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Studies on the Osmotic Change Caused by Auxin Treatment

By

Shizuko YODA

Botanical Institute, College of Science, University of Kyoto

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The author reported previously that the osmotic concentration of pea stem pieces was increased by auxin when they were smeared with its lanolin paste and kept at constant humidity (19, 21). This kind of treatment was adopted in order to exclude possibilities such as of leaching of solutes from cells when the tissue was soaked in solution for treatment. It was found later, however, that the effect of auxin increasing the osmotic value was conspicuous even with the solution treatment if the measurement was made within a limited period of treatment (23).

More detailed description of the latter experiment, together with some additional results, is presented here.

Material and Methods

Material. The third internode, 32 ± 1 mm in length, of seedlings of Alaska pea grown in the dark for 7 days at 25.5°C was selected, as in the preceding experiments (19, 21). Afer rejecting the apical 3 mm, a 5 mm piece was excised from the apical part of the remaining internode. Twenty-five pieces each were weighed and floated on 10 and 200 mg/l solutions of indole-3-acetic acid (IAA) and on water at 25.5°C. After suitable periods they were blotted with filter paper and packed in weighing tubes to prevent condensation and loss of moisture during freezing (at about −21°C) and thawing to follow. Tissue juice was expressed with a hand press from stem pieces killed by the freezing.

Representation of osmotic nature of tissue juice. Freezing point of tissue juice was measured with an accuracy of 0.025°C by means of a combination of copper-constantan thermojunction and potentiometer, as described previously (19). The molar concentration (w/v) of mannitol solution having the same freezing point as tissue juice was determined by referring to a standard curve prepared experimentally.

In order to know if the amount of osmotically active solutes has actually changed, the changes in osmotic value due to changes in water content should be eliminated. For this purpose, the solute change factor, α, was determined

according to the equation, $\alpha=\frac{C_1}{C_0}\beta$. C_0 and C_1 are the mannitol equivalent of tissue juice before and after the treatment, respectively. β, the water absorption factor, is the ratio of the water content of tissue after treatment to that before treatment, the water content being given by substracting dry weight from fresh weight.

Tensile force experiment. A stem piece was set in an apparatus (20, 22). Longitudinal tension of 2.5 g was applied on the piece as it was soaked in a solution of IAA or in distilled water, and the increase in distance between two marks on the piece was measured to the nearest 0.01 mm using a cathetometer.

Measurement of electric conductivity of tissue juice. A small conductivity cell, as shown in Fig. 1, was prepared by modifying CRAY's one (9). Conduc-

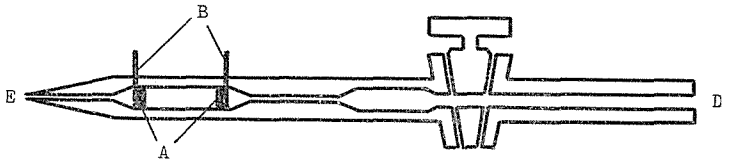

Fig. 1. The cell for electric conductivity measurement. A: Platinum plates, B: platinum leads, and C: cock. Tissue juice is sucked in through E to fill the cavity in which the platinum plates (A) are fixed, and the stop cock (C) is closed.

tivity could be measured with about 0.15 ml of tissue juice. The cell constant was 18.86 cm^{-1} as determined with 0.1 M potassium chloride solution.

Analytical methods. Sodium and potassium content of tissue juice was determined by means of flame photometry. Polyethylene vessels were used throughout.

For inorganic phosphorus: 0.1 ml of tissue juice was mixed with 1 ml of 4% cold perchloric acid[1], centrifuged and the deproteinized supernatant was diluted. Two ml of this was mixed with 0.5 ml of 70% perchloric acid, 2 ml of 5% aqueous ammonium molibdate and 5 ml butylacetate (18)[2]. The supernatant was cleared by centrifugation, and the absorption at 310 mμ was determined with a spectrophotometer.

For total phosphorus: BARLETT's method (1) was modified to use 70% perchloric acid instead of 10 N sulfuric acid. By this modification both blank value and digestion time were reduced. In this and the above methods, 0.5 μg–10 μg of phosphorus could be determined with errors not more than 1%.

For amides and ammonia: 0.3 ml of tissue juice was mixed with 2 ml of 2 N hydrochloric acid, heated for 2 hours at 100°C and diluted to 5 ml with

1) Perchloric acid is preferable as it does not absorb light at 310 mμ.

