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SUCROSE UPTAKE BY PEA STEM SEGMENTS AND ITS EFFECT ON GROWTH

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ABSTRACT

Longitudinal growth of etiolated pea stem segments has been shown to be strongly stimulated by indoleacetic acid. Lateral growth, on the other hand, was hardly stimulated either by high or low indoleacetic acid concentrations.

Addition of sucrose to high indoleacetic acid concentrations (10 μg/cc) enhanced lateral growth.

Although the 80% ethanol soluble sugar content increased greatly during the growth period, it was only slightly affected by indoleacetic acid.

Lateral growth is not, therefore, a result of an increased osmotic value of the cell sap, and the cells consequently do not swell.

Not more than 5% of the water absorbed by stem segments during longitudinal and lateral growth is released when the segments are incubated in solutions of high osmotic value.

It is concluded that lateral growth of etiolated pea stem segments in solutions of 10 μg/cc IAA and in the presence of sucrose requires, in the first instance, a weakening of the cell wall in a lateral direction and sucrose is somehow needed for this process.

INTRODUCTION

Sections from the growth zone of etiolated pea stems, when placed in solutions containing auxin, sugar and buffer, may increase both in diameter and length. The optimal concentrations of auxin for lateral growth and for elongation are not identical. The experiments of PURVES and GALSTON (1960) showed that in the presence of 2% sucrose, the increase in length of the sections was highest at 10^{-7} mol of indoleacetic acid, and the greatest increase in fresh weight was at 10^{-5} M of IAA.

BULT and VAN RAALTE (1961) found two maxima for the growth in length of sections in the presence of 2% sucrose, one at 10^{-1} μg/ml (5.7×10^{-7} M) IAA and a second at 10^{2} μg/ml (5.7×10^{-4} M). In between these maxima, at concentrations of around one μg/ml, the elongation was less, but the lateral growth of the sections showed a rather sharp maximum.

The explanations for the lateral growth maximum which have been suggested by Purves and Galston and by Bult and Van Raalte, are practically identical, *viz.* a weakening of the cell wall in a lateral direction is required.

A different explanation has been suggested by MILLER (1954). He says, "Sugar consistently causes an increase of cell volume but its

effect on cell elongation is variable. One interpretation of this is, that the effect of sugar on elongation depends upon the ability of the cell wall to increase its surface. If other factors are restricting the increase of cell wall surface, the sugar by increasing the cell volume, causes a rounding out of the cells of the segments and thereby causes an inhibition of elongation. If these other factors are less limiting, an increase of cell volume brings about an increase of cell length as well."

These two explanations differ in that the first suggests a weakening of the cell wall, whereas the second assumes a resistance of the wall towards extension.

The present experiments were carried out in order to try to determine which of these two interpretations is the more correct.

Miller's suggestion evidently requires an increase of the cell volume by increased osmotic pressure of the contents. Such an increase may be brought about either by uptake of sucrose from the surrounding solution or by anatonosis. Stimulation by indoleacetic acid of the uptake of amino-acids by tissue of *Helianthus* has been described by REINHOLD and POWELL (1958) and of potassium and rubidium ions by ILAN and REINHOLD (1963, 1964).

MATERIAL AND METHODS

Peas (*Pisum sativum*) of the variety "marktveroveraar" were grown in pans containing vermiculite. The pans were placed in a dark room at 25° C. After 7 days the third internode had reached a length of 1–3 cm, and from each plant a segment of 5.1 mm was cut at a distance of 1 mm from the tip. The segments were floated on 10–25 ml air-stirred 20 mM phosphate buffer solutions (pH 6.0). After 24 hours the segments were rinsed in de-ionised water and blotted with filter paper. The increase in fresh weight, length and width was then measured. Samples consisting of 10 segments were frozen in liquid nitrogen, ground in a mortar and extracted with 80% ethanol on a boiling water bath. All extracts were concentrated to a small volume, then diluted with ethanol to known volumes. The 80% ethanol soluble sugars were determined with anthrone reagent after a modification of FALES (1951).

In some of the experiments C^{14} labelled sucrose was used for measuring sucrose uptake by the pea stem segments. Aliquots of the extracts were plated on aluminum planchets and counted in a thin-window gas-flow geiger counter. The radioactivity is expressed in terms of the original amount of sucrose.

For measuring the length and width of the stem segments, an enlarged image of the segments was projected on to a positive film.

EXPERIMENTAL

Sets of 10 stem segments were floated for different periods of time on solutions containing 2% C^{14} labelled sucrose (U) and 10 μg/cc IAA. After the incubation period the segments were rinsed with de-ionised water and subsequently analysed for radioactivity in the 80% ethanol

soluble extract. Fig. 1 shows the result of two experiments, one ranging from 0 to 24 hrs. and the other from 18 hrs. to 66 hrs. It is clear that within 24 hrs. the rate of C^{14} fixation rises almost linearly with time. After about 24 hrs., when growth (fresh weight increase) has stopped, the fixation still goes on but the rate falls off.

