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Turgor-dependent Changes in *Avena* Coleoptile Cell Wall Composition

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WAYNE H. LOESCHER AND DONALD J. NEVINS
Department of Botany and Plant Pathology, Iowa State University, Ames, Iowa 50010

ABSTRACT

The effects of reduced turgor pressure on growth, as measured by cell elongation, and on auxin-mediated changes in cell walls, as measured by analyses of wall composition, were examined using *Avena* coleoptile segments. Although moderate (1–4 bar) decreases in turgor resulted in a progressive decline in growth proportional to the decrease in turgor, the major auxin-induced change in wall composition, a decrease in noncellulosic wall glucose, was unaffected. Severe (5–8 bar) decreases, however, did inhibit this auxin effect on the wall, and with turgor decreases of 9 bars or more this auxin effect was no longer apparent. The results show that turgor pressure is required for this auxin-mediated wall modification and also that this modification of wall glucose occurs at turgor pressures less than those required for wall extension. Changes in other wall components were generally unaffected by altering turgor pressure.

Growth of *Avena* coleoptile sections is regulated by wall extensibility and turgor pressure. These two requirements are not independent; there is evidence that a complex interrelationship may exist. Exposing growing tissues to solutions at various osmotic pressures shows not only that elongation rates drop rapidly as turgor values decrease, but also that growth rates are apparently zero at substantial turgor pressures (5, 6, 19). The results suggest that there is a critical turgor pressure or a yield threshold of the wall that must be exceeded in order for growth to occur. Other reports show that increases in wall extensibility do not occur at low turgor pressures (2, 20). These results likewise suggest that a critical turgor pressure must be exceeded in order for the wall modifications to occur.

The specific nature of the auxin-induced wall modifications involved in the growth process is unknown. Auxin-induced changes in the composition of *Avena* coleoptile cell wall preparations have been reported (9, 12, 16, 17, 21). The results reported in this paper demonstrate that an auxin-induced change in *Avena* coleoptile cell wall composition is dependent upon turgor pressure.

METHODS AND MATERIALS

Preparation of Tissue and Analyses of Wall Composition. Oats (*Avena sativa* var. Victory) were grown and prepared as before (12). Coleoptile sections were floated on distilled water 30 to 60 min, then transferred to 43- × 20-mm Stender dishes containing 5 ml of 2.5 mM potassium citrate buffer, pH 5.4, plus various concentrations of mannitol, and, in some experiments, 50 mM glucose. Sections were equilibrated in the osmoticum 1 hr, then an additional 5 ml of medium with or without 25 μM IAA were added. All equilibrations and incubations were conducted using a Dubnoff shaker, 60 oscillations per min at 26 C in the dark. Osmotic pressures were measured plasmolytically by estimating the point of incipient plasmolysis in intact sections after a 2-hr incubation in various concentrations of mannitol.

Coleoptile sections were removed after incubation, measured using a 10× dissecting microscope fitted with an ocular micrometer, and frozen at −24 C. Wall samples from 20 to 30 coleoptile sections were prepared as described earlier (12). All wall samples were hydrolyzed in 2 N trifluoroacetic acid at 121 C for 1 hr. This procedure liberates nearly all the arabinose, xylose, and galactose in *Avena* coleoptile wall polysaccharides (12). Since the same procedure liberates only small amounts of glucose from relatively pure sources of cellulose, *i.e.*, 4.6 mg of anhydroglucose per 100 mg of Munktell's cellulose powder and 6.5 mg/100 mg of Whatman No. 3 filter paper, the glucose released from *Avena* coleoptile wall preparations is predominantly noncellulosic in nature. The possibility that the wall preparations contain a small amount of starch relative to the glucose originating from wall polysaccharides has been discussed (12).

Determination of Respiration Rates. Standard manometric techniques were used to measure respiration rates. Fifteen to 20 coleoptile sections were placed in Warburg flasks containing 2.9 ml of 2.5 mM potassium citrate buffer, pH 5.4, with or without 200 mM or 400 mM mannitol. The center well contained 0.2 ml of 20% KOH, and the sidearm contained 0.1 ml of buffer with the appropriate concentration of mannitol with or without 0.375 mM IAA. The temperature was maintained at 26 C. After a 1-hr equilibration, readings were taken at intervals for the next 2 hr. The contents of the sidearm were then added, the sections were equilibrated for 1 hr, and readings were again taken. Respiration determinations were also made 18 hr after equilibration. Each experiment was repeated two or three times with two or three replicates per treatment.