2) For purification, commercial butylacetate was washed with an equal volume of 5% sodium carbonate solution, dried over anhydrous calcium chloride, and distilled.

water. Ammonia of the diluted hydrolysate (1 ml) was analysed using CONWAY's diffusion method (5). For free ammonia in the tissue juice, the same method was used without the procedure for hydrolysis.

For reducing and total sugars: 0.1 ml of tissue juice was deproteinized by mixing 5.9 ml of water, 2 ml of 0.3 N barium hydroxide solution and 2 ml of zinc sulfate solution (5 g of $ZnSO_4 \cdot 7H_2O$ in 100 ml of solution), and the solution was cleared by centrifugation. Reducing sugar was determined by applying SOMOGYI's method (15) to the deproteinized solution. For total sugar, 2 ml of the deproteinized solution was hydrolysed by adding 0.5 ml of 5 N hydrochloric acid and heating at 100°C for 2 hours, and SOMOGYI's method was applied after neutralization with 2.5 N sodium hydroxide and dilution.

Results

1. *Effect of IAA solution on osmotic value and water absorption.*

Stem pieces were floated on 10 and 200 mg/l solutions, optimal and supra-optimal concentrations, respectively, of indoleacetic acid (IAA). Samples being withdrawn at various periods, fresh weight and freezing point were determined. The results are represented in Fig. 2. The osmotic concentration of tissue juice increased very rapidly following exposure to the IAA solutions. It, however, began to decrease at about 30 minutes. The rise and fall of osmotic concentration were more conspicuous with 200 mg/l than with 10 mg/l. And no increase in osmotic concentration was observed in water control.

The rate of water absorption did not differ significantly between the two auxin levels in the first 30 minutes, in spite of that the osmotic values differed very much between the two. Presumably the water permeability may have been limiting the water absorption.

The osmotic concentration may drop due to (I) dilution by water absorption, (II) leaking of solutes from cells to outer solution and (III) catatonosis. The first factor can be eliminated by calculating the solute change factor, α. Effect of auxin on α is shown in Fig. 3. The rise of α in the first 30 minutes in the presence of IAA may be owing to anatonosis, and the fall of α may be owing to either or both of solute leakage and catatonosis. The rapid drop in the presence of 200 mg/l IAA may probably be ascribed to an unusual rise in permeability (6, 7, 11, 12 and 13).

It may be questioned whether the observed effect of IAA on the osmotic condition of the tissue is nothing but an effect owing to an acidic nature of IAA. So stem pieces were treated with phenylacetic acid and acetic acid in the molar concentration equivalent to 200 mg/l of IAA. Freezing point and water content after 30 and 60 minutes' treatments were measured. The results as represented in Table 1 show that these acids had no significant effect on osmotic concentration and water absorption. Consequently, the rapid osmotic effect of IAA described above may be considered as an effect of auxin, which promotes elongation of shoot cells.