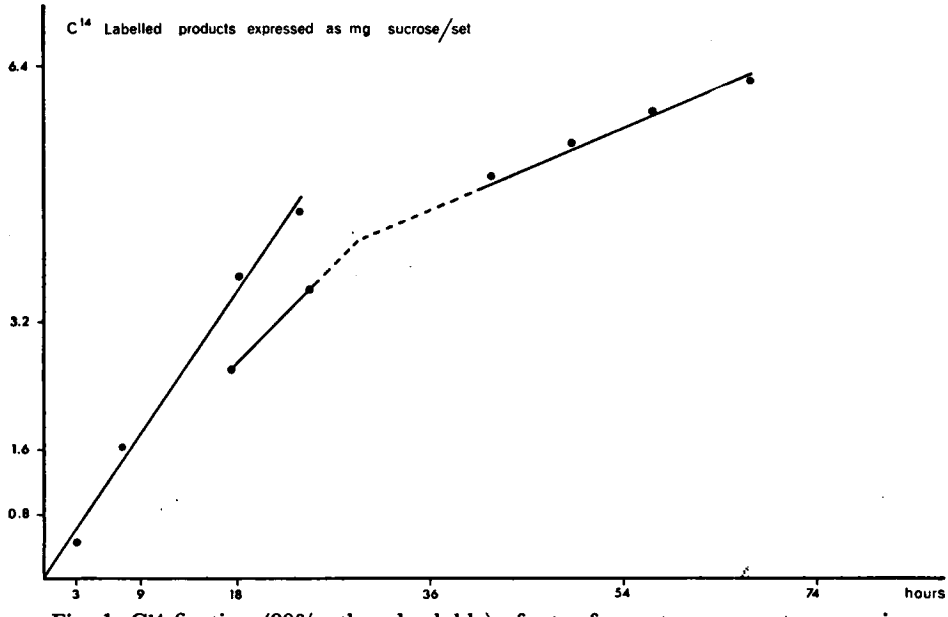

Fig. 1. C^{14} fixation (80% ethanol soluble) of sets of pea stem segments grown in solutions containing 2% C^{14} labelled sucrose and 10 μg/cc IAA.

In the next experiment sets of 10 stem segments were floated for 24 hrs. either on a 2% sucrose solution or on a sucrose solution to which IAA was added (10 μg/cc).

Table 1 shows an increase in fresh weight of about 40% in sets floated on a sucrose solution, whereas the increase rises to 150% when IAA is added to the sucrose solution. The increase in 80% ethanol soluble C^{14} labelled compounds, however, differs only slightly whether IAA is added or not. The increase in these compounds, which is brought about by IAA, is not more than 5%.

TABLE 1

The effect of IAA on the absorption of sucrose by stem segments of *Pisum*

Medium solution	Initial fr. wt.	Increase fr. wt.	C^{14} labelled products* in 80% ethanol extract
Sucrose 2%	125 mg	187 mg	3.28 mg
IAA (10 μg/cc) Sucrose 2%	125 mg	50 mg	3.15 mg

*The C^{14} labelled products are expressed as the amount of sucrose absorbed.

A third type of experiment was conducted in order to get an idea as to whether or not the increase in fresh weight in 24 hrs. is due to irreversible growth.

Ten sets of 10 stem segments with an initial weight of about 115 mg were floated on IAA solutions (10μg/cc). To half of the solutions sucrose was added up to a concentration of 2%. After the growth period of 24 hrs., the increase in fresh weight was measured.

Fig. 2 shows the relative size of the stem segments before and after growth in the two media. When incubated in a solution of 2% sucrose and 10 μg/cc IAA lateral growth is considerable.

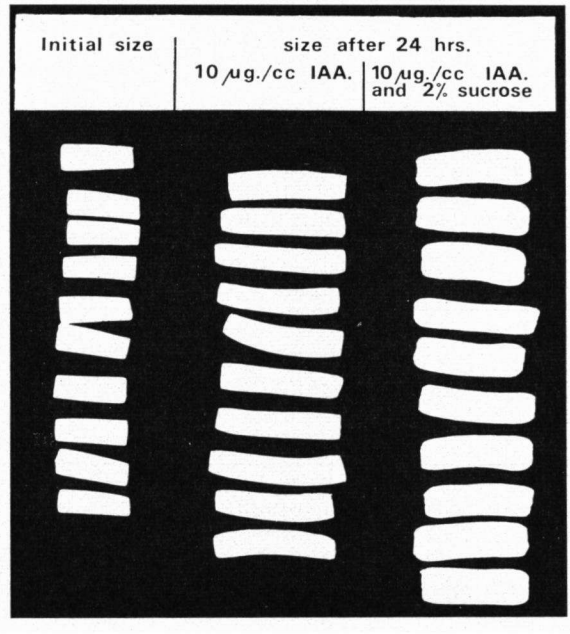

Fig. 2. The relative size of the etiolated pea stem segments before and after an incubation period of 24 hrs. in two different media.

