination of the chemical, and because of this the amounts of the derivatives decrease. This was shown by the amounts of 5-hydroxyindole in the cases of broad bean, pearl bean and cucumber. The hydroxylation is intensive for both musk melon and water melon; various derivatives are formed, the amounts of these being lower at the low 2.4-D concentration. For pea a large amount of 5-hydroxyindole was formed in comparison to the control; higher concentration of 2.4-D decreased this amount.

Table 3. Change in peroxidase activity on preemergent 2,4-D treatment. (As percentage of control).

Species and age in days	Treated with low conc.			Treated with high conc.		
	Shoot	Root	Cotyledon	Shoot	Root	Cotyledon
Pearl bean						
4	110	118	254	135	214	281
6	121	192	62	228	432	218
8	125	405	85	334	667	177
10	128	471	105	334	667	145
Cucumber						
6	100	91	—	100	121	—
16	96	108	—	122	191	—
Musk melon						
7	144	267	—	+	+	—
14	181	318	—	+	+	—

\+ Died

The variations in the natures and quantities of the hydroxyindoles are listed in Tables 4, 4/1 and 4/2.

Table 4. Changes in amounts of hydroxyindoles on the action of 2.4-D. (Derivatives given in γ/g fresh weight).

Species	Treatment	Age in days	5–OH–indole in	
			Shoot	Root
	Control		2.15	1.25
Pearl bean	Low conc.	4	1.10	1.10
	High conc.		1.90	1.40
	Control		5.00	40.00
Pearl bean	Low conc.	8	40.00	43.00
	High conc.		3.00	4.00
	Control		3.50	3.00
Pearl bean	Low conc.	12	17.00	20.00
	High conc.		2.00	4.00
	Control		4.65	33.00
Pea	Low conc.	7	20.00	30.00
	High conc.		43.00	66.00
	Control		4.60	56.00
Pea	Low conc.	14	28.00	56.00
	High conc.		10.00	28.00

Table 4/1. Change in the amoung of 5-hydroxyindole on 2,4-D treatment (γ/g fresh weight).

Broad bean	Shoot			Root		
	Control	Low conc.	High conc.	Control	Low conc.	High conc.
T_0 generation	16	4	4.5	5	4.5	28
	Treatment of seeds*			Treatment of seeds*		
T_1 generation	4.5	12	11.5	12	12	17

* Part of the produce of the T_0 generation emerged unchanged in the T_1 generation; the indicated samples received a repeated 2,4-D treatment.

Table 4/2. Changes in the amounts of hydroxyindoles formed on the action of 2,4-D in cucumber, musk melon and water melon seedlings (γ/g fresh weight).

Plant species and age in days	Indole deriv.	Shoot			Root		
		Control	Low conc.	High conc.	Control	Low conc.	High conc.
Cucumber							
10	5-OH	110.00	71.00	95.00	75.00	35.00	49.00
16	5-OH	76.00	50.00	76.00	48.00	27.00	27.00
20	5-OH	51.00	44.00	67.00	41.00	21.00	24.00
Musk melon							
6	4-OH	—	—	—	135.00	120.00	—
	5-OH	—	—	—	5.00	4.30	—
12	4-OH	2.35	3.50	—	120.00	105.00	—
	5-OH	2.60	2.00	—	3.00	3.00	—
20	4-OH	2.30	3.00	—	4.90	27.00	—
	5-OH	2.60	2.50	—	1.50	2.00	—
Water melon							
15	4-OH	530.00	310.00	—	540.00	390.00	—
	5-OH	52.00	41.00	—	57.00	40.00	—
	6-OH	40.00	20.00	—	30.00	27.00	—
20	4-OH	530.00	300.00	—	530.00	355.00	—
	5-OH	65.00	20.00	—	60.00	21.00	—
	6-OH	41.00	17.00	—	24.00	4.70	—

References

GRANT, W. F. (1972): Pesticides — subtle promoters of evolution. — Symp. Biol. Hung. *12*, 43—50.

HORVÁTH, M.—LONTAI, I. (1970): Investigation of physiological changes in roots and shoots as a result of a herbicide treatment (II) Development of the pigment content of shoots. — Acta Biol. Szeged *16*, 95—98.

HORVÁTH, M.—GUBA, F.—HANUSZ, B.—KIRÁLY, L. (1975): Indol hidroxilálás különböző növényfajoknál (Indole hydroxylation in various plant species). —(in press). Acta Phytopathologica, Budapest.

KERTÉSZ, J.—MOLNÁR, A.—HORVÁTH, M. (1972): The effect of the sodium salt of 2,4-dichlorophenoxyacetic acid on the mitotic growth of yellow corn. — Acta Biol. Szeged *18*, 131—133.

KERTÉSZ, J. (1974): 2,4-diklórfenoxiecetsav Na sójának hatása kultúrnövények T_0, T_1 nemzedékének morfológiájára és anyagcseréjére (Effects of sodium 2,4-dichlorophenoxyacetic acid on the morphology and metabolism of T_0 and T_1 generations of domesticated plants). — Doctoral dissertation, Szeged.

KOVÁCS, R. ZSUZSANNA (1972): Morfológiai és anyagcsereélettani vizsgálatok kultúrnövényeken 2,4-diklórfenoxiecetsav hatására (Morphological and metabolic-physiological examinations of the effects of 2,4-dichlorophenoxyacetic acid on domesticated plants). — Doctoral dissertation, Szeged.

LOWRY, O. H. — ROSELRENGHT, N. J. — FARR, A. L. (11951): Colorimetric determination of TCA precipitable protein content with a combined Folin-Biuret reaction. — J. Biol. Chem *139*, 265—275.

ROJIK, I. — HORVÁTH, M. — LONTAI, I. (1973): A herbicide effect in the meiosis of Vicia faba. — Acta Bot. Acad. Sci. Hung. *18*, 163—169.

SMITH, J. A. C. — BENITES, A. (1963): The protochlorophyll — chlorophyll transformation. The nature of protochlorophyll in leaves. — Cornegie Inst. Year Book *52*, 149—153.

VEINBRANTS, N. (1972): Effect of succinic acid-2,2-dimethyl hydrazide (Alar) or scoring on growth and flower initiation of young apple trees. — Aust. J. of Exptl. Agricult. and Animal Husbandry *12*, 88—95.

VORT, D. J. (1964): Effect of herbicides on plant composition and metabolism. — London—New York.

WUU, K. D. — GRANT, W. R. (1966a): Morphological and somatic chromosomal aberrations induced by pesticides in barley *Hordeum vulgare* L.). — Can. J. Genet. Cytol. *8*, 481—501.

WUU, K. D. — GRANT, W. R. (1966b): Induced abnormal meiotic behavior in a barley plant *Hordeum vulgare* L.) with the herbicide Lorox. — Fyton. Vicente. Coper. *23*, 63—67.

Adbress of the authors:
Dr. MÁRIA M. HORVÁTH
J. KERTÉSZ
Genetics Group,
A. J. University,
H—6701 Szeged, P. O. Box 428, Hungary