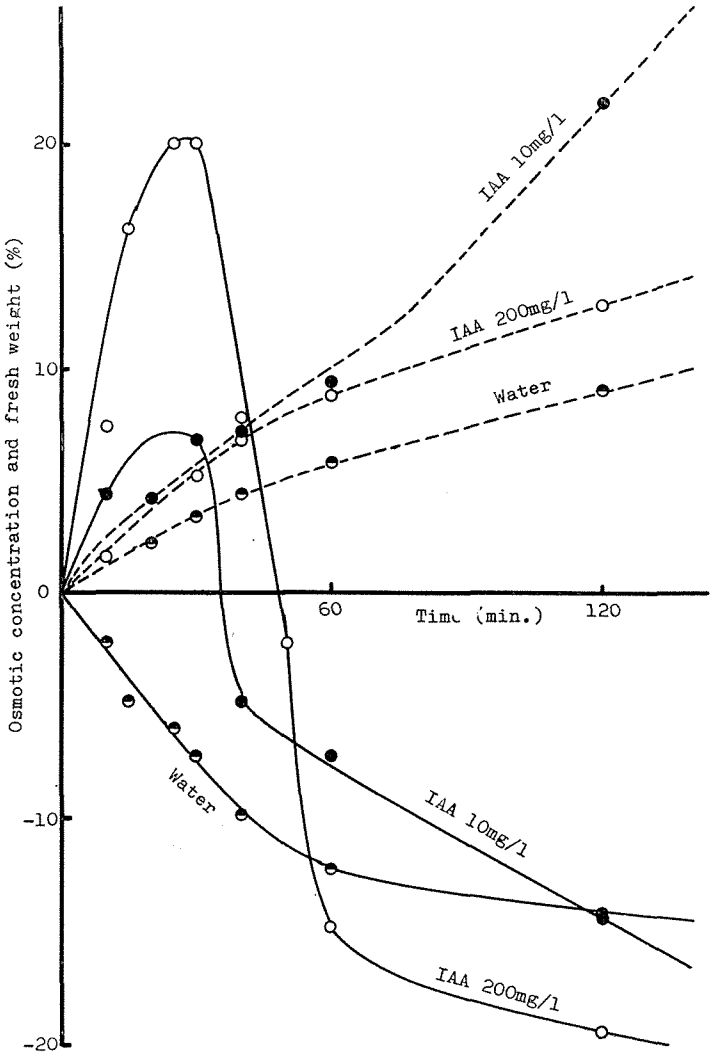

Fig. 2. Effect of IAA on the osmotic value (——) and water absorption (----) of pea stem sections floated on IAA solutions and water. The values after the various periods of treatment are expressed in percentage of the values at 0 time.

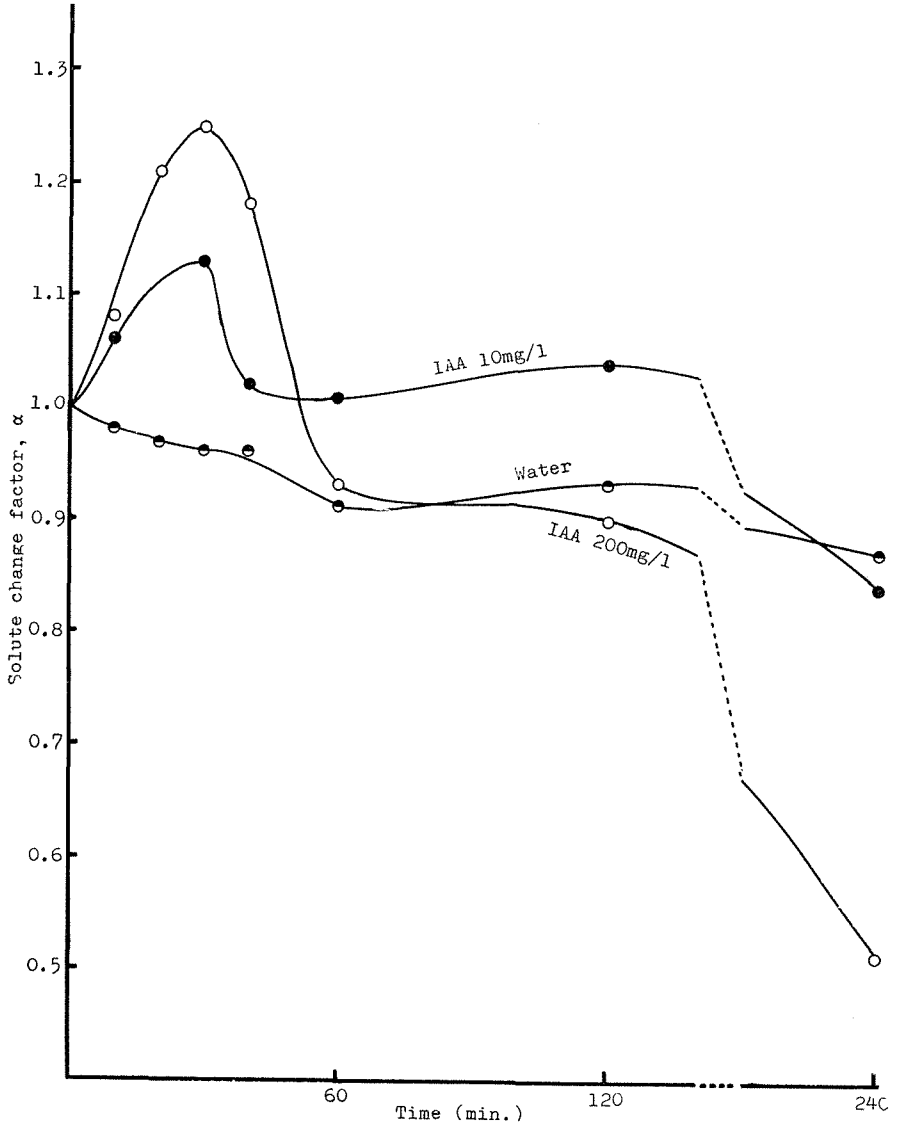

Fig. 3. Change of the solute change factor, α, with the time of exposure to IAA solutions and water. Calculation from Fig. 2.