In one experiment coleoptile sections were preincubated with buffer with or without 400 mM mannitol. After 2 hr the medium was removed and the sections were washed several times with buffer. After buffer without mannitol was added and the sections were equilibrated for 1 hr, the sidearm contents with or without IAA were added, and readings were taken 1 hr later.

RESULTS

IAA treatment of *Avena* coleoptile sections promotes a striking decrease in that glucose which is liberated from cell walls by 2 N trifluoroacetic acid. We consider this glucose to be primarily of noncellulosic origin. This decrease, however, is evident only when precursors for wall synthesis are not provided

in the incubation medium (12). Similar responses to IAA treatment were observed here in coleoptile sections incubated at two reduced turgor levels in the presence and absence of exogenous glucose. When 50 mM glucose was provided in the incubation medium, a time course study of compositional changes in coleoptile cell walls showed no reduction in noncellulosic wall glucose in response to IAA treatment when incubated in 200 mM or 400 mM mannitol (Fig. 1). In a similar experiment where exogenous glucose was not provided in the incubation medium, IAA effects on wall glucose were dependent on mannitol concentration (Fig. 2). Although decreases in wall glucose content were observed in all treatments, in sections treated with 200 mM mannitol and IAA, response was apparent at the end of 4 hr, and at the end of 8 hr glucose content of walls of IAA-treated sections had decreased 52%, while the control had decreased only 16%. At 20 hr glucose content in IAA-treated sections had decreased 73% while the control had decreased 54%. In contrast, IAA had no effect on walls of sections treated with 400 mM mannitol (Fig. 2). This lack of an IAA effect in sections treated with 400 mM mannitol was not due to plasmolysis. The measured osmotic pressure of the sections was 9.9 bars initially, and the osmotic pressure of a 400 mM mannitol solution is 9.0 bars. Direct microscopic examination indicated that after 1 hr of incubation only those cells immediately adjacent to the cut ends of the sections were plasmolyzed by 400 mM mannitol. With longer incubations no plasmolyzed cells were observed. Also, even though there were no IAA effects on wall glucose, other aspects of wall metabolism were relatively unaffected by 400 mM mannitol as increases in wall xylose and wall arabinose were observed (11).

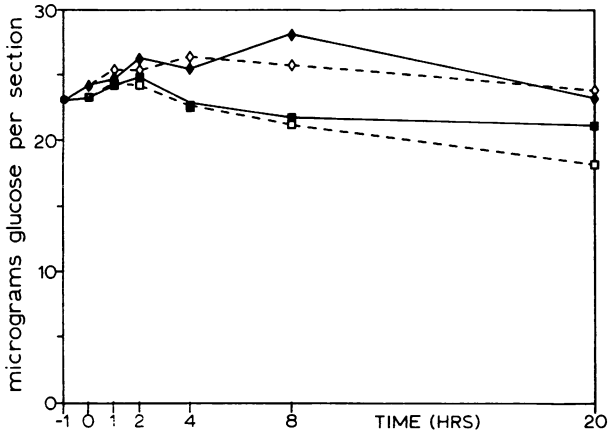

FIG. 1. Changes in noncellulosic glucose content of cell walls of coleoptile sections treated with 50 mM glucose plus 200 or 400 mM mannitol with and without 12.5 μM IAA. Samples were pretreated with the appropriate concentration of mannitol 1 hr prior to addition of IAA at time zero. 200 mM mannitol plus IAA (□); 200 mM mannitol minus IAA (■); 400 mM mannitol plus IAA (◇); 400 mM mannitol minus IAA (◆).

