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THE EFFECT OF AUXIN AND SUCROSE ON GROWTH AND FORM OF PEA STEM SECTIONS

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INTRODUCTION

The results, reported in literature, of experiments on the effect of indoleacetic acid, in the presence of sucrose, on the growth of excised sections of pea epicotyls are rather inconsistent. GALSTON and HAND (1949) and GALSTON and BAKER (1951) report that sucrose enhances the growth of etiolated epicotyl sections in the presence of indoleacetic acid. CHRISTIANSEN and THIMANN (1950) found no effect of sucrose on growth of etiolated sections. With pea stem sections in light, BRIAN and HEMMING (1958) obtained increased growth by the addition of 2 % sucrose to near-optimal concentrations of indoleacetic acid. In the experiments of GALSTON and BAKER (1951) sucrose retarded the growth of sections in light.

These inconsistencies have been ascribed to different causes. AUDUS (1952) has pointed out that, under different conditions, the time course of growth may be different. If only total growth is determined the result obtained may depend upon the duration of the experiment. DE ROPP and MARKLEY (1950) found that sections from the apical and from the basal end of sunflower hypocotyls reacted in a different way to indoleacetic acid; PURVES and HILLMAN (1958) reported that sections of pea epicotyls of different length reacted differently.

In several investigations it has been found that, in the presence of sucrose, the optimal concentrations of indoleacetic acid for growth in length and for increase in fresh weight are different (e.g. GALSTON, BAKER and KING, 1953; DE ROPP and MARKLEY, 1953). This may give rise to confusion, for increase in weight has been used as a measure for growth in length (AUDUS, 1952) as well as for lateral growth (PURVES and GALSTON, 1960).

It seems difficult to understand why the optimal concentrations of IAA for longitudinal and for lateral growth are different. PURVES and GALSTON (1960) have suggested that these types of growth were not independently affected by auxin, but that high lateral growth will inhibit growth in length. In work in our laboratory, with the dwarf pea variety "Rondo", a rather uncommon curve of the effect of IAA concentration in the presence of sugar on longitudinal growth was obtained. We believe that this curve illustrates the dependency of longitudinal growth on lateral extension in a better way than most curves, that have been published.

Material and Methods

Peas of the dwarf variety Rondo were soaked in tap water for 3–4 hours. The seeds were sown in pans containing vermiculite. The pans were placed in a dark room at 28° C. The light that was necessary when handling the plants came from a 80 W incandescent bulb screened by a green glass. After 6–7 days the third internode grew out.

For the experiments plants were chosen the third internode of which had a length of 2–4 cm. By means of a cutter consisting of two parallel razor blades a cylinder of 5.1 mm. was cut from the third internode at a distance of 1 mm. from the tip. The cylinders were floated on the surface of the solutions in 50 ml. glass beakers. The solutions always contained .02 M. phosphate buffer of a pH of 5.9–6.0. The solutions were stirred by a gentle air stream. After 24 hours the sections were placed in holes which had been drilled in a perspex block. The block was placed under water in a cuvette in front of an incandescent lamp. By means of a lens system the shadow of the sections was projected on the wall. The enlargement was exactly ten times. The length and the largest diameter of the sections were measured with a ruler. Unless stated otherwise the figures in the tables are the average of the measurements of 10 sections. For microscopical inspection the sections were fixed in Rawlin's solution: alcohol 50 % 100 ml., formalin 40 % 6.5 ml., glacial acetic acid 2.5 ml.

Results

1. *Effect of auxin and 2 % sucrose*

The effect on growth of different concentrations of indoleacetic acid in buffer solution and of the same concentrations in the presence of 2 % socrose is shown in Fig. 1. The data from which the curves have been drawn are given in table 1 and in tables 2–4. The numbers in figure 1 give the water absorption of the sections. This was determined by weighing of the sections before and after the experiment. In the experiments with 2 % sucrose water absorption has been determined in 11 experiments, viz. in expts nrs 34–45.

Fig. 1 shows that water absorption is not always a reliable measure for lateral growth. For instance, the diameter of sections grown in 2 % sucrose without auxin and of sections grown in 2 % sucrose in the presence of 10^2 µg./ml. of indoleacetic acid is the same (2.3 mm.) while the water absorption is different (58 % and 114 %).

Lateral growth is only slightly affected by indoleacetic acid when sucrose is absent. Under these conditions growth in length is enhanced by concentrations of auxin between 10^{-2}µg./ml. and 10 µg./ml. A concentration of 10^2 µg./ml. is supra-optimal. A similar flat curve for the effect of indoleacetic acid on longitudinal growth of Avena sections has been published by Schneider (1938).