Table 1. Effect of phenylacetic acid and acetic acid on water absorption and freezing point depression of pea stem sections.

	Freezing point depression (°C)	Water absorption (%)
After treatment for 30 minutes with:		
Water	0.48	4.6
Phenylacetic acid (1.15 mM)	0.48	4.4
Acetic acid (1.15 mM)	0.48	4.7
After treatment for 1 hour with:		
Water	0.46	5.4
Phenylacetic acid (1.15 mM)	0.46	4.6
Acetic acid (1.15 mM)	0.46	4.9

2. *Time relation between the auxin effects on cell wall and osmotic value.*

In order to see whether the cell wall extensibility is affected by auxin so soon as the osmotic value, longitudinal tension by 2.5 g weight was applied to stem pieces which had been soaked for various periods in 200 mg/l solution of IAA. By this method, an increase in extensibility became observable after 15 minutes' soaking, but not yet in 10 minutes. So the osmotic change became significant earlier than an observable change in the cell wall extensibility.

3. *Substances contributing to the osmotic change caused by IAA.*

Some dissolved substance(s) must be increased in tissue juice when its α value is raised. To find such substance(s) may give a clue to the action mechanism of auxin. From this point of view, several substances were analysed for their quantitative changes in the tissue juice when pea stem sections were treated with IAA.

Electric conductivity and ions. In order to see if the osmotic change caused by IAA is due to a change in the total concentration of ions, electric conductivity of tissue juice was measured.

A number of stem pieces were smeared at their both ends with 0.5% lanolin paste of IAA, and others with pure lanolin as control. They were incubated in the air of 100% humidity at 25.5° ±0.01°C, using the apparatus as described elsewhere (19). No leaching of solutes and no significant change in water content were expected, as reported before (19). After 3.5 hours of incubation, juice was obtained from the part of tissue not contaminated with lanolin. Specific conductivity was 0.00290 $cm^{-1}ohm^{-1}$ in the juice from the IAA-treated tissue and 0.00294 $cm^{-1}ohm^{-1}$ in that from the control tissue. So, no significant difference was found between the two.

In the next place, stem pieces were treated with 200 mg/l IAA solution by

floating on it. Juice was expressed from them after 30 and 120 minutes of treatment. Sampled at 30 minutes, the juice of the IAA-treated tissue showed an electric conductivity rather lower than that of water-treated one (Table 2),

Table 2. Effect of IAA solution on specific electric conductivity and pH of expressed juice of etiolated pea stem sections.

	Specific electric conductivity ($ohm^{-1}\,cm^{-1}$)	pH
After treatment for 30 minutes with:		
Water	0.00305	6.16
IAA (200 mg/l)	0.00285	6.18
After treatment for 2 hours with:		
Water	0.00269	——
IAA (200 mg/l)	0.00203	——

in spite of that the osmotic concentration of the former was much higher than the latter as shown in Fig. 2. Hence the rise in osmotic concentration in this case is not ascribable to an increase in the total ionic concentration. As to the pH of tissue juice measured by means of a BECKMANN's glass electrode pH meter, no significant difference was found between the IAA-treated tissue and control.

By floating for 2 hours, the conductivity of tissue juice became lower than that in the case of 30 minutes' floating, and the lowering was more conspicuous in IAA than in water.

In order to see whether ions actually leaked from cells, groups of 25 stem sections were floated on 10 ml each of 200 mg/l IAA solution and distilled water, and sodium and potassium contents of the tissue juice and the treating media were measured after 30 and 120 minutes of floating. The results as represented in Table 3 show that sodium and potassium leaked from the tissues, and that the leakage was larger in the IAA solution than in water. Hence, the drop of α in tissue juice as seen in Fig. 3 seems to be due, at least partly, to leakage of ions, especially of potassium. The potassium ion seems to contribute much to the electric conductivity and its lowering, as shown in Table 2.