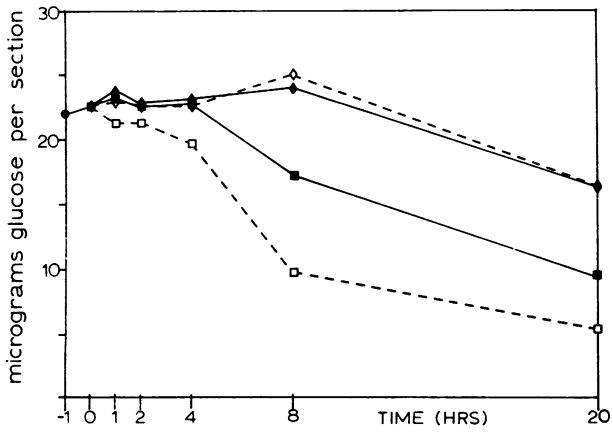

FIG. 2. Changes in noncellulosic glucose content of cell walls of coleoptile sections treated with 200 or 400 mM mannitol with and without 12.5 μM IAA and with no exogenous glucose. Samples were pretreated with the appropriate concentration of mannitol 1 hr prior to addition of IAA at time zero. 200 mM mannitol plus IAA (□); 200 mM mannitol minus IAA (■); 400 mM mannitol plus IAA (◇); 400 mM mannitol minus IAA (◆).

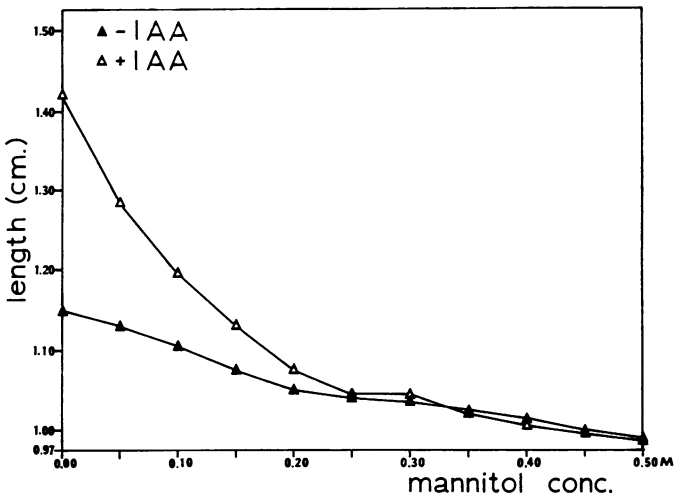

FIG. 3. Length of coleoptile sections after an 8-hr treatment with various concentrations of mannitol with and without 12.5 μM IAA.

Coleoptile sections initially 1.07 cm grew to 1.19 cm by the end of 20 hr of IAA treatment when incubated in 200 mM mannitol. The IAA-free controls grew to 1.10 cm. Sections incubated without mannitol, however, grow to 1.20 cm without IAA and to 1.54 cm with IAA. Although the presence of exogenous glucose resulted in more growth in mannitol-free controls (IAA-treated sections were 1.98 cm; the IAA-free controls, 1.29 cm), sections treated with 200 mM mannitol and 50 mM glucose for 20 hr grew to only 1.12 cm without IAA and to 1.23 cm with IAA. Sections treated with 400 mM mannitol did not grow, regardless of the presence or absence of exogenous glucose.

Although these experiments demonstrated the kinetic aspects of IAA effects on wall glucose at two levels of reduced turgor, they provided little information on the precise turgor sensitivity of the IAA effects. In subsequent experiments, coleoptile sections were incubated with concentrations of mannitol ranging from 0 to 500 mM. No exogenous glucose was provided. All sections were equilibrated for 1 hr prior to adding IAA, and then incubated for 8 hr. Figure 3 shows that increasing mannitol concentrations progressively inhibited growth in both IAA-treated and control sections. At the higher concentrations, some shrinkage was observed. No significant IAA-promoted growth was apparent at mannitol concentrations greater than 200 mM. Figure 4 shows the glucose content in walls of these sections. At low concentrations mannitol had no effect on the extent to which IAA caused an additional decrease in wall glucose. At concentrations greater than 200 mM, however, further increases progressively inhibited the IAA effect, and at 400 mM and higher concentrations there was no IAA effect on wall glucose.

From the results presented in Figures 3 and 4 a comparison can be made (Figure 5) of the extent to which IAA-induced in-

crements of growth and IAA-induced decreases in noncellulosic wall glucose were inhibited by increasing mannitol concentration. Although mannitol concentrations less than 250 mM had no effect on IAA-induced decreases in noncellulosic wall glucose, IAA-induced growth was inhibited severely. The extent of the IAA-induced change in noncellulosic wall glucose was clearly independent of the increment of IAA-induced growth.

Preliminary results showed that a 1-hr pre-equilibration in mannitol was necessary prior to adding IAA. For example, with no pre-equilibration, IAA treatment resulted in an 18% decrease in wall glucose in sections treated 20 hr with 400 mM mannitol without exogenous glucose.