Lateral growth is enhanced by indoleacetic acid in the presence of 2 % sucrose. The effect is maximal at concentrations of 1 µg./ml. and 10 µg./ml. At these concentrations the sections become barrel-shaped owing to the fact that lateral growth is higher in the middle than at

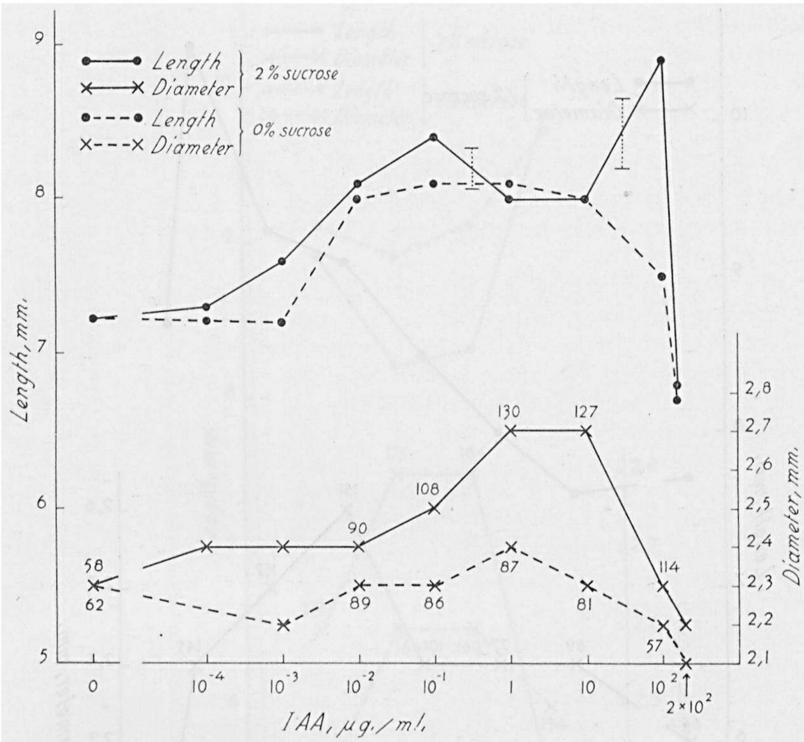

Fig. 1. Effect of indoleacetic acid on growth in the absence of sucrose and in the presence of 2 % sucrose. The two dotted vertical lines indicate the least significant difference between longitudinal growth in the presence of 2 % sucrose at 10^{-1} µg/ml and 1 µg/ml and at 10 µg/ml and 10^2 µg/ml respectively. (P = 0,01). The numbers indicate the increase in fresh weight in percents of the original weight.

both ends. The same phenomenon has been reported by PURVES and GALSTON (1960).

The curve which represents the effect of auxin concentration on longitudinal growth shows two peaks separated by a depression. This depression is situated at concentrations of indoleacetic acid that are optimal for lateral growth. The difference between the maximum at 10^{-1}µg./ml. and the decreased growth at 1 µg./ml. of I.A.A. is 0.35 ± 0.1 mm. The difference between the growth at 10 µg./ml. and the second growth maximum at 10^2 µg./ml. is 0.83 ± 0.18 mm. These differences are highly significant.

2. *Effect of auxin and 1 % sucrose*

In the presence of 1 % sucrose the effect of indoleacetic acid was not the same in different experiments. Probably the state of the plants, e.g. the sugar content of the cells, affected the results.

Fig. 2 shows the result of one experiment. The diameter of the sections was maximal at 1 µg./ml. of IAA, as in the experiments with