Phosphorus. The tissue juice and the media floating the tissue were analysed for total and inorganic phosphorus. The difference between the two was regarded as organic phosphorus. In Table 4 are presented the values obtained.

At 30 minutes, the total phosphorus was less in the expressed juice of the IAA-treated tissue than in the control. This difference reflects that, in organic phosphorus, inorganic phosphorus is practically the same between the two. It may be supposed that, in the presence of IAA, degradation of organic phosphorus

Table 3. Effect of IAA on sodium and potassium contents of tissue juice and of media floating the tissue. Values for each medium represent the amounts of sodium and potassium which have come out to 10 ml of the medium from 25 stem pieces and the leakage per original fresh weight of the tissue.

Sodium

	In tissue juice (μ equiv./ml)	In medium	
		(μ equiv.)	(μ equiv. from 1 g tissue)
After floating for 30 minutes on:			
Water	1.9	0.014	0.60
IAA (200 mg/l)	1.6	0.030	1.26
After floating for 2 hours on:			
Water	1.6	0.017	0.72
IAA (200 mg/l)	1.4	0.030	1.26

Potassium

After floating for 30 minutes on:			
Water	19.3	0.017	1.14
IAA (200 mg/l)	18.2	0.043	3.10
After floating for 2 hours on:			
Water	17.2	0.030	2.08
IAA (200 mg/l)	12.6	0.127	9.16

is accelerated and inorganic phosphorus leaks out to the medium. This is supported by the analytic results of the media.

The above mentioned tendency became more remarkable in the 2-hour treatment. Organic and inorganic phosphorus in tissue juice did not decrease much in water control, but decreased considerably in the presence of IAA, the main type of phosphorus found in the medium being inorganic.

Amides. It is reported that a large quantity of amides is contained in etiolated seedlings (2). Hence, effect of IAA on the amides content was observed.

Juice was expressed from the stem pieces floated on 200 mg/l IAA solution and distilled water for 30 and 120 minutes, and amides and free ammonia contained in the juice were determined. As shown in Table 5, 30 minutes' treatment with IAA increased the amides content about 16% over water control. And amides decreased conspicuously in 2 hours of IAA treatment, while the amount did not change in water. It seems, therefore, that the rise and fall of α occur-

Table 4. Effect of IAA on phosphorus contents of tissue juice and media floating the tissue. Phosphorus found in the treating media (10 ml) came out from 25 pieces (about 0.5 g fresh weight) of excised stem. Phosphorus concentration of juice of IAA-treated tissue would be about 2% higher if the water absorption were the same as water control.

Tissue juice

	Total phosphorus (μg)	Inorganic phosphorus (μg)	Organic phosphorus (μg)
After treatment for 30 minutes with:			
Water	250	180	72
IAA (200 mg/l)	230	180	56
After treatment for 2 hours with:			
Water	240	170	68
IAA (200 mg/l)	190	140	47

Treating medium

After treatment for 30 minutes with:			
Water	4.0	2.1	1.9
IAA (200 mg/l)	6.8	5.6	1.2
After treatment for 2 hours with:			
Water	5.6	2.8	2.8
IAA (200 mg/l)	21	18	3.4

Table 5. Effect of IAA solution on amide and free ammonia contents of tissue juice.

	Amide (M)	Ammonia (M)
After treatment for 30 minutes with:		
Water	0.0507	0.0063
IAA (200 mg/l)	0.0587	0.0063
After treatment for 2 hours with:		
Water	0.0507	0.0063
IAA (200 mg/l)	0.0413	0.0066

ring in the IAA solution is partly related to changes in the amount of amides.

Free ammonia increased in the juice of 2-hour IAA treatment. Degradation of amides seems to be stimulated by IAA.

Reducing sugar and total sugar. Results of sugar analysis are represented in Table 6. In 30 minutes of treatment, reducing sugar was increased by IAA. If it is assumed that the difference between the total sugar and the reducing sugar represents the amount of disaccharoses and the reducing sugar represents monosaccharoses, the decrease in disaccharoses corresponds, though very roughly, to the increase (over control) in monosaccharoses. The fall in sugars content after 2 hours' treatment may be due to leakage.

Table 6. Effect of IAA solution on reducing sugar and total sugar contents of tissue juice.