The sets were then transferred to water or to sucrose solutions of different molarity. The temperature of the solutions was held at 4°C.

Table 2 shows the change in fresh weight of sets previously grown for 24 hrs. in a 10 μg/cc IAA solution.

It is clear that in a concentration of up to 150 mM sucrose no water is released by the stem segments. On the contrary a small amount of water is absorbed. At still higher concentrations water is released but the greatest release of 14 mg water is only 12% of the increase in fresh weight obtained during the 24 hrs. growth period.

TABLE 2

The change in fresh weight of stem segments, previously grown for 24 hrs. in an IAA solution (10 μg/cc), when incubated in sucrose solutions of different molarity at a temperature of 4°C

Sucrose conc. (mM)	Fr. wt. after 24 hrs. (mg)	Change in fr. wt. (mg)
0	227	+ 12
50	234	+ 7
150	233	+ 3
200	225	— 3
400	230	— 14

Table 3 shows the same for sets previously grown in 10 μg/cc IAA solutions to which sucrose had been added.

TABLE 3

The change in fresh weight of stem segments previously grown for 24 hrs. in a solution containing IAA (10 μg/cc) and 2% sucrose. The stem segments were incubated in sucrose solutions of different molarity at a temperature of 4°C

Sucrose conc. (mM)	Fr. wt. after 24 hrs. (mg)	Change in fr. wt. (mg)
0	321	+ 12
50	314	+ 7
150	314	+ 1
200	326	— 5
400	346	— 16

Although the increase in fresh weight of the stem segments is about doubled when sucrose had been added to the IAA solutions, only the same amount of water is released to a 400 mM sucrose solution. The suction force of the cells of both types of segments is about equal, since they are in equilibrium with the same sucrose solution of the outer solution.

The experiments conducted indicated that:

1°. sucrose is absorbed by the stem segments and at a constant rate.

2°. IAA only slightly enhances the amount of C^{14} labelled compounds recovered in the 80% ethanol soluble fraction.

3°. the increase in fresh weight of sets either grown in IAA solutions or in sucrose containing IAA solutions is irreversible.

DISCUSSION

As already stated in the introduction, the lateral growth of stem segments when incubated in a solution containing 10 μg/cc IAA and 2% sucrose may be brought about by an increased osmotic value of the cell sap. A stimulated sucrose absorption by IAA may be responsible

for the increase of the osmotic value of the cell sap. Fig. 1. showed that the C^{14} content of the 80% ethanol soluble extract of the stem segments increases linearly with time during an experimental period of 24 hrs. This C^{14} fixation may be regarded as a mesaure of sucrose absorption by the stem segments.

Part of the absorbed sucrose may be transferred into other 80% ethanol soluble compounds for instance amino-acids and organic acids, part of it may be transferred to 80% ethanol insoluble compounds and finally, part of it may be lost in respiration. However, the main part of the 80% ethanol soluble C^{14} labelled compounds are sugars, for the increase in 80% ethanol soluble sugars as determined by the Anthrone method is in fairly close agreement with the amount calculated from the radioactivity measurements.

The 80% ethanol soluble C^{14} labelled compounds contribute to the osmotic value of the cell sap and are therefore important for determining the change in the osmotic value due to sucrose absorption of the stem segments.

If the increased lateral growth in IAA + sugar is due to an osmotic phenomenon, sucrose absorption should be stimulated by IAA. Table 1, however, shows that sucrose absorption is hardly effected in the presence of IAA. This means that sucrose contributes equally to the osmotic value of the cells whether or not they do grow in a lateral direction.

It may be concluded that lateral growth in the presence of sucrose is not a rounding out of the cells caused by an increased osmotic value of the cell sap.

A further argument against this interpretation is based on the fact that the growth of the stem segments both in a predominantly longitudinal direction (Table 2, Fig. 2) and in a combined longitudinal and lateral direction (Table 3, Fig. 2), is irreversible.

Lateral growth as a result of a rounding out of the cells caused by an increased osmotic value of the cell sap, with no change in the resistance of the cell wall towards extension, would be reversible in high osmotic values of the surrounding medium.

The general conclusion is that lateral growth primarily requires a weakening of the cell wall in a lateral direction and that sucrose is needed for this process.

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