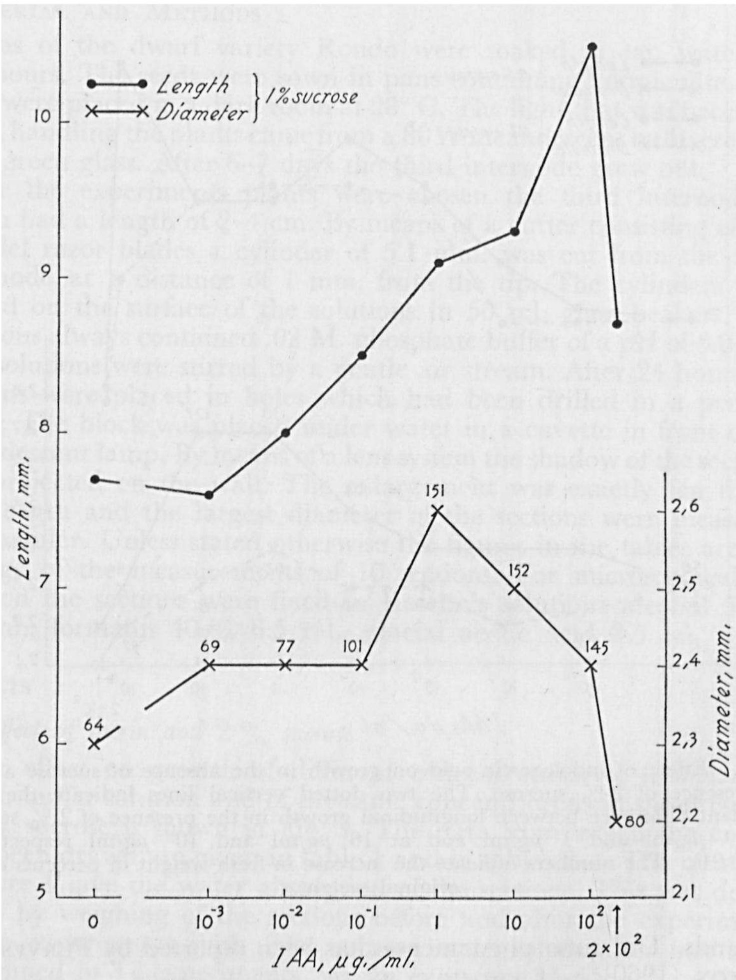

Fig. 2. Effect of indoleacetic acid on growth in the presence of 1 % sucrose. The numbers indicate the increase in fresh weight in percents of the original weight.

2 % sucrose, but the maximum is .1 mm lower. From 1 μg./ml. towards higher concentrations the curve representing the diameter falls off. In the curve of longitudinal growth a depression is practically absent. The curve shows an optimum at 10^2 μg./ml.

In a second experiment the effect of 1 % and 2 % of sucrose was compared at concentrations of indoleacetic acid that cause the depression in the curve of longitudinal growth. Fig. 3 shows the results. In this experiment growth in length was decreased at 1 μg./ml. and at 10 μg./ml. of IAA in the presence of both, 1 % and 2 % sucrose. The diameter of the sections was maximal at these concentrations. In 2 % sucrose the depression in the curve of longitudinal growth is deeper and the maximum of the curve representing the diameter of the sections is higher than in 1 % sucrose.

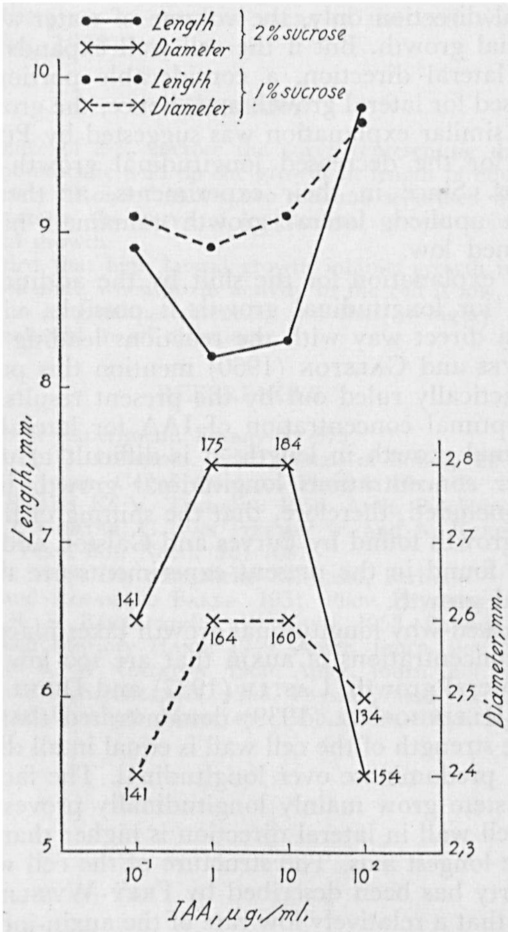

Fig. 3. Effect on growth of 1 % and of 2 % of sucrose in the presence of 4 con centrations of indoleacetic acid. The numbers indicate the increase in fresh weight in percents of the original weight.

Discussion

The present results differ in mainly one respect from those obtained by previous authors, viz. the effect of auxin concentration on growth in length in the presence of 2 % sucrose. Usually, the graph representing this relationship shows one optimum. In the present experiments a curve with two maxima, separated by a depression, was obtained. The optimum for lateral growth in the presence of 2 % sucrose lies at the same concentrations of auxin as the depression in the curve representing longitudinal growth. The following explanation is suggested for this coincidence.

