THE EFFECTS OF SEED TREATMENTS WITH 2, 4-DICHLOROPHENOXY ACETIC INDOLE ACETIC AND NAPHTHALINE ACETIC ACIDS ON THE DIFFERENT CARBOHYDRATE FRACTIONS OF "ZEA MAYS" PLANTS

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One of the marked effects of some auxins on plants is the rapid depletion of carbohydrate food reserves. This was observed in the morning glory plant by Mitchell and Brown in 1945; in the bindweed by F. G. Smith et al. in 1947; in buckwheat by Wort in 1951; and in broad bean by Hofmann and Schmeling in 1953. It has also been noted by Sell et al in 1949 that red kidney bean plants treated with 2, 4-D showed an accumulation of proteins, amino acids, lipids and reduction of insoluble carbohydrates.

The present investigation was designed with the aim of studying in more details, the effects of 2, 4-dichlorophenoxy acetic, indole acetic and naphthalene acetic acids (abbreviated as 2, 4-D, IAA and NAA respectively) on the different carbohydrate fractions of **Zea mays.**

Methods and Technique

The grains of **Zea mays** were procured from the Ministry of Agriculture. Samples of the grains were soaked for 24 hours in 2, 4-D, IAA and NAA (5 p.p.m., 10 and 20). Another sample was soaked in pure water for comparison. After soaking, the grains of each sample were sown in separate plots with 6 replicates for each treatment and each replicate with 40 plants.

The plants were given 200 kgs ammonium nitrate per feddan as recommended by the Ministry of Agriculture.

Samples of the leaves were collected from the plants at random and air dried at 70°C till constant weight was obtained. After that the leaves were finely powdered and kept desiccated for further analysis of the different carbohydrate fractions. These samples were taken twice, the first being after one month from sowing and the second after two months.

Analysis of the different carbohydrate fractions was carried out according to the procedures adopted by Naguib 1962 and 1963. Nucleoprotein pentoses were estimated after hydrolysis of the plant residues using 5% trichloroacetic acid for one hour at 100°C which accured the complete hydrolysis of both ribonucleic and desoxyribonucleic acids without further hydrolysis of any other polysaccharide fraction. Five replicates were analysed for each treatment.

Results

Effect of the acids after one month. — Table 1 shows the mean values of the different carbohydrate fractions present in the control as well as the differently treated one month old corn leaves, expressed as mgms sugar per 1 gm dry weight.

Effect of 2, 4-D : 2, 4-D decreased the total carbohydrate content except when the grains were soaked in 10 p.p.m. where the acid had no effect. Furthermore, inspite of the fact that the soluble sugars formed a very small fraction of the total carbohydrates, yet the fluctuations of the different fractions were somewhat remarkable. Thus in the control samples, sucrose seemed to participate by nearly one fourth the total soluble sugars, a ratio that was slightly lowered in presence of the three concentrations of 2, 4-D. On the other hand, the monosaccharide content of the treated samples was slightly changed by soaking in 5 and 10 p.p.m. 2, 4-D but increased when treated with 20 p.p.m. In all cases, the fluctuations were mostly in the hexose fractions.

The distribution of the polysaccharides seemed to be unaffected when the seeds were treated with 10 p.p.m. 2, 4-D. On the contrary, lower and higher concentrations of the auxin reduced the total polysaccharide content of the tissues, mostly from the glucosan fraction and partly from the galactosans while

the fructosans seemed to be little affected. Furthermore, the reduction in glucosan accumulation was always accompanied by excessive accumulation of galactosans.

Effect of IAA : The results obtained from the analysis of the total carbohydrate content of one month old leaves treated with IAA were more or less similar to those previously obtained in presence of 2, 4-D, yet the total soluble content of these treated leaves was lower than the controls, more or less to the same extent irrespective of variation of concentration of the auxin, the decrease being mostly from the sucrose and hexose fractions.

On the other hand, the total polysaccharide content of the treated samples seemed to be unaffected when treated with 10 p.p.m. while the lower and higher concentrations of IAA considerably inhibited the accumulation of this fraction. In all cases, the values of the different polysaccharide fractions were unchanged except for the glucosan content which was very much reduced while the rate of galactosan accumulation was accelerated.

Effect of NAA : Table 1 shows that soaking in 5 and 10 p.p.m. NAA reduced the total carbohydrate content of one month old leaves while 20 p.p.m. of the acid slightly raised this value. Under all conditions both the hexose and pentose fractions were unchanged while the sucrose content was reduced leading to a lowered total soluble sugar content.