	Total sugar (mg/ml)	Reducing sugar (mg/ml)	(M)*
After treatment for 30 minutes with:			
Water	16.7	14.9	0.0830
IAA (200 mg/l)	17.5	17.0	0.0945
After treatment for 2 hours with:			
Water	13.7	13.0	0.0722
IAA (200 mg/l)	13.2	10.6	0.0572

* Calculated as hexose.

Discussion

Potato tuber tissue has often been used in the study of osmotic effect of auxin (4, 8, 10, 14 and 17). This tissue may be more convenient than small materials in that, among other respects, much tissue juice is easily obtainable. Potato tuber, however, is far less sensitive to auxin than *Avena* coleoptile and pea seedling. Effect of auxin has often been determined after treating the material for a long period, even a number of days. However, since auxin must act even in a short period, the author tried to determine a short term effect by using pea seedlings as the material and measuring the freezing point depression of expressed tissue juice in a small quantity. The experimental result reported in this paper, that the osmotic value is raised by treatment with IAA solution, appears to be inconsistent with many others (3, 8, 10, 14, 16 and 17).

When tissues which have been in air are put in an aqueous solution, much water absorption and leaching of cellular substances would occur. So the author formerly treated the stem piece with lanolin paste and found that the osmotic concentration of expressed tissue juice was raised by IAA. However, as the time of IAA action can not be exactly known in the lanolin paste treatment,

the solution treatment was used in the present study. And osmotic concentration was found to be raised by IAA solution if measurement was made before much solutes leached out. The effect of IAA on the osmotic value became observable rather earlier than that on the extensibility of cell wall.

In order to determine what kinds of substances are responsible for the IAA-induced increase of osmotic value, analysis were conducted at the most conspicuous phase, namely at 30 minutes of treatment with 200 mg/l, a supraoptimal concentration, of IAA. The osmotic value of juice of the treated tissue was about 40 mM (or even 60 mM according to conditions) of mannitol equivalent higher than that of control. And increases of amides and reducing sugars were estimated to account for only a half or less of the total osmotic rise, even if the increases of these classes of substances were assumed to contribute in full to raising the osmotic value. Potassium and sodium ions, and also total ions, are not increased when the osmotic value was at its maximum. Hence some other substances should also be considered to account for the whole osmotic increase caused by IAA.

The fall of osmotic value after 30 minutes' IAA treatment seems to be largely due to leaking of various solutes from the tissue. The leakage of K, Na and phosphate into the treating medium was demonstrated quantitatively. It was measurable even in 30 minutes. Yet, α, the net amount of osmotically active solutes in the tissue juice, namely the osmotic concentration corrected for the dilution by absorbed water, was maintained for a certain period above the original value when IAA concentration was optimal (10 mg/l). And even with supraoptimal IAA concentration which made much solutes leak out, α was maintained at about the same level until 2 hours of treatment.

It may be concluded that the water absorption, which underlies the increase of cell volume, is made more active by auxin through an increase in the amount of solutes acting osmotically in the cell, besides the increase in cell wall extensibility reported elsewhere (20, 22) and treated also in this paper briefly.

Summary

1. Excised internodes of Alaska pea seedlings were floated for various periods on 10 and 200 mg/l solutions of indole-3-acetic acid (IAA) and distilled water, and the freezing point depression of expressed juice of the tissue was determined by cryoscopy. Following exposure to IAA the osmotic concentration increased rapidly, then to decrease after 30 minutes or so. No increase in osmotic concentration was observed in the water control, even if the correction was made for the dilution due to water absorption.

2. Extensibility of the stem piece was measured by applying a force of 2.5 g at various periods after soaking the piece in 200 mg/l solution of IAA. An increase in the extensibility became detectable after 15 minutes of IAA action, but not in 10 minutes, when an increase in osmotic value was already observable.

3. No increase in osmotic value was observed when stem pieces were treated with phenylacetic acid and acetic acid solutions equimolar to 200 mg/l IAA. Hence IAA is not acting merely as an acid when it increases the osmotic concentration.

4. Expressed tissue juice was analysed when its osmotic concentration became maximal due to the effect of IAA. K, Na and phosphorus derivatives do not contribute to the osmotic rise; electric conductance shows that the total ions concentration does not either. Increases in amides and reducing sugars were demonstrated, but quantitatively they can account for only a part of the osmotic rise.

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