It is fairly generally agreed upon that auxin induces a weakening of the cell wall. This causes the cell to absorb water osmotically and the osmotic pressure of the cell to decrease till it attains a new equilibrium with the tension of the cell wall. If the cell wall expands in a longitudinal direction only, the volume of water absorbed is used for longitudinal growth. But if the cell wall expands in both, longitudinal and lateral direction, a considerable portion of the water taken up is used for lateral growth and, hence, the growth in length is decreased. A similar explanation was suggested by PURVES and GALSTON (1960) for the decreased longitudinal growth at high auxin concentrations. Since in their experiments, at the highest auxin concentration applied, lateral growth remained high, growth in length remained low.

A different explanation for the shift by the addition of sucrose of the optimum for longitudinal growth is possible, viz. that sucrose interferes in a direct way with the reactions leading to longitudinal growth. PURVES and GALSTON (1960) mention this possibility. However, it is practically ruled out by the present results. For if in 2 % sucrose the optimal concentration of IAA for lateral growth is too high for optimal growth in length, it is difficult to understand why at still higher concentrations longitudinal growth increases again. It must be concluded, therefore, that the shifting of the optimum for longitudinal growth found by Purves and Galston and the depression in that curve found in the present experiments are the result of increased lateral growth.

It may be asked why longitudinal growth takes place at a considerable rate in concentrations of auxin that are too low or too high to cause much lateral growth. CASTLE (1937) and DIEHL, GORTER, VAN ITERSON and KLEINHOONTE (1939) demonstrated that if, in a cylindrical cell, the strength of the cell wall is equal in all directions lateral extension will predominate over longitudinal. The fact that the cells of the young stem grow mainly longitudinally proves that the resistance of the cell wall in lateral direction is higher than in a direction parallel to the longest axis. The structure of the cell wall responsible for this property has been described by FREY–WYSSLING (1959).

We suggest that a relatively low rate of the auxin-induced reactions will be suffcient to decrease the low resistance of the cell wall to longitudinal extension, and that a high auxin activity is required to decrease the resistance of the wall to lateral extension. This hypothesis explains why lateral growth takes place at a narrow range of near-optimal concentrations only. At these concentrations the lateral growth will inhibit longitudinal extension by the mechanism described above. At concentrations of indoleacetic acid that are farther away from the optimum auxin activity in the cell will be too low to induce weakening of the cell wall in lateral direction but it may be still high enough for longitudinal extension. For that reason growth in length will be highest at concentrations that are just too high or just too low for lateral growth to take place.

The hypothesis that the rate of lateral growth is high only when auxin activity is high implies that not only concentration, but also any other agent that increases auxin activity may induce increased lateral growth. This might be a simpler explanation for the effect of benzimidazole on lateral extension than the hypothesis of a selective effect of this substance on certain areas of the cell wall (GALSTON, BAKER and KING, 1953).

SUMMARY

1. In the presence of 2 % sucrose the curve representing the effect of the concentration of indoleacetic acid on the growth in length of etiolated pea stem sections of the variety "Rondo" shows two maxima separated by a depression.

2. The depression in the curve of longitudinal growth coincides with the optimum for lateral growth.

3. It is concluded that high lateral growth inhibits growth in length.

4. It is suggested that, when auxin activity in the cell is low, the cell wall is weakened mainly in longitudinal direction and that at higher auxin activities the cell wall is weakened in all directions.

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TABLE 1

Length, diameter and water absorption of sections in phosphate buffer and indoleacetic acid after 24 hours. Initial length: 5.1 mm

Conc. of IAA in μg/ml	0	10^{-3}	10^{-2}	10^{-1}	1	10	10^2	2×10^2
Average length in mm	7,2	7,2	8,0	8,1	8,1	8,0	7,5	6,7
Average diameter in mm	2,3	2,2	2,3	2,3	2,4	2,3	2,2	2,1
Waterabsorption in % of original weight .	62		89	86	87	81	57	

TABLE 2

Length of sections in mm in the presence of 2 % sucrose, indoleacetic acid and phosphate buffer after 24 hours. Initial length: 5.1 mm. The figures represent the average length of the number of sections indicated in the second column.