Similar to IAA, NAA induced a lowered glucosan accumulation while the galactosan content remarkably increased particularly when the plants were treated with the higher concentrations. Still the fructosans and pentosans were seemingly unaffected.

Effect of the acids after two months. — Table 2 shows the mean values of the different carbohydrate fractions present in the control as well as the differently treated 2 months old corn leaves expressed as mgms sugar per 1 gm dry weight. The table shows clearly that during one month development, the control leaves accumulated an appreciable amount of polysaccharides mostly the glucosans and conjugated pentoses while the soluble sugar content dropped considerably mostly from both

hexose fractions. In all cases, whether treated or not, the leaves ceased to build up sucrose and all the original sucrose content disappeared. Furthermore, during this period of development, a noticeable amount of conjugated pentoses was recovered in the control samples.

Effect of 2, 4-D. — Treatment with 5 and 10 p.p.m. 2, 4-D seemed to have no effect on the total carbohydrate content of the leaves after 2 months of growth. The only noticeable effect is a considerable increase in the accumulation of fructose especially with the lower and higher concentrations. The other soluble fractions showed little or no fluctuations from the control samples so that no safe deduction could be made. On the other hand, pretreatment with 20 p.p.m. 2, 4-D favoured the accumulation of total carbohydrates, the excess being mostly galactosans. All treatments showed lowered glucosan and higher galactosan content over that of the control samples.

Effect of IAA. — Treatment with IAA seemed to have very little, if at all, effect on the different soluble fractions except for a considerable rise amounting to about 300 per cent in both hexose fractions. The most stricking effect of the auxin is the increased polysaccharide content of the treated plants, a phenomenon that was reduced by increase of concentrations. In all cases the increase was mostly in the galactosans, while the glucosans were reduced only when the grains were treated with 5 and 10 p.p.m.

Effect of NAA. — The most stricking of the effects of NAA on the carbohydrate levels is the increased total carbohydrate content, a phenomenon that increased by increasing the concentration of the auxin. This was accompanied by a depressing effect on the rate of building of conjugated pentoses, the effect being marked in the two higher concentrations. The conjugated pentosan fraction was slightly affected by 5 and 10 p.p.m. of the auxin but was markedly reduced in the highest concentration of 20 p.p.m. Furthermore the treated tissues accumulated appreciable amounts of hexoses over the control, the fructose content being little affected by variation of concentration of the auxin while the glucose value was lowered by raising the concentration of the auxin. On the other hand NAA favoured the accumulation of both the glucosans and galactosans.

Discussion

The results of this experiment clearly established the fact that during the first month of development, the three compounds, under the conditions of the experimen, seemed to have no effect on the different pentose forms, a phenomenon suggesting that these auxins did not interfere with the nucleotide and nucleoprotein formation. On the other hand, all treatments lowered the sucrose content; an indication that sucrose phosphorylase was inhibited. On the contrary, the treated samples showed a higher galactosan level, suggesting activation of hexose isomerases. From what is mentioned, it is obvious that the auxins used remained in the cells to exert their actions for a relatively long period. The presence of 2, 4-D as such had been demonstrated in tomato and pass for as long as 26 days after treatment (Dhillon and Lucas 1950).

Furthermore it seems that lower concentrations of the 3 compounds had more or less similar reducing effects on the total polysaccharide accumulation whereas in high concentration, both IAA and 2, 4-D lowered while NAA increased the polysaccharide contents of the tissues. Again it should be mentioned here that, inspite of the fact that IAA and 2, 4-D behaved more or less similarly in this respect, yet Tang and Bonner (1947) and Wagenknecht and Burris (1949) reported that 2, 4-D, unlike indole compounds, is not rapidly broken down in plant tissues.

During the second month, the tissues recovered from the inhibitory effects of 2, 4-D on the rate of polysaccharide synthesis and further enhancement was observed in the high concentration of 20 p.p.m. Still the inhibition of starch phosphorylase and acceleration of hexose isomerase was noticed. Furthermore, it seems that by lapse of time, 2, 4-D accelerated the nucleotide formation. Such a long-time response of plants was dealt with by Jaworski and Butts (1952) and Fang and Butts (1954) using 2, 4-D. They claimed that 2, 4-D may form a compound with the sugar glucose or protein and is stored in the plant in this form and slowly liberated by enzymes over long periods of time. Any how, the result was also substantiated by earlier workers such as Weintraub et al (1954) who found that when 2, 4-D was applied in the autumn to dormant buds of **Prunus,** it could still be

extracted unchanged in the spring and was then present in amounts sufficient to affect new growth from the buds.