The various wall analyses also indicated that mannitol had effects aside from those on turgor. Table I compares the effects of various treatments on content of trifluoroacetic acid-liberated mannose of coleoptile cell walls. In sections treated with both mannitol and glucose, mannose content increased strikingly, 10-fold in one treatment. In the absence of either glucose or mannitol only slight or moderate increases were observed.

Because of reports of reduced turgor pressures affecting respiration rates (15), the possibility that turgor effects on auxin-induced wall changes were the result of modified respiration rates was investigated. Effects of mannitol and IAA on respiration are listed in Table II. As reported by Ordin *et al.* (15), the presence of 400 mM mannitol initially depressed the respiration rate and inhibited the IAA-enhanced increment of respiration. This, however, proved to be a transient effect since with further incubation respiration rates recovered, and after 19 hr incubation 400 mM mannitol-treated sections were respiring at rates greater than either the controls or 200 mM mannitol-treated sections. In another experiment the initial inhibitory effect of 400 mM mannitol appeared to be readily reversible. Preincubation of sections in 400 mM mannitol followed by restoration of high turgor had no effect on subsequent response of respiration to IAA (Table III).

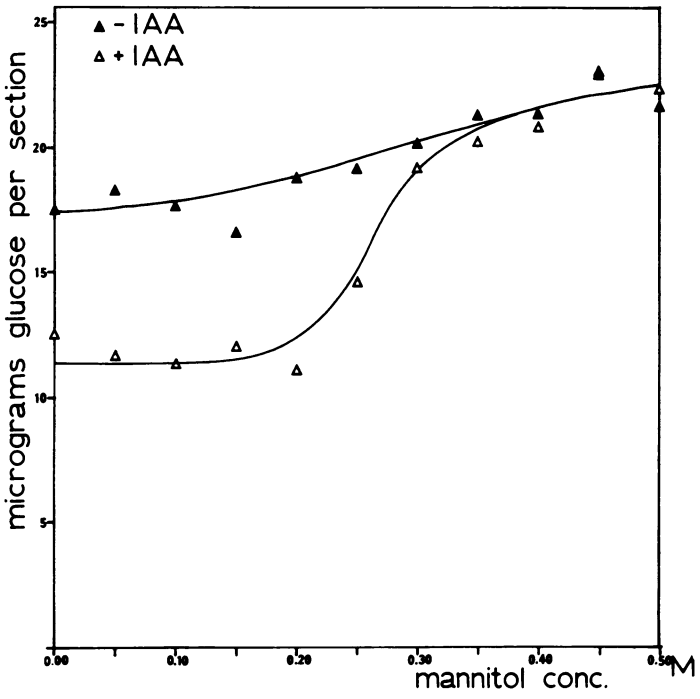

FIG. 4. Noncellulosic glucose content of cell walls of coleoptile sections after an 8-hr treatment with various concentrations of mannitol with and without 12.5 μM IAA.

DISCUSSION

Many investigators (5–7, 19) have shown that turgor pressure is required for cell elongation. Turgor pressure is also

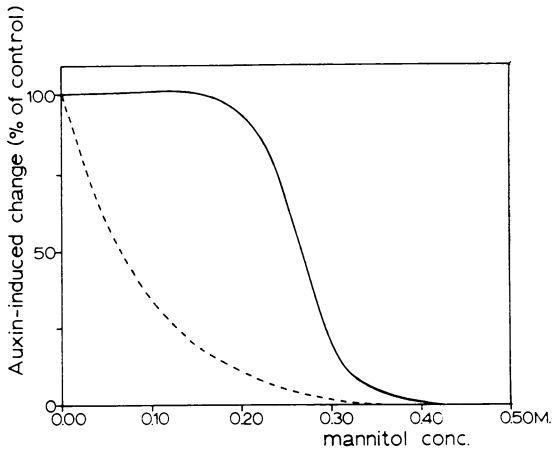

FIG. 5. Effects of increasing mannitol concentration on auxin-enhanced growth (----) and auxin-enhanced decreases in noncellulosic glucose (——).

Table I. *Trifluoroacetic Acid-liberated Mannose of Avena Coleoptile Cell Walls after 20-hr Treatments with and without Glucose, IAA, and Mannitol*

Initial mannose content was 0.7 μg/section.