Conc. of IAA in g/ml										
Expt. number	Number of sections	0	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1	10	10^2	2×10^2
8	10	7,2	7,0	7,5	7,8	8,2	8,0	7,8	8,5	6,7
10	10	6,7		7,8	8,4	7,4	8,1	8,2	6,6	6,6
11	10	7,3	8,7	9,4	9,2	9,6	8,5	8,6	10,2	7,9
12	10	6,8	7,0	7,6	7,1	8,1	8,4	8,4	7,6	6,1
14	10	7,3	7,0	7,3	7,9	8,3	8,1	8,0	8,6	6,1
15	9	6,7	6,7	6,8	7 3	7,4	7,9	8,5	8,7	6,2
16	10	7,6	7,4	8,2	8,7	9,5	8,2	8,0	9,2	7,5
17	7	7,0		7,1	8,8	9,0	7,9	8,3	8,8	6,0
18	10					8,4	8,0	8,4	9,3	
18	10					8,5	8,3	8,4	9,3	
19	7				7,5	8,3	7,9	8,1	8,7	6,3
20	10	7,8	7,3	7,7	7,9	7,8	7,8	7,7	8,6	6,3
22	10	6,7		6,5	8,0	7,9	7,4	7,9	8,1	6,8
25	9	7,6		7,9	8,3	8,9	8,8	8,2	9,9	7,3
32	10	8,3		7,8	8,5	8,8	8,0	8,1	9,7	8,2
33	10					8,1	8,2	7,1	9,3	
34	9					9,3	8,3	8,1	9,2	
35	7					7,9	7,4	7,6	8,4	
36	5					7,9	7,7	7,6	9,0	
37	10					7,9	7,6	7,9	9,3	
38	10					7,8	7,8	7,8	8,5	
42	10					8,9	8,2	8,3	9,8	
44	7	7,1			8,1	8,4	8,1			
45	8	7,4			8,5	9,0	8,3			
Total:		101,5	51,1	91,6	122,0	201,3	192,9	177,0	195,3	88,0
Average of all the experiments		7,2	7,3	7,6	8,1	8,4	8,0	8,0	8,9	6,8

TABLE 3

Largest diameter of the sections of table 1 in the presence of 2 % sucrose, indoleacetic acid and phosphate buffer after 24 hours. The initial diameter has not been determined

Conc. of IAA in gr/ml		0	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1	10	10^2	2×10^2
Expt. number	Number of sections									
10	10	2,2		2,3	2,5	2,6	2,6	2,6	2,2	2,3
11	10	2,4	2,6	2,7	2,5	2,7	2,8	2,9	2,4	2,2
12	10	2,5	2,4	2,3	2,4	2,5	2,6	2,6	2,3	2,2
14	10	2,5	2,5	2,4	2,5	2,5	2,8	2,6	2,4	2,2
15	9	2,3	2,4	2,4	2,4	2,5	2,7	2,7	2,2	2,1
16	10	2,3	2,5	2,5	2,5	2,5	2,8	2,8	2,4	2,3
17	7	2,2		2,2	2,2	2,4	2,6	2,8	2,4	2,2
18	10					2,6	2,8	2,7	2,5	
18	10					2,3	2,4	2,6	2,1	
19	7				2,4	2,6	2,9	2,9	2,4	2,3
20	10	2,2	2,2	2,2	2,1	2,4	2,4	2,4	2,2	2,0
22	10	2,2		2,2	2,1	2,2	2,4	2,6	2,1	2,0
25	9	2,6		2,6	2,6	2,8	2,8	3,0	2,6	2,3
32	10	2,3		2,3	2,4	2,6	2,7	3,0	2,4	2,3
33	10					2,3	2,5	2,3	2,2	
34	9					2,4	2,5	2,3	2,2	
35	7					2,4	2,6	2,7	2,4	
36	5					2,5	2,8	2,5	2,4	
37	10					2,7	2,9	2,8	2,5	
38	10					2,6	2,8	2,8	2,5	
42	10					2,6	2,8	2,8	2,4	
44	7	2,4			2,6	2,8	3,0			
45	8	2,3			2,5	2,5	2,8			
Total:		30,4	14,6	26,1	33,7	58,0	62,0	56,4	49,2	26,4
Average of all the experiments		2,3	2,4	2,4	2,4	2,5	2,7	2,7	2,3	2,2

TABLE 4

Length and largest diameter of the sections of tables 2 and 3 in the presence of 2 % sucrose, indoleacetic acid and phosphate buffer after 24 hours

Conc. of IAA in g/ml	0	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1	10	10^2	2×10^2
Average length in mm (from table 1)	7,2	7,3	7,6	8,1	8,4	8,0	8,0	8,9	6,8
Average diam. in mm (from table 2)	2,3	2,4	2,4	2,4	2,5	2,7	2,7	2,3	2,2
Waterabsorption % of original weight	58			90	108	130	127	114	