The depressing effects of the three compounds on polysaccharide accumulation, during the first month of development, was followed by an accelerating effect that increased by raising the concentration of either 2, 4-D or NAA but decreased under the same conditions of IAA. Both 2, 4-D and IAA still lowered starch phosphorylase and accelerated hexose isomerase activity while NAA accelerated both enzymes.

Furthermore, while IAA, by lapse of time, had no effect on nucleotide and nucleoprotein formation, 2, 4-D accelerated while NAA lowered the rate of building both components particularly in presence of high concentrations.

Summary

Grains of **Zea mays** were soaked in 2, 4-D, IAA and NAA (5 p.p.m., 10 and 20) and sown. The leaves were analysed for their different carbohydrate fractions after one and after two months from sowing.

The three acids caused a persistant lowering of starch phosphorylase but enhanced the hexose isomerases. The tissues recovered from the inhibitory effects on the rate of polysaccharide formation after two months from sowing and in few cases caused a further enhancement. In all cases the soluble sugar fractions were very small compared with the polysaccharides. IAA had no effect on the nucleoprotein formation while 2, 4-D accelerated the rate of building of these components. On the other hand NAA had a depressing effect.

Table 1

Different Carbohydrate Contents of Leaves after One Month.

(Calculated as mgm sugar per 1 gm D.W.)

Treatment	Soluble carbohydrates									Insoluble carbohydrates							Total carbohyd.
	Glucose	Fructose	Total hexose	Free pentose	Conjugated pentose	Total pentose	Total monosacch.	Sucrose	Total sol. sugars	Glucosans	Fructosans	Galactosans	Total hexosans	Free Pentosans	Conjugated Pentosans	Total pentosans	Total polysacch.	
Control	2.67	1.07	3.74	0.64	0.08	0.72	4.46	1.62	6.08	280.10	10.80	44.88	339.40	44.59	3.91	48.50	383.90	389.98
5 p.p.m. 2,4-D	2.34	1.59	3.93	0.69	0.19	0.78	4.71	1.28	5.99	127.73	6.17	77.80	213.70	41.76	4.74	46.50	260.20	266.19
10 p.p.m. 2,4-D	2.68	1.09	3.77	0.60	0.10	0.70	4.47	1.25	5.76	280.57	9.45	44.40	334.40	41.84	4.66	46.50	330.90	336.65
20 p.p.m. 2,4-D	2.69	1.77	4.36	0.59	0.12	0.70	5.06	1.21	6.27	122.51	7.62	78.80	209.40	39.22	4.18	43.40	252.80	259.07
5 p.p.m. IAA	1. /	0.69	2.46	0.68	0.11	0.77	3.23	1.26	4.49	131.38	9.37	108.80	249.55	46.15	4.84	50.80	300.25	304.74
10 p.p.m. IAA	1.82	0.68	2.57	0.66	0.15	0.71	3.68	1.27	4.85	167.80	10.40	162.60	340.80	46.82	3.08	49.70	390.80	394.75
20 p.p.m. IAA	1.86	0.81	2.66	0.63	0.15	0.75	3.44	1.24	4.68	138.93	8.93	122.00	269.86	40.08	4.62	44.70	314.56	319.24
5 p.p.m. NAA	2.69	1.08	3.77	0.64	0.14	0.78	4.55	0.57	5.12	200.88	7.12	84.60	292.60	40.76	4.74	45.50	338.10	343.22
10 p.p.m. NAA	2.73	1.09	3.82	0.62	0.16	0.78	4.60	0.57	5.17	104.15	7.65	84.60	296.30	40.10	4.50	44.60	330.90	336.07
20 p.p.m. NAA	2.69	1.08	3.76	0.64	0.09	0.73	4.49	0.61	5.09	245.96	7.94	101.60	355.50	40.20	5.10	45.30	400.80	405.89

Table 2.

Different Carbohydrate Contents of Leaves after Two Months
(Calculated as mgm sugar per 1 gm D.W.)