Mannitol Treatment	Mannose Content			
	−Glucose		+ 50 mM Glucose	
	−IAA	+ 12.5 μM IAA	−IAA	+ 12.5 μM IAA
mM	*μg/coleoptile μg/section*			
0	0.9	0.9	0.7	1.0
200	1.2	0.9	2.2	2.1
400	1.5	1.4	5.4	8.0

Table II. *Effects of IAA and Mannitol on Rate of Respiration of Avena Coleoptile Sections*

Total Incubation Time	Rate of Oxygen Uptake					
	0 mannitol		200 mM mannitol		400 mM mannitol	
	−IAA	+IAA	−IAA	+IAA	−IAA	+IAA
hr	*μl/hr coleoptile section*					
1	2.04 ± 0.10		2.00 ± 0.09		1.77 ± 0.08	
4	2.13 ± 0.07	2.68 ± 0.09	2.38 ± 0.03	2.58 ± 0.24	2.08 ± 0.08	2.15 ± 0.05
19	1.42 ± 0.11	1.36 ± 0.14	1.34 ± 0.06	1.48 ± 0.03	2.18 ± 0.15	2.29 ± 0.05

Table III. *Effect of IAA on Oxygen Uptake of Avena Coleoptile Sections after a 2-hr Pretreatment with or without Mannitol*

Pretreatment	Rate of Oxygen Uptake	
	−IAA	+ 12.5 μM IAA
	μl/hr·coleoptile section	
Buffer	2.39 ± 0.05	2.68 ± 0.06
Buffer + 400 mM mannitol	2.37 ± 0.06	2.74 ± 0.05

required for auxin-induced wall modifications which result in increased wall extensibility (2). The results presented in this paper provide evidence that turgor pressure is required for an auxin-induced modification of wall composition.

This turgor requirement suggests several possible explanations for the mechanism of IAA-induced wall modifications which are consistent with ideas of other investigators in explaining the apparent requirement for a critical turgor in growth and also the turgor requirement for auxin-enhanced wall extensibility (1, 2, 13, 19).

One possible explanation is that turgor results in some sort of conformational change in the wall structure, exposing susceptible sites and allowing wall-modifying factors to come into contact with specific wall components.

A second possible explanation is that wall modifications occur only when specific linkages within the wall are under stress (20). The results reported here are consistent with such a mechanism in that the wall must be under tension in order for the decreases in glucose to occur. The decreases in glucose, however, are not proportional to the increases in turgor, a feature that is inconsistent with a simple stress-relaxation mechanism.

A third possible explanation is that auxin-induced wall modifications involve reversible reactions. Stresses on the wall would modify the reversibility of the reactions involved either by physically separating the components of a linkage once that linkage is broken or by causing the original components of a linkage to reform in an altered configuration. The reversible reactions involved in such a mechanism might actually involve two separate processes: one a turgor-dependent auxin-enhanced cleavage of load-bearing crosslinks in the wall, and the other a wall-stabilizing process responsible for reformation of crosslinks in new configurations. The wall modifications observed here could be a result of the first of these two processes, cleavages of linkages involving glucose resulting in loss of glucose from the noncellulosic fraction of the wall. The second process would stabilize the modified wall by reformation of altered crosslinks. The reformation of crosslinks would account for the apparent absence of auxin-induced stored growth in *Avena* coleoptile tissue during periods of reduced turgor (4). Also, while cleavage of a crosslink might not initiate a certain amount of extension at reduced turgor, the same crosslink in the absence of displacement could result in changes in the physical properties of the wall. These changes might account for the discrepancies between auxin-induced wall loosening, as measured by the Instron technique, and actual cell wall extension (3).

It may be that the effects of reduced turgor are not on the wall itself, but on events leading to production or activation of wall-modifying factors. Increases on osmotica have been shown to suppress some of the hormonal effects of GA on metabolism (8, 10). Results reported here show inhibition of oxygen uptake. Increased mannitol concentrations have also been shown to inhibit the incorporation of labeled glucose into the wall (2, 14, 18).

Whatever the explanation for the role of turgor in controlling the extent of auxin effects on the wall, it is clear that there is a correlation of the turgor requirement of these compositional changes in the wall with previous reports of turgor requirements for growth and auxin-enhanced wall extensibility. These similarities suggest a possible specific role of these particular wall modifications in wall extension.

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