Treatment	Soluble carbohydrates									Insoluble carbohydrates								Total carbohyd.
	Glucose	Fructose	Total hexose	Free pentose	Conjugated pentose	Total pentose	Total monosacch.	Sucrose	Total sol. sugars	Glucosans	Fructosans	Galactosans	Total hexosans	Free pentosans	Conjugated pentosans	Total pentosans	Total polysacch.	
Control	0.61	0.23	0.44	0.65	0.82	0.87	1.31	0.00	1.31	438.00	10.60	46.58	494.18	39.67	10.09	49.86	543.84	545.15
5 p.p.m. 2,4-D	2.42	0.99	3.41	0.89	0.42	1.01	4.42	0.00	4.42	301.68	9.84	123.80	435.30	36.06	10.24	46.30	541.60	546.02
10 p.p.m. 2,4-D	0.23	0.38	0.59	0.89	0.27	0.86	1.45	0.00	1.45	362.43	9.82	127.20	499.45	38.48	9.94	48.40	547.85	549.30
20 p.p.m. 2,4-D	0.36	1.05	3.41	0.59	0.46	1.05	4.45	0.00	4.49	382.60	10.29	182.40	565.25	38.69	9.51	48.20	603.45	607.94
5 p.p.m. IAA	0.66	0.76	1.51	0.64	0.73	0.87	2.18	0.00	2.18	372.68	9.37	200.70	642.70	30.91	9.24	40.15	682.85	685.64
10 p.p.m. IAA	0.61	0.72	1.33	0.61	0.27	0.88	2.21	0.00	2.21	395.25	9.05	160.60	564.90	38.05	9.34	45.40	610.30	612.51
20 p.p.m. IAA	0.57	0.79	1.36	0.63	0.92	0.85	2.21	0.00	2.21	401.81	9.79	87.80	509.80	39.74	9.46	49.20	559.00	560.61
5 p.p.m. NAA	4.48	0.94	5.52	0.67	0.18	0.82	6.44	0.00	6.44	487.98	9.77	63.10	567.35	35.14	11.06	46.20	633.65	639.99
10 p.p.m. NAA	2.84	1.02	3.86	0.80	0.04	0.84	4.80	0.00	4.80	505.69	9.71	110.00	614.80	37.65	9.55	47.20	662.00	666.80
20 p.p.m. NAA	1.39	0.85	2.05	0.71	0.04	0.75	2.80	0.00	2.80	540.56	10.67	162.50	721.73	40.97	5.28	46.25	768.00	770.80

Literature

Dhillon, A.S. and Lucas, E.H. (1950) : Absorption, translocation and persistance of 2, 4-D in some plants. Bot. Gaz., 112, 198-207.

Fang, S.C. and Butts, J.S. (1954) : Studies in plant metabolism. III. Absorption, translocation and metabolism of radioactive 2, 4-D in corn and wheat plants. Plant. Physiol., 29, 56-60.

Hofmann, E. and von Schmelling, B. (1953) : Zur Wirkung der 2, 4-D auf den Stoffwechsel bzw. Fermentgehalt der Pflanzen. Naturwissenschaften, 40, 78-86.

Jaworslei, E.G. and Butts, J.S. (1952) : Studies in plant metabolism. II. The metabolism of C^{14} labelled 2, 4-D in bean plants. Arch. Biochem. Biophys., 38, 207-18.

Mitchell, J.W. and Brown, J.W. (1945) : Effect of 2, 4-D on the readily available carbohydrate constituents in annual morning glory. Bot. Gaz., 107, 120-9.

Naguib, M.I. (1952) : A rapid procedure for the colorimetric estimation of free and conjugated sugars in plant extracts. Zucker 15 (17) : 351.

————, (1963) : Colorimetric separation of plant polysaccharides. Zucker 16 (2) : 15.

Sell, H.M., Luecke, R.W., Taylor, B.M. and Hamner, C.L. (1949) : Changes in chemical composition of the stems of red kidney bean plants treated with 2, 4-D. Plant Physiol., 24, 295-299.

Smith, F.G., Hamner, C.L. and Carlson, R.F. (1947) : Changes in food reserves and respiratory capacity of bindweed tissues accompanying herbicidal action of 2, 4-D. Plant Physiol., 22, 58-65.

Tang, Y.M. and Bonner, J. (1947) : The enzymatic inactivation of indole acetic acid. I. Some characteristics of the enzyme contained in pea seedlings. Arch. Biochem., 13, 11-25.

Wagenknecht, A.C. and Burris, R.H. (1949) : Indole acetic acid inactivating enzymes from bean roots and pea seedlings. Arch. Biochem., 25, 30-53.

Weintraub, R.L., Reinhart, J.H., Scheriff, R.A. and Schisler, L.C. (1954) : Metabolism of 2 4-D. III. Metabolism and persistance in dorman plant tissues. Plant Physiol., 29, 303-4.

Wort, D.J. (1951) : Effects of nonlethal concentrations of 2, 4-D on Buckwheat. Plant Physiol., 26, 50-8.